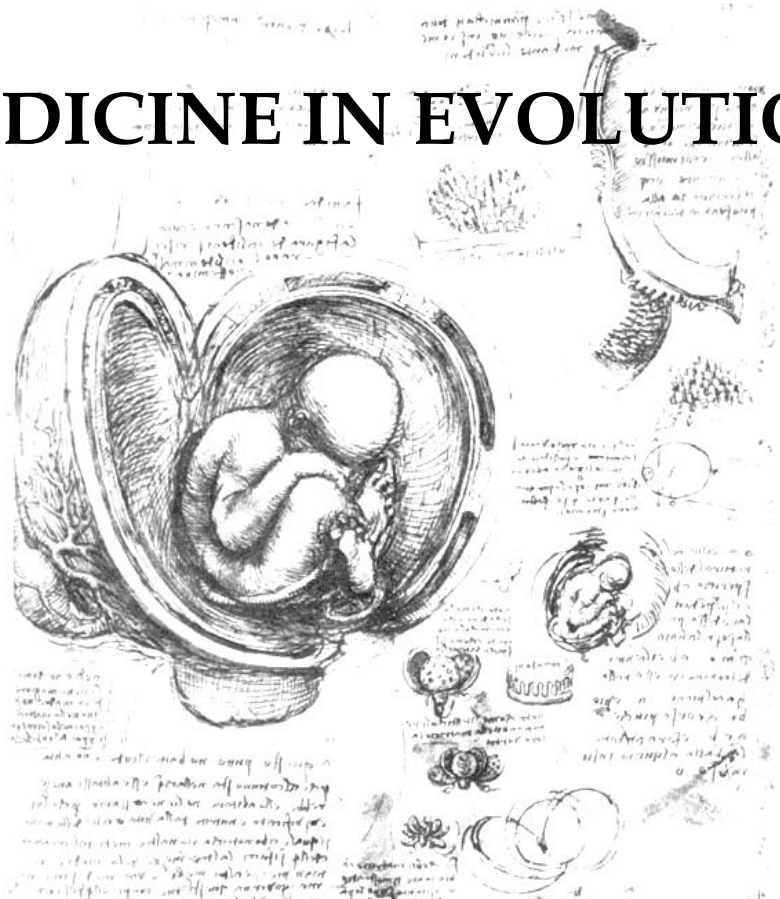
Volume XVIII, Nr. 1, 2012, Timişoara, Romania

ISSN 2065-376X

MEDICINE IN EVOLUTION



CENTER OF PROMOTING HEALTH EDUCATION AND MOTIVATION FOR PREVENTION IN DENTISTRY

CENTER FOR CONTINUOUS MEDICAL EDUCATION

http://umft.ro/newpage/medicineinevolution

Journal edited with the support of the

Timis County Council

Main sponsor

Mr. ROLF MARUHN

Consul of Germany in Timisoara

(2004 – 2009)

Printed at: WALDPRESS, Timisoara,
17 Brandusei Street,
Phone/Fax: 0040256422247

Edited at: EUROSTAMPA, Timisoara
26, Revolutiei 1989 Street,
Phone: 0040256204816

EDITORIAL BOARD

CONTENTS

ARTICLES

Recomandări privind rezumatele lucrărilor:

- rezumatele prezentărilor vor fi publicate în suplimentul special al revistei Medicine in Evolution şi se vor conforma următoarelor standarde:
  - rezumatele comunicărilor orale vor fi redactate în limba română şi engleză
  - vor fi precizate titlul prezentării (cu majuscule), numele complet al autorilor şi afilierea acestora
  - rezumatele se vor limita la 250 de cuvinte
  - rezumatele vor fi strucurate astfel: obiectivul studiului, material şi metodă, rezultate şi discuţii, concluzii
  - rezumatele conferinţelor vor fi de maxim 400 de cuvinte şi vor fi redactate în limba engleză.
  - se admite o prezentare orală în calitate de prim autor
- rezumatele vor fi redactate în Microsoft Word cu caractere Times New Roman RO, normal, 10
- timpul alocat comunicărilor orale este de 10 minute
- rezumatele vor fi expediate prin email la adresa boarium@yahoo.com
- Comitetul ştiinţific al Congresului îşi rezervă dreptul respingerii rezumatelor care nu îndeplinesc criteriile ştiinţifice şi de tehnoredactare.
- Comitetul ştiinţific al Congresului va notifica autorilor prin email acceptarea rezumatelor şi includerea lor în programul ştiinţific al congresului.
- Nu se admit rezumate fără dovada de achitare a taxei de participare.
- **Termenul limită pentru trimiterea rezumatelor este 11 aprilie 2012. După această dată, din raţiuni tehnice, niciun rezumat nu va mai fi acceptat.**
- **Organizatorul nu îşi asumă responsabilitatea expedierii mapei şi diplomei de participare. Acestea pot fi ridicate doar de la Secretariatul Congresului.**

CMDR
COLEGIUL MEDICILOR DENTIŞTI DIN ROMÂNIA
COLEGIUL JUDEŢEAN TIMIŞ

COLEGIUL MEDICILOR DENTIŞTI DIN ROMÂNIA
FILIALA TIMIŞ

organizează

CONGRESUL CU PARTICIPARE INTERNAŢIONALĂ ZILELE MEDICALE BĂNĂŢENE EDIŢIA a XVII - a MEDICINA MILENIULUI III

3rd Millenium Medicine The International Congress of Medical Days in Banat 17th EDITION Timişoara, 9-11 Mai 2012

Al doilea anunţ

Hotel Club Senator
Timişoara, Calea Lugojului

Preşedinte:

Prof. Univ. Dr. Mihai Romînu
Preşedintele Colegiului Medicilor Dentişti Timiş

Preşedinte de onoare:

Prof. Univ. Dr. Ioana Nica

Lectori invitaţi din străinătate:

Prof. Dr. Holger Jentsch - Parodontologie
Leipzig, Germania

Prof. Dr. Andrej Kielbassa - Prevenţie
Krems, Austria

Prof. Dr. Denis Lynch - Chirurgie, Patologie orală
Milwaukee, Wisconsin, SUA

Prof. Dr. Nagy Gábor - Patologie orală
Budapesta, Ungaria

Conf. Dr. Madléna Melinda - Ortodonţie
Budapesta, Ungaria

P. D. Dr. med. dent. Sigrun Eick - Microbiologie orală
Berna, Elvetia

P. D. Dr. Dr. med. dent. Adrian Kasaj - Parodontologie
Mainz, Germania

Dr. med. dent. Peter Kiefner - Endodonţie
Stuttgart, Germania

Dr. med. dent. Tom Schloss - Endodonţie
München, Germania

Dr. med. dent. Mark Antal - Odontoterapie-Endodonţie
Szeged, Ungaria

• INFORMAŢII GENERALE

LOCAŢIA:
Timişoara, Hotel Club Senator – Calea Lugojului
Limbile oficiale ale congresului: română şi engleză

TERMENE LIMITĂ:
Trimitere rezumate: 11.04.2012
Trimiterea scrisorilor de acceptare sau respingere: 20.04.2012

Rezumatele vor fi trimise pe urmatoarea adresa de e-mail: boarium@yahoo.com, Dr. Marius Boariu, tel. 0722-701871

• DATE DE CONTACT:

În comunicarea cu organizatorul, vă rugăm să folosiţi următoarea adresă de email: timis@cmdr.ro sau
Tel: 0741 – 010252 Piticea Eugenia
0356 – 881199 CMDR Timiş
Tel/Fax: 0356 – 442530 CMDR Timiş

Manifestarea are un profund caracter ştiinţific şi formativ, fiind creditată cu 50 puncte E.M.C. de către Colegiul Medicilor Dentişti din România.

• PROGRAM PRELIMINAR

Miercuri 9 mai 2012
- Orele 08.00 – 10.00
  - Informaţii
  - Înscrieri
- Ora 10.00 – Deschiderea lucrărilor Congresului cu Participare Internaţională „Zilele Medicale Bănăţene”
- Orele 10.30 – 13.00 Conferinţe, comunicări ştiinţifice
- Orele 13.00 – 14.30 - Pauză de prânz
- Orele 14.30 – 18.30 Conferinţe, comunicări ştiinţifice

Joi 10 mai 2012

- Orele 09.00 - 13.00 - Conferinţe, comunicări ştiinţifice
- Orele 13.00 - 14.30 - Pauză de prânz
- Orele 14.30 - 18.30 - Conferinţe, comunicări ştiinţifice
- Ora 20.30 – Cocktail (Restaurant Hotel Club Senator)

Vineri 11 mai 2012

- Orele 09.00 - 13.00 - Conferinţe, comunicări ştiinţifice
- Orele 13.00 - 14.30 - Pauză de prânz
- Orele 14.30 - 18.30 - Conferinţe, comunicări ştiinţifice

• TAXA DE PARTICIPARE

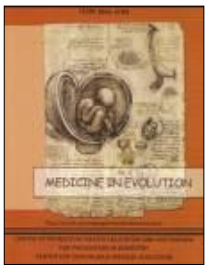
Taxa de participare pentru participanţii la „Zilele Medicale Bănăţene” se va achita în contul nr. RO79BPOS36306935448RON01 BANCPOST Timişoara, beneficiar COLEGIUL MEDICILOR DENTIŞTI DIN ROMÂNIA FILIALA TIMIŞ, C.U.I.: 17094018.

Talonul de achitare a taxei va cuprinde: Numele, Prenumele medicului pentru care se plăteşte taxa, Denumirea cabinetului, Judeţul şi Localitatea.

Taxa de participare	
Medici dentişti	350 RON
Medici rezidenţi	150 RON
Medici pensionari şi peste 65 de ani	150 RON
Studenţi	gratuit

Taxa de participare include:
- diploma de participare
- mapa cu documentele congresului şi ecuson
- accesul la programul ştiinţific
- cocktailul de bun-venit oferit de organizatori

BENEFICIAL EFFECTS OF INTENSIVE STATIN THERAPY AND STATIN-EZETIMIBE COMBINATION THERAPY IN CARDIO-VASCULAR DISEASES



GLIGOR LAVINIA CODRUTA[1] , GLIGOR SERBAN[2]

1. **Selfmed Polyclinic, Timisoara,**
2. **West University of Timisoara, Physical Education and Sport Faculty, Kinetotherapy Department**

ABSTRACT

The increased morbidity risk as well as cardio-vascular death risk is related to increased LDL cholesterol. Recent trials on patients suffering of coronary artery disease proved a reduction of arteriosclerosis progress by using intensive statin treatment compared to the case when moderate treatment has been applied.

The reduction of arteriosclerosis progression related to intensive statins treatment is caused by significant reduction of both lipoproteins level and C-protein level, suggesting a connection between those two biomarkers and disease progress; PCR is a marker of atherosclerosis inflammation and is useful to predict pathogen cardio-vascular incidents.

In order to reduce cardio-vascular incidents, cholesterol has to be within the targeted limits which are lowered in every new therapy guidelines.

Side effects often occur and are much more serious in high doses, while at low and medium concentrations statins side effects are well tolerated. For this reason, the statin-ezetimib combination therapy, could be a viable alternative to high dosage statins monotherapy and also better results in lowering the cholesterol level can be achieved since the LDL level reduction is more significant when using ezetimib combined with statins.

Decisions regarding treatment alternatives should consider not only the serum lipids level, but also the risk assessment of patients regarding cardio-vascular incidents.

***Key Words**: LDL Cholesterol (LDLc), C reactive protein (CRP), ezetimibe, statin-ezetimibe combination therapy*

Correspondence to:

Gligor Lavinia Codruța, MD, PhD candidate
Adress: Selfmed Polyclinic, Timişoara, Behelei str., No. 2
Phone: 0356265091, 0728331059
E-mail address: lavinia_linta@yahoo.com

INTRODUCTION

Nowadays, the frequencies of cardio-vascular diseases occurence increased, especially in youngsters, the main causes (which can be easily modifiable) being dyslipidemia, high blood pressure as well as obesity.

These are the main causes of death in adult population and if these would be removed or at least reduced, the middle life duration expectations could be raised up to 7 years. Increased cardio-vascular morbidity and mortality is strictly related to increased serum level of LDLc. Statins therapy is the basic treatment in reducing and prevention of cardio-vascular events by selective activity over HMG-CoA reductase, key enzyme in cholesterol synthesis, thereby reducing serum level of LDLc.

MATERIALS AND METHODS

Recent studies proved that statins have pleiotropic, beneficial, activity, useful in endothelial function, plaque stabilization, angiogenesis, vascular cytoprotection and also antioxidizing, anti-inflammatory, immuno-modulating and antithrombotic properties, beside hypolipemiant activity [5].

The plaque stabilization is accomplished by reducing the inflammatory cell infiltration and macrophage synthesis and also by increasing the collagen synthesis and the content of vascular smooth muscle cells. Endothelial function is influenced by NO endothelial synthase expression increase, endothelial 1 expression decrease, with the preservation of endothelial cell coronary function, myocardic perfusion and coronary vasa vasorum.

The antioxidizing effect is achieved by NADPH oxydase inhibition, decreasing the oxidation of LDL and of the superoxide radical formation, and by increasing O^2 free radicals concentration. The immuno-modulator effect is done by decreasing the γ interferon, decreasing the T-cells activation, decreasing the macrophages activation and by increasing the antigen 1 inhibition of leukocyte function. The anti-inflammatory function is done by increasing the nitric oxide concentration and decreasing the nuclear kB factor activation, decreasing the endothelial cells activation, decreasing the activity of proinflammatory cytokines, C reactive protein (CRP) and leukocyte-endothelial cells adhesion. The antithrombotic effect is achieved by increasing the fibrinolytic activity of endothelial cells, increased Ecot-5′ nucleotidase and decreasing the tissue factor expression, the thrombocytes activation and as a result reducing cerebral ischemia and strokes occurs [6]. Additionally, statins treatment has superior effect than other classes of hypolipemiant drugs because of the good therapeutic efficiency / side effect ratio. Moreover, this therapy is associated with decreased level of C reactive protein in patients without clinical symptoms of atherosclerosis, because of the statin antiinflammatory effect. This effect can be very useful in prevention of acute cardio-vascular events, independent to decreasing serum level of lipids. The reduction of atherosclerosis progression associated to high statin doses is connected to significant reduction of lipoprotein and C reactive protein levels, suggesting a connection between these two biomarkers and disease progression [3].

The 3rd Edition of NCEP/ATP III guide ("National Cholesterol Education

Programe-Adult Treatment Panel III") [4] recommends the correlation of aggressive therapy for reducing the LDLc to the absolute patients risk of coronary heart disease. This guide is based on Framingham risk factors, according to which the LDLc levels vary with the patients risk category (Table I).

Table 1 Variation of LDLC levels with patient risk category (NCEP-ATP III)

Risk category	LDLc target (mg/dL)	Level of LDLc (mg/dL) at which the lifestyle needs to be changed	Levels of LDLc (mg/dL) at which drug therapy is required
Coronary heart disease or equivalent (diabetes mellitus, stroke, arteriopathy a.a.)	< 100	≥ 100	≥ 130 (between 100-129 - optional drug therapy)
2 or more risk factors	< 130	≥ 130	≥ 160
None or only one risk factor	< 160	≥ 160	≥ 190 (between 160-189 - optional drug therapy)

Thus, patients with a history of coronary heart disease or equivalents (diabetes mellitus, symptomatic carotid artery disease, abdominal aortic aneurysm, peripheral artery disease) and those with two or more risk factors having the highest risk of acute cardio-vascular events in the next 10 years (over 20%).

Patients without risk factors or with one risk factor presents a small possibility (under 10%) to have an acute cardio-vascular event over the next 10 years.

The physician assesses the patients risk for cardio-vascular events, based not only on the increased LDLc level – this being only one clinical parameter. The higher the risk is (by the presence of atherosclerosis, diabetes mellitus, high blood pressure, smoking, old age), the more aggressive the statins therapy must be, with high doses of statins [12].

Disadvantage is that high doses of statins increase the frequency of adverse effects, such: elevated transaminases, myositis, rabdomiolisis, a.a.

Muscle damage is dose dependent and occurs frequently in statins drug interactions; severe forms of muscle damage, such myopathies and rabdomiolisis, having an incidence of 1 per 100.000 patients.

RESULTS

PROSPER and CARE ("The Cholesterol and Reccurent Events") studies (using Pravastatin) as well as HPS ("Heart Protection Study") and 4S

(„Scandinavian Survival Study") (using Simvastatin) showed a carcinogenic potential of statins treatment, although no significant statistic evidence could be provided.

Breast cancer incidence occurred in female patients treated with Pravastatin during PROSPER and CARE ("the Cholesterol And Recurrent Events trial") studies and the large number of non-melanoma skin cancers seen in patients treated with Simvastatin, during HPS and 4S trials, were assigned to hazard. Also, the increased frequency of neoplasms in PROSPER trials ("PROspective Study of Pravastatin in the Elderly at Risk") have been attributed to the fact that aggressive statine treatment accelerated the symptoms of neoplasia to the patients with increased neoplasm risk, how the elders are [10].

Patients without a high risk of cardio-vascular events should not be treated with hypolipemiants only because of an abnormal lipid profile (increased), because no significant reduction of mortality will occur.

Clinical trials have prouved that high statin doses therapy reduced ischemia at patients with acute coronary syndrome (compared to placebo) as well as in patients with stable coronary artery disease [9].

A comparative randomized, double-blind, multicentric, active controlled trial with statins is the REVERSAL study („The REVERSing Atherosclerosis with Aggressive Lipid Lowering"), performed in the USA on patients using two of the most familiar statines: Atorvastatin – 80 mg/day and moderate doses of Pravastatin – 40 mg/day. Unlike Pravastatin, the intensive treatment using Atorvastatin reduced the progression of the coronary atherosclerosis in patients with ischemic cardiac disease; the monitoring of atherosclerosis has been performed by intravascular ultrasound. No progression in atheroma was noticed in patients treated with Atorvastatin, but in patients treated with moderate doses of Pravastatin, the atheromatous injuries evolved.

These differences could be explained by the higher reduction of atherogenic lipoproteins (LDLC) and C reactive protein in patients treated with Atorvastatin [1]. Another randomized, placebo controlled, double-blind trial showed that the mortality and frequency of major cardio-vascular events were reduced in patients with coronary syndrome treated with Atorvastatin (80 mg/day).

A recent study, published in the first months of 2007, proved the effects of intensive statin therapy compared to the moderate one in decreasing the level of plasma lipids in older patients with coronary heart disease [11].

AVERT Study („Atorvastatin versus Revascularization treatment") proves that intensive statin therapy (Atorvastatin 80 mg/day) is as good as percutaneous coronary angioplasty in reducing the ischemic events in patients with stable angina. After 18 months of treatment, patients under 80 mg/day Atorvastatin had a 31% total cholesterol reduction and 46% LDLc reduction and 13.4% had ischemic events compared to 20.9% of patients with percutaneous coronary angioplasty. Also, the time until a new ischemic event occurred was significantly higher in the group treated with Atorvastatin.

The comparative trials PROVE-IT („Pravastatin Or Atorvastatin Evaluation And Infection Therapy") and REVERSAL („Reversing Atherosclerosis with Aggressive Lipid Lowering"), ALLIANCE („Aggressive Lipid Lowering Initiation Abates New Cardiac Events") and TNT („Treating to New Targets Study") studies showed that high doses of statins will better reduce

the cardio-vascular events in high risk patients, although the side effects would be significant. Treatment of patients with high cardio-vascular risk using high doses of statins is useful, but treating the patients with low cardio-vascular risk will bring little benefints and significant side effects [10].

While small and medium doses of statins are well tolerated, in high dosage the side effects are significant. For this reason the statin-ezetimibe combination therapy can be an alternative.

Ezetimibe is the first drug of a new class of hypolipemiants called SCAII (Selective Cholesterol Apsorption Inhibitor). It selective inhibits the transportation and intestinal apsorbtion of endogenous and exogenous cholesterol, without interfering in the intestinal apsorption of fatty acids, bile acids, triglycerides or liposoluble vitamins (A, D, E, K) [2]. Ezetimibe exerts its effect through it's active metabolite – the glucuronidate – which affects the chilomicrons (which have an important role in atherosclerosis appearance).

The inhibition of cholesterol apsorbtion by ezetimibe monotherapy (10 mg/day) produces a significant reduction of 18% in LDLc, after 9-12 weeks of treatment.

The statin – ezetimibe combination therapy produced an additional 18% reduction of LDLc levels. This is very important, since the major LDL cholesterol reduction takes place at the first doses of statins, increasing doses giving only a small benefit, but also an increase in side effects. Beside the reduction of LDLc, ezetimibe has a good effect on triglycerides and HDLC. The statin – ezetimibe combination therapy, in patients with hypercholesterolemia, causes the reduction of cholesterol to the target values, without the increase of side effects risks, which occur in case of high doses statins therapy [7].

A study conducted during 2003-2005 period in Long Beach (USA), on 218 patients treated with ezetimibe and other hypolipemiants compared the efficiency of the association of ezetimibe in reduction of LDLc levels.

They found an overall reduction of total LDLc of 23% in the patients treated with ezetimibe.

Patients treated with statins as monotherapy obtained a greater reduction of LDLc, after the administration of 10 mg ezetimibe, compared to those treated only with statins as monotherapy [8].

CONCLUSIONS

Currently, an increase of statins usage occurs, because of the high frequency of cardio-vascular diseases and because of the favorable risk / benefit ratio compared to other drug classes. The statin – ezetimibe combination therapy could be a better alternative than high doses statins monotherapy and could provide a better solution to reduce cholesterol levels to the target values.

Ezetimibe treatment or the statin-ezetimibe combination therapy has good effects on mortality and morbidity reduction in patients with coronary heart disease. Ezetimibe monotherapy in 10 mg dosage is recommended in patients with low statins tolerance

The statins – ezetimibe combination therapy is recommended in patients with high LDLc and/or cholesterol levels under appropriate statins monotherapy and in patients with familial hypercholesterolemia (ezetimibe 10 mg can be associated with medium and high doses of statins).

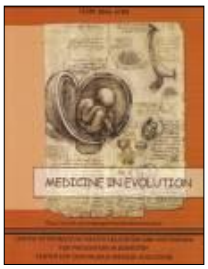
High doses of statins are recommended in treating patients with acute coronary syndrome, as well as in severe hypercholesterolemia, homozygous forms (in combination with ezetimibe).

Treating patients with high doses of statins is useful in those with the highest risk of cardio-vascular events, but is less efficients and with less benefits in patients with low risk of cardio-vascular disease.

REFERENCES

1. Cannon C.P., Steinberg B.A. et al. Meta-analysis of cardiovascular outcomes trials comparing intensive versus moderate statin therapy, J. Am. Coll. Cardiol. 2006; 48(3): 438-445.
2. Cristodorescu R. Actualităţi în farmacoterapia hipolipemiantă în prevenţia secundară a cardiopatiei ischemice şi modalităţi de creştere a aplicării ei în teren. Medicină Internă. 2006; Vol. III, nr.1, pag. 49-58
3. Kapoor A.S., Kanji H. et al. Strengh of evidence for perioperative use of statins to reduce cardiovascular risk: systematic review of controlled studies, BMJ, 2006; 333 (7579):1149
4. NCEP – ATP III. Expert panel on detection, evaluation, and treatment of high blood pressure in adults. Executive summary of the third report of the National Cholesterol Education Program (Adult Treatment Panel III), JAMA, 2001; 285: 2486-2497
5. Lablanche J.M. Place des statines dans les accidents coronaires aigus, Therapie, 2003; vol. 58 (4), No 1, pp. 59-62
6. Mason J.C. Statins and their role in vascular protection, Clinical Science, 2003; 105: 251-266
7. Mead Mike. Ezetimib (Ezetrol) un nou medicament, Medical UPDATE, decembrie, 2005; pag. 198–199
8. Meyers Cd, Moon YSK, Ghanem H., Wong ND. Type of preexisting lipid therapy predicts LDLc response to Ezetimibe, Ann. Pharmacother. 2006; 40: 818-823
9. Morozova S., Suc-Rofer I., Auwerx J. Modulateurs du metabolism du cholesterol et avenir du traitment de l'atherosclerose, Médecine Science, 2004; Vol. 20, No. 6-7, Juin-Juillet, 685-690
10. Ong H. T. The statin studies: from targeting hypercholesterolaemia to targeting the high risk patient, QJM, 2005; 98(8): 599-614
11. PRAKASH D., STONE P.H. et al. Effects of intensive versus moderate lipid lowering therapy on myocardial ischemia in older patients with coronary heart disease, Ciculation, 2007; 115: 700-707
12. SPRATT K.A. Reducing the risk of coronary heart disease via lipid reduction, JAOA, 2004; vol. 104, No 9, Suppl., 9-13

EVALUATION OF THE SEVERE MITRAL REGURGITATION MECHANISM USING TRANSTHORACIC THREE-DIMENSIONAL ECHOCARDIOGRAPHY: A COMPARATIVE STUDY WITH THE INTRAOPERATIVE FINDING



PENTERIDIS IOANNIS[1], MORNOS CRISTIAN[1], IONAC ADINA[1], FEIER HORIA[2], GASPAR MARIAN[2], DRAGULESCU STEFAN IOSIF[1]

1. **II-nd Cardiology Clinic, Victor Babes University of Medicine and Pharmacy Timisoara,**
2. **IInd Cardiovascular Surgery Clinic, Victor Babes University of Medicine and Pharmacy Timisoara**

ABSTRACT

We investigated three-dimensional (3D) live transthoracic echocardiography (TTE) in the assessment of mitral regurgitation (MR) mechanism. Bidimensional (2D) TTE, 2D transesophageal echocardiography (2D TEE) and 3D TTE were performed in 51 patients with severe MR, prior to valve surgery. Using a surgical scoring protocol for recognition of the valvular segments, 2D and 3D methods were compared. Adequate echocardiographic visualization of the mitral segments was more frequently obtained by 3D TTE than by 2D TTE or 2D TEE. Total 3D TTE score were significantly better than 2D TTE or 2D TEE score. Using surgical classification as gold standard, the sensibility and specificity were 95% and 91 % for 3D TTE, 87% and 81% for 2D TTE, and 90% and 86% for 2D TEE, respectively. In conclusions, 3D TTE provides accurate analysis of MR mechanism and appears to be superior to 2D echocardiographic technique.

Keywords: mitral valve, mitral regurgitation, three-dimensional transthoracic echocardiography

Correspondence to:

Mornos Cristian MD
Adress: Victor Babes University of Medicine and Pharmacy - Timişoara, Institute of Cardiovascular Disease,13 A Gh. Adam st., Romania
Phone: +40 256207363, fax: +40 256207362, mobile: +40 744790381
E-mail address: mornoscristi@yahoo.com

INTRODUCTION

Conventional two-dimensional (2D) echocardiography can evaluate cardiac structure and function by providing crosssectional views of the heart. The examination requires one to mentally conceive a series of orthogonal, planar, or tomographic images as a multidimensional reconstruction. The mental reconstruction is often difficult and inadequate in understanding of complex cardiac structures. As repair techniques have advanced, so has the need to obtain accurate information prior to surgery. Some studies suggest that adding three-dimensional (3 D) imaging to standard 2D echocardiography could be helpful in the quantification of valvular diseases. Three-dimensional echocardiography is a new emerging technique that allows recording of volumetric echographic data [1]. This technique can provide views of the entire valve, allowing a complete assessment of the valve leaflets and commissures. Several authors recommended already to integrate this new technique in the standard preoperative examination and become an important tool in the decision to process valvular repair/replacement [1, 2].

AIM AND OBJECTIVES

The aim of the present study was to evaluate the diagnostic value of 3 D live transthoracic echocardiography (3 D TTE) compared to 2 D imaging (2 D TTE and 2D transesophageal echocardiography - TEE) for mitral valve anatomy assessment and mitral regurgitation (MR) mechanism using as a standard the surgical findings in a group of patients with different etiologies of severe MR.

MATERIALS AND METHODS

Sixty consecutive patients with severe MR referred to surgery were evaluated by 2D TTE 2D TEE and 3D TTE, respectively. Exclusion criteria were represented by inadequate echocardiografic images, movement artifacts caused by coughing or hiccough, difficulty with ECG registering, and equipment failure.

A complete TTE study (2D and 3D) and 2D TEE study were obtained using Vivid 9 GE echocardiographic system, prior to surgery in a time gap <7 days. Three-dimensional and 2D data were compared with surgical data. The local ethics committee approved the study. Informed consent was obtained from all patients.

The Carpentier nomenclature was applied to the mitral leaflets [3]. The so-called posterior leaflet scallops were classified as lateral (P1), middle (P2), and medial (P3) starting from the anterolateral commissure. The anterior leaflet segments were classified as lateral (A1), middle (A2), and medial (A3) using the facing segment of the posterior leaflet as the referral points. All valvular segments were classified as normal, prolapsing, flail, tenting, perforation, erosion, fibrosis or calcification. The fusion of the commissures, vegetations and ruptured chordae were also noted. Gross etiology was classified as degenerative, congenital, rheumatic disease, infective, or functional. Three-dimen-

sional acquisition and reconstruction times were measured in each patient. A single echocardiographer acquired and measured the 2D and 3D echo studies.

The surgeon described the anatomy of the mitral valve. He was aware of the 2D findings, but not of the 3D analysis. All valvular segments were classified as normal, prolapsing, flail, tenting, perforation, erosion, fibrosis or calcification. For the mitral valve the Carpentier classification was used[3]. The fusion of the commissures, vegetations and ruptured chordae were noted.

The 2D and 3D images were analysed off-line separately and blinded to the surgical findings. Segments considered inadequate were not included for the analysis. Valvular morphology data described by the operating surgeon was used as gold standard. He described the anatomy of the valve using the same proforma like for echocardiographic analysis. The surgeon was aware of the 2D findings but not the 3D analysis. Segments were counted as accurately imaged if they matched surgical findings precisely in terms of pathology description and correct location (score = 1). The segments that were not adequately recognized (score = 0) were coded as inaccurate.

Statistical analysis

The sensitivity and specificity of the echocardiographic evaluation were calculated with surgical data as a reference. Variables are expressed as proportions, mean, and standard deviation. The chi-squared test and the student t-test with subsequent two-tailed t-tests were used to compare differences between groups. Receiver operating characteristic (ROC) curves were constructed to determine the performance of the echocardiographic techniques in detecting MR mechanism as defined by intraoperative findings.

Differences were considered statistically significant at the two-sided $p < 0.05$ level. All computations were carried out with the software SPSS 17.0 for Windows (SPSS Inc. Chicago, IL, USA).

RESULTS

From 60 patients with severe MR screened between January 2010 and June 2011, 51 were enrolled in the study (27 women, 53%). Mean age was 52 ± 13 years, and 23 patients (45%) presented atrial fibrillation. Characteristics of the patients study group are presented in Table I. For each patient, 1-3 acquisitions were realized and the best one chosen for imaging and quantification. Acquisition time ranged between 23 s and 4 min 19 s, depending on the basal heart rate and rhythm disturbances. The elapsed time for completion 3D TTE (13.5 ± 3.2 min) was shorter than for 2D TEE (23.4 ± 5.2 min, $p < 0.001$) and 2D TTE (17.0 ± 4.1 min, $p < 0.001$). Adequate echocardiographic visualization of the valve segments was more frequently obtained by 3D TTE than by 2D TTE and 2D TEE imaging, respectively (387/408 by 3D TTE, 351 / 408 by 2D TTE, and 369/408 by 2D TEE, respectively, each $p < 0.05$). The valve leaflets segments were more clearly identified by 3D TTE than by 2D TTE or 2D TEE (293/306 by 3D TTE, 362/306 by 2D TTE and 277/306 by 2D TEE, respectively, each $p<0.05$). For adjacent commissures the results were similar by the two (94/102 by 3D TTE, 89/102 by 2D TTE and 92/102 by 2D TEE, respectively, each $p >0.05$).

The etiology of the MR was degenerative in 32 patients (62.7%), infectious endocarditis in 10 patients (19.6%)

and functional in 9 patients (17.7%). The mean mechanism of MR was represented by mitral flail (22 patients, 43.2%), leaflet prolaps (17 patients, 33.3%), leaflet tenting (4 patients, 7.8%), leaflet perforation (3 patients, 5.9%), annular dilatation (4 patients, 7.8%) and leaflet erosion (1 patient, 2%). The most frequent MR mechanism affected the P2 segment (fig. 1) of the posterior mitral leaflet (22 patients, 39.2%). The most of our patients presented one or two diseased mitral valve segments (one segment in 21 patients and two segments in 13 patients).

The surgeon description of the mitral valve anatomy was used as gold standard.

The 3D TTE technique identified the primary pathology in 93% of our patients, 2D TTE in 85% and 2D TEE in 88% of the patients. The 2D examination requires one to mentally conceive a series of orthogonal, planar, or tomographic images as a multidimensional reconstruction. Total 3D TTE score (fig. 2) for the mitral valve was significantly better than 2D TTE or 2D TEE score (mean score 7.51 ± 0.52 by 3D TTE versus 5.48 ± 0.95 by 2D TTE and 6.44 ± 0.72 by 2D TEE, each $p < 0.05$). This superiority of 3D TTE was irrespective of rhythm ($p<0.05$ for both, sinus rhythm and atrial fibrillation).

The superiority of 3D TTE is kept for each mitral leaflet: 2.74 ± 0.22 by 3D TTE vs 2.15 ± 0.35 by 2D TTE and 2.32 ± 0.29 by 2D TEE (each $p < 0.05$) for the anterior mitral leaflet, and 2.52 ± 0.41 by 3D TTE vs 1.91 ± 0.38 by 2D TTE and 2.08 ± 0.21 by 2D TEE (each $p < 0.05$) for the posterior mitral leaflet.

Table 1 Caries experience indexes at the first dental visit Baseline characteristics of the study group (data are presented as mean +/- standard deviation or number (%).

Characteristics	**Date**
Number of patients	51
Age, years	52 +/- 13
Woman/Male	27 (53%) / 24 (47%)
Body mass index, kg/m^2	26.3 +/- 4.8
Heart rate, beats/min	81 +/- 13
Atrial fibrillation	23 (45%)
Mean blood pressure, mmHg	98.3 +/- 13.7
New York Heart Association class	2.6 +/- 0.7
LV ejection fraction (%)	46 ± 12
PSAP, mmHg	45.6 +/-16.8

LV = left ventricle; PSAP = pulmonary systolic artery pressure.

For adjacent commissures the results were similar by the three echocardiographic methods (each $p > 0.05$). The receiver operating characteristic (ROC) curve areas for assessment MR mechanism (fig. 3) using 3D TEE (ROC area = 0.95) was higher than for those using 2D TEE and 2D TTE (ROC areas 0.91 and 0.87, respectively). Using surgical classification as gold standard, the sensibility and specificity were 95% and 91 % for 3D TTE, 87% and 81% for 2D TTE and 90% and 86% for 2D TEE, respectively.

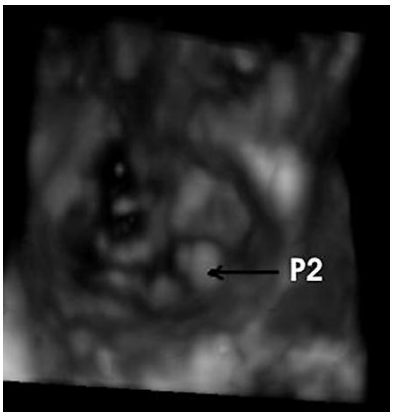


Fig. 1 Transthoracic three-dimensional echocardiography: view of the mitral valve prolapse as seen from the left atrium. P2 = the middle scallop of the posterior mitral leaflet.

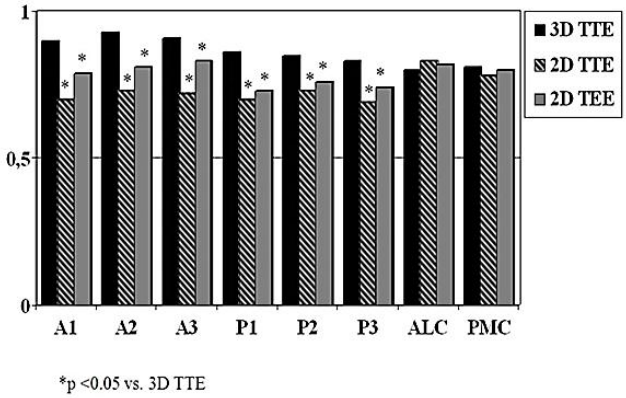


Fig. 2 Comparison between 3D live transthoracic echocardiography (3D TTE) score versus 2D transthoracic echocardiography (2D TTE) score and 2D transesophageal echocardiography (2D TEE) score for each mitral valve segment, using the intraoperative findings as gold standard. The posterior leaflet scallops are: lateral (P1), middle (P2), and medial (P3); the anterior leaflet segments are: lateral (A1), middle (A2), and medial (A3); ALC = antero-lateral commissure; PMC = postero-medial commissure.

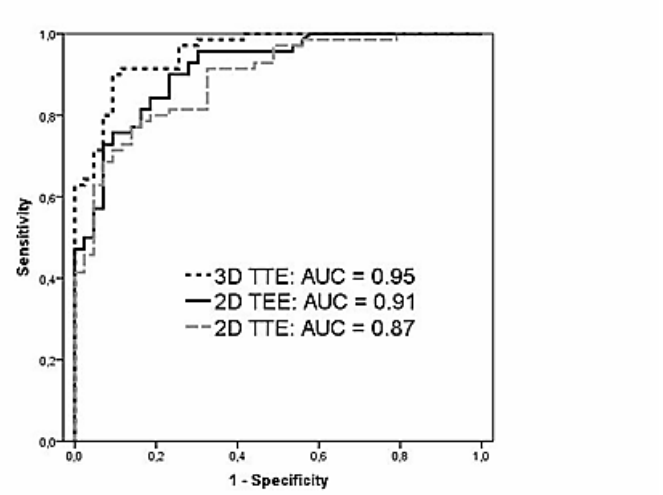


Fig. 3 The receiver operating characteristic (ROC) curve for assessment mitral regurgitation mechanism using 3D live transthoracic echocardiography (3D TTE), 2D transthoracic echocardiography (2D TTE) and 2D transesophageal echocardiography (2D TEE). AUC = area under the ROC curve.

DISCUSSION

The results of this study fully validate the role of transthoracic three-dimensional live echocardiographic reconstruction in preoperative evaluation of MR mechanism and clinical decision-making. We studied an unselected cohort of patients with severe MR undergoing surgery, irrespective of heart rhythm, with a representative array of etiologies typically encountered at a surgical centre and we observed a valuable incremental role of 3D TTE over 2D TTE and 2D TEE in the complete and accurate evaluation of mitral valve morphology prior to valve surgery. Conventional 2D echocardiography can diagnose valvular diseases, but can not show en face views of the leaflets,

which can lead to difficulty defining the exact location of defect, and may result in difficulties communicating with surgeons[1]. The detection of valvular lesion location in 2D examination may be influenced by the change of blood pressure. As shown in our study, 2D TTE is limited in its ability to completely visualize the mitral valve, in particular the leaflets. The operator expertise-dependent accuracy of 2D echocardiography when imaging mitral valve anatomy, using non-simultaneous multiple views, could be considered a weak point in mitral regurgitation repair planning [1, 2]. Accurate localization of the valve lesion is crucial when planning the surgical repair strategy for prolapse-related mitral regurgitation1.

One of the most important advances in echocardiography during the last decade has been the development of 3D techniques. This method allows instant acquisition of a complete 3D data set without complex post-processing [1, 2]. 3D TTE reduces the potential sources of error and relies only on the quality of the apical acoustic window. New generation 3D technology reduces the acquisition and reconstruction time to few minutes and facilitates the visualization of the valves [4]. In the majority of cases in our study, imaging quality was good or optimal and permitted 3D reconstruction. Using surgical classification as gold standard, we found a sensibility of 95% and a specificity of 91% for the identification of valvular pathology using 3D TTE, a percentage comparable to previous studies [1,4-6]. Using these diagnostic criteria, our large study population, characterized by a relevant percentage of patients with complex mitral valve disease, found that 3D TEE was both more accurate than 2D imaging for the assessment of mitral valve lesions. According to the per-patient analysis, 3D TEE provided incremental diagnostic value, especially for the assessment of complex multisegment mitral valve disease involving one or both leaflets. Interestingly, in our patients with adequate echografic window, 3D TTE was not different to 2D TTE in the identification of commissural pathologies, but was significantly superior in the evaluation of leaflet segments. There are several studies that showed similar results [4, 6]; in contrast, Hoole et al[2] found that 3D method was excellent in identifying the commissures morphology of mitral valve, in particular if the transesophageal echocardiography was used. Therefore, this study confirms recent data showing that 3D TTE may be integrated in the standard examination facilitating the exact spatial localization of pathological structures and avoiding the need for mental reconstruction of 3D valve anatomy by the examiner.

Our results should be considered in the context of several limitations. The number of patients in this study was relatively small; however, we were able to reach several significant observations.

Patients were consecutively enrolled, but referral bias is possible and patients may not represent the whole population with severe MR. In addition, the non-physiologic load conditions from anesthesia might have influenced the dynamic findings of mitral valve segments and related echocardiographic findings.

CONCLUSIONS

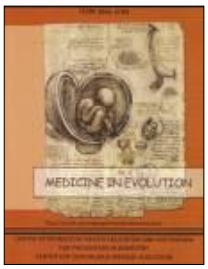
Live 3D TTE provides accurate analysis of MR mechanism and appears to be superior to conventional 2D echocardiographic technique. Live 3D TTE

could be used not only for complete recognition of the valvular morphology but also for planning the surgical repair strategy. 3D TTE analysis is simple and can be performed within a few minutes.

REFERENCES

1. Hirata K, Pulerwitz T, Sciacca R, Otsuka R, Oe Y, Fujikura K, Oe Het al. Clinical utility of new real time three-dimensional transthoracic echocardiography in assessment of mitral valve prolapse. Echocardiography 2008; 25(5): 482-488.
2. Hoole SP, Liew TV, Boyd J, Wells FC, Rusk RA. Transthoracic real-time three-dimensional echocardiography offers additional value in the assessment of mitral valve morphology and area following mitral valve repair. Eur J Echocardiogr 2008; 9(5): 625-630.
3. Carpentier A., Cardiac valve surgery-the French correction. J Thorac Cardiovasc Surg 1983; 86: 323–337.
4. Grewal J, Mankad S, Freeman WK, Click RL, Suri RM, Abel MD, et al. Real-time three-dimensional transesophageal echocardiography in the intraoperative assessment of mitral valve disease. J Am Soc Echocardiogr 2009; 22(1): 34-41.
5. Wei J, Hsiung MC, Tsai SK, Ou CH, Chang CY, Chang YC, et al. The routine use of live three-dimensional transesophageal echocardiography in mitral valve surgery: clinical experience. Eur J Echocardiogr 2010; 11: 14–18.
6. Tamborini G, Muratori M, Maltagliati A, Galli CA, Naliato M, Zanobini M, et al. Pre-operative transthoracic real-time three-dimensional echocardiography in patients undergoing mitral valve repair: accuracy in cases with simple vs. complex prolapse lesions, Eur J Echocardiogr 2010; 11(9): 778-85.

HYPERGLICEMIA EFFECT ON ENDOTHELIAL PROGENITOR CELLS SUBPOPULATIONS IN HYPERTENSIVE NON-DIABETIC PATIENTS



CRISTIANA BUJOR[1], STEFAN IOAN STRATUL[4], LIVIU TAMAS[1], CORINA SAMOILA[1], FLORINA BOJIN[2], SORIN URSONIU[3], ANDREI ANGHEL[1]

1. **Department of Biochemistry, University of Medicine and Pharmacy "Victor Babes" Timisoara**
2. **Department of Physiology, University of Medicine and Pharmacy "Victor Babes" Timisoara**
3. **Department of Public Health, University of Medicine and Pharmacy "Victor Babes" Timisoara**
4. **Department 1 Dental Medicine – Periodontology, University of Medicine and Pharmacy "Victor Babes" Timişoara**

ABSTRACT

Endothelial progenitor cells (EPCs) are a subtype of bone marrow-derived progenitor cells which contribute to neovasculogenesis and the repair of endothelium. Hypertension, diabetes or impaired fasting glucose (pre-diabetes) as well as various cardiovascular diseases are associated with EPC impairment, both in number and function.

The aim of this study was to quantify different EPC subpopulations in hypertensive treated patients with impaired glucose homeostasis and IFG in order to evaluate the effect of hyperglycemia on hypotensive therapy outcome and to identify a possible reduction of EPCs number as a biomarker for severe endothelial dysfunction.

The study includes 27 normoglycemic subjects without any medication (control group) and 20 hypertensive non-diabetic patients following hypotensive therapy.

EPCs were quantified by flow cytometry from whole blood

The results showed no significant difference between EPCs number in patients group vs control group, however higher percentages were recorded for all EPCs subpopulations in patients group comparative with healthy controls.

In pre-diabetic patients with hypertension, the hypotensive therapy could positively influence the number of EPCs and appears to have a protective effect on vascular endothelium.

***Key words**: endothelial progenitor cells, hypertension, pre-diabetes, impaired fasting glucose, EPCs*

Correspondence to:

Andrei Anghel
Adress: Department of Biochemistry, University of Medicine and Pharmacy "Victor Babes" Timişoara, Piata Eftimie Murgu nr. 2
E-mail address: biochim@umft.ro

INTRODUCTION

Endothelial progenitor cells (EPCs) are a subtype of bone marrow-derived progenitor cells expressing surface antigens of both hematopoietic stem cells and endothelial cells [1]. The cells are mobilized from bone marrow into the blood stream and home to sites of vascular injury induced by endothelial dysfunction or other causes, where are physically integrated into the nascent vasculature (neovasculogenesis and compensatory angiogenesis) or contribute to the continuous turnover and repair of endothelium [2].

Circulating EPCs are characterized by the expression of several antigens: CD1 33, CD 34, and vascular endothelial growth factor receptor-2 (VEGFR 2). Several distinct EPCs subpopulations can be distinguished by the expression of these surface antigens: CD 34+ / CD1 33+, CD 34+ / VEGFR 2+, CD 34+ /CD1 33+ / VEGFR 2+. The CD 34+ / CD1 33+ and CD 34+/ VEGFR 2+ EPCs are considered to be less differentiated EPCs ("early" EPCs) and the CD 34+ /CD1 33+ / VEGFR 2+ are defined as "more mature" EPCs being more differentiated and with a higher reparatory and vasculogenetic potential [3, 4].

The most frequent cardiovascular risk factors (hypertension, diabetes, dyslipidemia, and smoking) as well as various cardiovascular diseases are associated with EPC impairment, both in number and function.

Diabetes has a negative effect on the number and function of EPCs which contribute to the severity of cardiovascular disease in diabetic patients [5]. A study by Fadini et al. showed that individuals with impaired glucose tolerance (pre-diabetic) compared with individuals with normal glucose tolerance (NGT) had significantly lower levels of CD 34+ cells and CD 34 + VEGFR 2+ cells also declined progressively with worsening glucose tolerance, but they were not significantly reduced in pre-diabetic individuals, as they were in diabetic individuals [6, 7, 8]. Emerging evidence shows that periodontal disease is also associated with endothelial dysfunction. A study from 2009 on 86 non-smoking subjects showed for the first time that moderate to severe CP is associated with an increased level of circulating EPC9. On the other hand, many studies have reported that periodontal disease is associated with diabetes, but its relation with impaired fasting glucose (IFG) has been understudied. Choi et al. demonstrated in a very recent study on 12,254 participants in the NHNE Survey III, that chronic periodontitis measured by CAL and pocket depth was positively associated in a linear relation with IFG and diabetes in U.S. adults [10].

Impaired glucose tolerance (IGT) was originally defined to identify individuals at high risk of developing diabetes and was also recognized as an independent risk predictor for cardiovascular mortality and morbidity. The diagnosis of IGT requires an oral glucose tolerance test (OGTT), which consists of the administration of 75 g glucose and the measuring of blood glucose values initially, after one and two hours. The test is time-consuming, inconvenient for the patient and expensive compared with a fasting glucose testing [11].

Impaired fasting glucose (IFG) is defined by The American Diabetes Association (ADA) as a state of hyperglycemia in which glucose levels do not meet criteria for diabetes but are too high to be considered normal [12].

World Health Organization (WHO) and ADA have approved new diagnostic criteria for the diagnosis of

diabetes and the pre-diabetic state. The diagnostic cut-off value for fasting glucose has been lowered from 140 mg/dL to 126 mg/dL based on the evidence that the cut-off point has the best agreement with the diagnostic cut-off value of 200 mg/dL for 2-h glucose, and on the observation that the risk of retinopathy increased sharply at fasting glucose of 126 mg/dL or higher. In addition, a new category, impaired fasting glycemia (IFG) of 110 mg/dL – 125 mg/dL, was introduced in order to define impaired glucose homeostasis, an intermediate state between diabetes and normal glucose homeostasis considered to be analogous to IGT [11, 12].

ADA suggested with these new definitions that fasting glucose testing could be used alone because it is a simple and equally accurate test compared with the OGTT; the OGTT was thus no longer recommended. Furthermore, ADA lowered recently the limits for IFG to 100 mg/dL but WHO kept the limit of 110 mg/dL. The patients with IFG are called by ADA "pre-diabetic" patients, but reported estimates of diabetes development in IFG patients vary widely [11, 12, 13].

Hypertension was shown to be associated with a decline in number and function of EPCs, however the hypertension treatment improved the dynamics of EPCs.

Several studies showed that hypotensive drugs (ACE-inhibitors, angiotensin II receptor blockers, beta blockers, calcium channel blockers, nitrates), lipid-lowering drugs (statins), aspirin (low dose), diuretics (spironolactone) have a positive effect on circulating EPCs number and function (proliferation, differentiation, migratory function, adhesion) [14, 15].

The aim of this study was to quantify different EPC subpopulations in hypertensive treated patients with impaired glucose homeostasis and IFG in order to evaluate the effect of hyperglycemia on hypotensive therapy outcome and to identify a possible reduction of EPCs number as a biomarker for severe endothelial dysfunction.

MATERIALS AND METHODS

Subjects – The study comprised 27 normoglycemic subjects without any medication (control group) and 20 hypertensive non-diabetic patients with values of glycemia ≥110mg/dL and ≤ 126 mg/dL (patients group).

Hypertensive patients were recruited at the Institute of Cardiovascular Diseases of Timisoara. All hypertensive patients were following standard hypotensive therapy (ACE-inhibitors and/or beta blockers).

Healthy controls were selected as individuals who had no sign of acute illness or infection, no immunological disease, no history of recent surgery, no uncontrolled hypertension and no established cardiovascular disease and were recruited upon obtaining informed consent. All participants didn't have any previously known abnormalities in glucose homeostasis.

For all participants the following parameters were recorded: age, sex, family history for diabetes or cardiovascular disease and blood pressure (systolic and diastolic).

The study was approved by the local Ethical Committee and all subjects have given their written consent.

Quantification of epcs by flow cytometry – A sample of 2 ml of fasting state blood was collected in EDTA collection tubes and processed within 2-3 hours. 100 µl of whole blood was lysed with

Blood FACSTM Lysis Solution* (Beckton Dickinson), washed with phosphate – buffered saline solution (PBS) and then was incubated with 10 µl of each of the human CD34 FITC (Miltenyi Biotec), CD133/1 (AC 133)-APC (Miltenyi Biotec) and hVEGF R2-PerCP (R&D Systems).

All staining procedures were performed on ice. An unstained sample was analyzed as negative control for each sample. BD FACS Calibur Flow Cytometer was used to analyze the samples. A number of 200 000 events were acquired for each sample and negative control.

Statistical Analysis

All analyses were conducted with Stata 9.2 (Statacorp, Texas, USA). Student's t-test or non-parametric Mann-Whitney U test were used for comparison of continuous variables between the groups, as appropriate. Simple and multiple regression analysis were used to assess the significance of the relation between the number of progenitor cells (CD34+/KDR- and CD34+/KDR+) and glucose concentration.

The P values for all hypothesis tests were two-sided, and statistical significance was set at $P<0.05$.

RESULTS AND DISCUSSIONS

As expected, the t test results have shown a significant difference of glucose and triglycerides level between the patients and control groups ($p = 0.004$ respectively $p = 0.007$), even if the triglycerides level was normal (Table I).

Table 1 Baseline characteristics of subjects

	Age (years)	Glucose	Total cholesterol	Triglycerides	Sex	Blood pressure	
						Systolic	Diastolic
Patients n=20	53±3	115±7	163±14	120±17	M:15 F:5	142±30	85±16
Controls n=27	45±9	88±5	161±11	89±15	M:17 F:10	123±11	74±8

Table 2 The percent of cells / PBMC in control and patients group

	%CD133$^+$	%VEGFR2$^+$	%CD 34$^+$CD133$^+$	%CD 34$^+$VEGFR$^+$	%CD34$^+$ CD133$^+$VEGFR $^+$
Controls (n=27)	0.4376± 0.3577	0.4093± 0.3220	0.3063± 0.2528	0.3174± 0.2895	0.0376± 0.0782
Patients (n=20)	0.6580± 0.6739	0.6148± 0.6562	0.5001± 0.4836	0.5895± 0.8263	0.0422± 0.0540
P value	0.2366	0.4014	0.1524	0.1162	0.3016

The results showed no significant difference between EPCs number in patients group vs control group, however higher percentages were recorded for all EPCs subpopulations in patients group comparative with healthy controls (Table II).

The EPCs subpopulations were qu-antified as cells/106 events and then re-ported as the percentage from peri-pheral blood mononuclear cells (PBM Cs) number in order to reduce the po-ssible quantification errors.The onset of atherosclerosic car-diovascular disease in patients group associated with hypertension could in-duce a minimal endothelial dysfuncti-on and an consecutive mobilization of bone marrow EPCs followed by an increased number of circulating EPCs. In addition, the hypotensive therapy has a positive effect on EPCs number and function as was shown in numerous studies and could induce an increase of EPCs levels [16, 17, 18].

This hypothesis could be supported by the fact that the percentages of EPCs subpopulations are not significantly different between the patients and control groups, but they are increased for all EPCs subpopulations in patients group even if they presented impaired fasting glucose.

Hyperglycemia in diabetic patients has a negative effect on EPCs, as was demonstrated by two recent studies Fadini et al, which showed that type 2 diabetes is associated with a significant decline of EPC levels and also with a decrease of functional capabilities of EPCs [19, 20].

Other studies showed that the number of circulating EPCs is decreased in both type 1 and type 2 diabetes and is likely to play an important role in the pathogenesis of cardiovascular complications. These clinical complications can be observed even in diabetics who achieved a good long-term glycemic control [21, 22, 23].

Therefore it is possible that there is still EPC dysfunction in diabetics with good glycemic control. Furthermore, the EPCs number and function is impaired in pre-diabetic patients with IFG, but not all the EPCs subpopulations are affected [24, 25, 26].

Fadini et al. showed that only CD34+ cells were significantly decreased in patients with IGT but no other EPCs subpopulation was affected. Only the CD34+VEGFR2+ subpopulation declined progressively with worsening glucose tolerance, but they were not significantly reduced in pre-diabetic individuals, as they were in diabetic individuals [6]. Also, in diabetic patients with a good glycemia control, the number and function of EPCs was improved, but did not reach the level in healthy controls [27]. Another study showed that mild glucose intolerance during pregnancy can be associated with a decrease in the number of circulating EPCs [28].

Nevertheless, subjects from previous studies either were not following hypotensive therapy or had a mixed therapy for diabetes and cardiovascular diseases.

The hypotensive therapy may positively influence the number of EPCs in hypertensive patients. Therefore, even if a pre-diabetic state is present in these patients, and hyperglycemia has a negative effect on EPCs number, even if not all EPCs subpopulations seem to be altered, the hypotensive treatment appears to have a protective effect and could increase the EPCs number to values higher then in healthy controls.

Many pre-diabetic patients with impaired fastening glucose have or will develop hypertension and an early administration of a hypotensive treatment even in low doses, should be considered as a preventive treatment for slowing the progression of endothelial dysfunction and atherosclerotic cardiovascular disease.

The association of long-term hypotensive therapy with local complications as gingival overgrowth and inflammation, especially in pre-diabetic patients, should be also taken into con-

sideration. EPCs quantification could evaluate the efficacy of hypotensive therapy and the risk for cardiovascular complications.

However, the functionality of these EPCs could be altered and functional characterization of EPCs should be performed in order to eliminate this study limitation. Also, the reduced number of investigated subjects represented another limitation of our study and a further enhancement of our study subjects' number in the future is required.

CONCLUSIONS

Treated hypertensive patients with impaired fasting glucose (110 mg/dL – 125 mg/dL) seem to have normal levels of EPCs, considering all the EPCs subpopulations. These levels were higher than in healthy controls but were not statistically significant. Early initiation of hypotensive treatment, even in low doses could decrease the development of later cardiovascular complications. The effect of therapy could be evaluated by EPCs quantification.

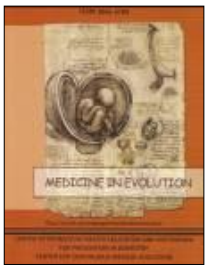
Funding Acknowledgement:
This work was supported by Romanian CNCSIS Idei grant no. 2494/2008-2011.

REFERENCES

1. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science. 1997; 275(5302): 964-7.
2. Fadini GP, Pucci L, Vanacore R, Baesso I, Penno G, Balbarini A, et al. Glucose tolerance is negatively associated with circulating progenitor cell levels. Diabetologia. 2007; 50(10): 2156-63.
3. Fadini GP, Agostini C, Sartore S, Avogaro A. Endothelial progenitor cells in the natural history of atherosclerosis. Atherosclerosis. 2007; 194(1): 46-54.
4. Miller-Kasprzak E, Jagodzinski PP. Endothelial progenitor cells as a new agent contributing to vascular repair. Arch Immunol Ther Exp (Warsz). 2007; 55(4): 247-59.
5. Ding H, Triggle CR. Endothelial cell dysfunction and the vascular complications associated with type 2 diabetes: assessing the health of the endothelium. Vasc Health Risk Manag. 2005; 1(1): 55-71.
6. Fadini GP, Pucci L, Vanacore R, Baesso I, Penno G, Balbarini A, et al. Glucose tolerance is negatively associated with circulating progenitor cell levels. Diabetologia. 2007; 50(10): 2156-63.
7. Chen YH, Lin SJ, Lin FY, Wu TC, Tsao CR, Huang PH, et al. High glucose impairs early and late endothelial progenitor cells by modifying nitric oxide-related but not oxidative stress-mediated mechanisms. Diabetes. 2007; 56(6): 1559-68.
8. Krankel N, Adams V, Linke A, Gielen S, Erbs S, Lenk K, et al. Hyperglycemia reduces survival and impairs function of circulating blood-derived progenitor cells. Arterioscler Thromb Vasc Biol. 2005; 25(4): 698-703.
9. Li X, Tse HF, Yiu KH, Jia N, Chen H, Li LS, Jin L. Increased levels of circulating endothelial progenitor cells in subjects with moderate to severe chronic periodontitis. J Clin Periodontol. 2009 Nov; 36(11): 933-9. Epub 2009 Oct 2.
10. Choi YH, McKeown RE, Mayer-Davis EJ, Liese AD, Song KB, Merchant AT. Association between periodontitis and impaired fasting glucose and diabetes. Diabetes Care. 2011 Feb; 34(2): 381-6. Epub 2011 Jan 7.
11. Qiao Q, Lindstrom J, Valle TT, Tuomilehto J. Progression to clinically diagnosed and treated diabetes from impaired glucose tolerance and impaired fasting glycaemia. Diabet Med. 2003; 20(12): 1027-33.
12. American Diabetes Association (ADA): Clinical practice recommendation. Diabetes Care 2011, Diabetes Care. 2011; 34 (Suppl 1): S1-S69.
13. Yeboah J, Bertoni AG, Herrington DM, Post WS, Burke GL. Impaired fasting glucose and the risk of incident diabetes mellitus and cardiovascular events in an adult population: MESA (Multi-Ethnic Study of Atherosclerosis). J Am Coll Cardiol. 2011; 58(2): 140-6.
14. Antonio N, Fernandes R, Rodriguez-Losada N, Jimenez-Navarro MF, Paiva A, de Teresa Galvan E, et al. Stimulation of endothelial progenitor

cells: a new putative effect of several cardiovascular drugs. Eur J Clin Pharmacol. 2010; 66(3): 219-30.
15. Vasa M, Fichtlscherer S, Adler K, Aicher A, Martin H, Zeiher AM, et al. Increase in circulating endothelial progenitor cells by statin therapy in patients with stable coronary artery disease. Circulation. 2001; 103(24): 2885-90.
16. Werner N, Kosiol S, Schiegl T, Ahlers P, Walenta K, Link A, et al. Circulating endothelial progenitor cells and cardiovascular outcomes. N Engl J Med. 2005; 353(10): 999-1007.
17. Imanishi T, Hano T, Nishio I. Angiotensin II accelerates endothelial progenitor cell senescence through induction of oxidative stress. J Hypertens. 2005; 23(1): 97-104.
18. Levitzky YS, Pencina MJ, D'Agostino RB, Meigs JB, Murabito JM, Vasan RS, et al. Impact of impaired fasting glucose on cardiovascular disease: the Framingham Heart Study. J Am Coll Cardiol. 2008; 51(3): 264-70.
19. Fadini GP, Sartore S, Albiero M, Baesso I, Murphy E, Menegolo M, et al. Number and function of endothelial progenitor cells as a marker of severity for diabetic vasculopathy. Arterioscler Thromb Vasc Biol. 2006; 26(9): 2140-6.
20. Fadini GP, Sartore S, Schiavon M, Albiero M, Baesso I, Cabrelle A, et al. Diabetes impairs progenitor cell mobilisation after hindlimb ischaemia-reperfusion injury in rats. Diabetologia. 2006; 49(12): 3075-84.
21. Shantsila E, Watson T, Lip GY. Endothelial progenitor cells in cardiovascular disorders. J Am Coll Cardiol. 2007; 49(7): 741-52.
22. Reinhard H, Jacobsen PK, Lajer M, Pedersen N, Billestrup N, Mandrup-Poulsen T, et al. Multifactorial treatment increases endothelial progenitor cells in patients with type 2 diabetes. Diabetologia. 2010; 53(10): 2129-33.
23. Snyder RJ. Controversies regarding vascular disease in the patient with diabetes: A review of the literature. Ostomy Wound Management. 2007; 53(11): 40-8.
24. Tepper OM, Galiano RD, Capla JM, Kalka C, Gagne PJ, Jacobowitz GR, et al. Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. Circulation.2002; 106(22): 2781-6.
25. Loomans CJ, De Koning EJ, Staal FJ, Rabelink TJ, Zonneveld AJ. Endothelial progenitor cell dysfunction in type 1 diabetes: another consequence of oxidative stress? Antioxid Redox Signal. 2005; 7(11-12): 1468-75.
26. Loomans CJ, de Koning EJ, Staal FJ, Rookmaaker MB, Verseyden C, de Boer HC, et al. Endothelial progenitor cell dysfunction: a novel concept in the pathogenesis of vascular complications of type 1 diabetes. Diabetes. 2004; 53(1): 195-9.
27. Churdchomjan W, Kheolamai P, Manochantr S, Tapanadechopone P, Tantrawatpan C, Y UP, et al. Comparison of endothelial progenitor cell function in type 2 diabetes with good and poor glycemic control. BMC Endocr Disord. 2010; 10:5.
28. Penno G, Pucci L, Lucchesi D, Lencioni C, Iorio MC, Vanacore R, et al. Circulating endothelial progenitor cells in women with gestational alterations of glucose tolerance. Diab Vasc Dis Res. 2011; 8(3): 202-10.

COADMINISTRATION OF METFORMIN AND SITAGLIPTIN - THE EVIDENCE FOR ACHIEVING GLYCEMIC CONTROL IN TYPE 2 DIABETES



NARCISA DANIELA MIHAILESCU[1], MARIA PUSCHITA[1], LUMINITA STAMORAN[1], ROXANA HOMOLKA[3] , RODICA IOIART[2]

1. **"Vasile Goldis" West University Arad**
2. **Arad County Hospital**
3. **University of Medicine and Pharmacy "Victor Babes" Timisoara**

ABSTRACT

Classic hypoglycemic medications are unable to address all of the underlying pathophysiologic defects in patients with type 2 diabetes. It has been known for many years that administration of an oral glucose challenge elicits a stronger insulin response than an equivalent intravenous glucose challenge. This incretin effect was attributed to the insulinotropic action of gut hormones: GLP (glucagon like peptid1) and GIP (glucose-dependent insulinotropic polypeptide). The incretin hormones are released in response to food ingestion to stimulate insulin secretion and, in the case of GLP1, to inhibit glucagon secretion; thereby decreasing glucose production by the liver in a counter regulatory manner.70% of post-glucose insulin secretion is due to the incretin effect. In type 2 diabetes that effect are greatly diminished. There are 2 different strategies for enhancing GLP1 action in diabetes: extending the activity of GLP1 in the form of synthetic long-acting GLP1 analogues and inhibiting DPP4 (dipeptidyl peptidase-4) to prevent the inactivation of endogenous GLP1 and GIP.

Authors are proposing to evaluate the efficacy and safety of sitagliptin in patients with T2DM not adequately controlled with metformin (1500-2500 mg/d); primary endpoint: HbA1C change after 32 weeks, secondary endpoint: fasting plasma glucose, postprandial glucose, body weight change, hypoglycemia, adverse events.

The results aimed to provide data to help clinicians decide to use this novel agent.

***Keywords**: incretin effect, DPP-4, GLP-1, GIP, sitagliptin*

Correspondence to:

Narcisa Mihailescu
Adress: Arad County Hospital, Feleacului str. no. 1
E-mail address: narcisamihailescu@yahoo.com

INTRODUCTION

An recent rapport of International Diabetes Federation conclude that in 2007 at the global level were 246000000 persons that suffer of diabetes mellitus and till 2025 the number of the ill will be 380000000. Every year, 1.3 million people are diagnosed with type 2 diabetes. The rapid increase in new cases of type 2 diabetes in persons 30 to 39 years of age and in children and adolescents is of special concern. The increasing number of illnesses is caused by a sedentary way of life, increasing obesity (when discovered, 80% were overweight or obese) and by increasing age of population. In present the type 2 diabetes mellitus is the forth cause of death in the word. The pathogenesis of type 2 diabetes is characterized by a combination of factors that ultimately lead to loss of glycemic control: insulinresistence in liver, muscle and adipose tissues; a progressive decline in beta-cell mass and function resulting in relative insulin insufficiency and defective suppression of postprandial glucagon levels, leading to increased hepatic glucose production [8, 12].

Effects of classic antihyperglicemic medications: Classic antihyperglycemic medications are unable to address all of the underlying pathophysiologic defects in patients with type 2 diabetes [8]; furthermore, these agents frequently exhibit reduced efficacy over time, leading to inadequate glycemic control and may be associated with adverse side effects [3, 5, 18, 19, 24, 25, 29] (table 1).

Table 1 Effects of clasic antihyperglicemic medications and limitations

Medications	**Fasting glucose**	**Post prandial glucose**	**Hipoglycemia**	**Wheight gain**	**Renal insufficiency**	**Liver failure**	**Heart failure**	**others**
metformin	↓↓	↓	0	-	CI	CI	CI	Lactic acidosis, gastrointestinal symptoms
sulfonylurea	↓↓	↓↓	++	+	!!	!!		Drog interaction
glinide	↓	↓↓	+	+		!!		Drog interaction
thiazolidindione(pioglitasone)	↓↓	↓	0	++	!	!!	CI	Edema, fractures, mild anemia
alfa glucozidase inhibitor	0	↓↓	o	0				Gastro intestinal symptoms
insulin	↓↓↓	↓↓↓	+++	++	!			

CI=contraindicate; !=precaution

The Adult Diabetes Outcome Progression Trial (ADOPT) showed that the durability of glycemic control with orally administered hypoglycemic agents is limited, with rosiglitazone having a more sustained effect than metformin or sulfonylurea [16]. Another study suggested that rosiglitazone was associated with risk of myocardial infarction [25]. Nichols and col. discovered that elevated serum insulin levels promote the development of cardiac disease [24] and, consistent with the UKP DS (United Kingdom Prospective Dia-

betes Study), metformin may offer some protection from incident cardiac heart failure relative to sulfonylurea or insulin.

The increasing risk of heart fai-lure and the higher mortality risk associated with sulfonylureas, reported in the current study, further suggest that sulfonylureas terapy should perhaps be used sparingly [29]. None of these agents has been shown to prevent the progression of the disease it self. There are more and more studies that demonstrate the incapacity of present therapies to prevent the progressive destruction of pancreatic B cell mass. The majority of classic therapies meant to lower the plasmatic glucose levels are associated with weight gain, and obesity is the leading aspect that raises the incidence of diabetes [5]. The usual therapeutic agents used in type 2 diabetes cannot achieve these goals neither in high doses or combinations.

The incretins offers new perspectives in type 2 diabetes treatment.

Intestinal secretion of insulin: It has been known for many years that administration of an oral glucose challenge elicits a stronger insulin response than an equivalent intravenous glucose challenge [1, 2, 9]. This incretin effect was attributed to the insulinotropic action of gut hormones: GLP1 (glucagon like peptid1) and GIP (glucose-dependent insulinotropic polypeptide) [2]. The incretin hormones are released in response to food ingestion to stimulate insulin secretion and, in the case of GLP1, to inhibit glucagon secretion, thereby decreasing glucose production by the liver in a counter regulatory manner [10]. 70% of post-glucose insulin secretion is due to the incretin effect [22, 23]. In type 2 diabetes that effect are greatly diminished [4].

Biological effects of incretin hormo-nes: GLP-1 is synthesized and secreted by L cells of the ileum and colon and stimulates glucose-dependent insulin release with a concomitant suppression of hepatic glucose output by inhibiting glucagon secretion.

Furthermore, it improves beta-cell responsiveness to glucose by increasing the expression of glucose-transporter-2 and glucokinase together with a decrease in the pro-insulin to insulin ratio.

It also inhibits gastric emptying and has a central nervous system effect resulting in reduced food intake and a decrease in body weight (table 2).

Table 2 Biological effects of incretin hormones

GLP-1 (glucagon - like peptide 1)	**GIP (glucose-dependent insulinotropic polypeptide)**
Stimulates insulin release from beta-cell	Stimulates insulin release from beta-cell
Potent inhibition of gastric emptying	Modest inhibition of gastric emptying
Potent inhibition of glucagon secretion	-
Reduction food intake	-
Reduction body weight	-
Effects on beta-cell growth	Effects on beta-cell growth
(released from L cell-ileum and colon)	(released from K cell-duoden)

Dypeptidil Peptidase IV: A major disadvantage of using GLP-1 to treat T2DM patients is its short half-life (1-2 min) due to rapid catabolism by the ubiquitous enzyme dipeptidyl peptidase-IV (DPP-IV). DPP-4 is a glycoprotein consisting of 766 amino acids and is a catalytic enzyme. It is highly concentrated in the capillaries close to the intestinal cells where GLP-1 and GIP are produced, and it is widely distributed throughout the body.

DPP-4 can inactivate over 50% of GLP-1 in about 1 minute, and over 50% of GIP in about 7 minutes. Consequently, naturally occurring GLP-1 is precluded from use as a therapeutic agent, and other strategies to increase GLP-1 concentrations, mimic its effect, or prolong its half-life are required. This has been accomplished in a number of ways including use of GLP-1 receptor agonists or mimetics that are resistant to DPP-4 degradation; covalent or noncovalent binding of GLP-1 to a large protein like albumin, or inhibition of DPP-4 activity [1, 2, 6, 7, 9].

Incretin Therapies: There are 2 different strategies for enhancing GLP1 action in diabetes: extending the activity of GLP1 in the form of synthetic long-acting GLP1 analogues and inhibiting DPP4 (dipeptidyl peptidase-4) to prevent the inactivation of endogenous GLP1 and GIP [13, 14, 17, 23, 28]. Although both are incretin-targeted therapies, DPP-4 inhibitors and GLP-1 receptor agonists have a number of characteristics that distinguish them. DPP-4 inhibitors are oral agents that are given daily, while GLP-1 receptor agonists are injectable agents that can be given twice-daily. Investigational GLP-1 receptor agonists can be administered as little as once a week. DPP-4 inhibitors act to increase endogenous levels of GLP-1, while GLP-1 receptor agonists offer the possibility of supraphysiologic activation of GLP-1 receptors.

The impact of the incretins on weight is very important since 90% of people with type 2 diabetes are overweight, other agents promote weight gain (sulfonylureas, thiazolidindiones, and insulin), and weight loss has a positive effect on glycemic control [22, 27]. Another difference between the 2 therapies is that GLP-1 receptor agonists are associated with gastrointestinal (GI) adverse effects including nausea and vomiting. These effects tend to be seen at the higher doses whereas robust glycemic benefits are achievable with lower doses (table 3).

Table 3 Incretin agents-effects

DPP IV inhibitors	**Incretin mimetics**
Significant reduction HbA1c	Significant reduction HbA1c
Weight neutral	Weight loss
Almost no gastrointestinal side effects	Higher rate of gastrointestinal side effects
Very low rate of hypoglycaemia	Low rate of hypoglycaemia
Oral administration	Injectable
Multiple targets (GLP-1 and GIP)	Single target (GPL-1)

Two agents are currently available in Romania that act upon the incretin hormone system - exenatide , a twice-daily glucagon-like peptide-1 (GLP-1) receptor agonist, and sitagli-ptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor. Additional agents are currently under review by the US Food and Drug Administration (FDA) or in phase 3 clinical trials.

The roles of glucagon-like peptide-1 (GLP-1) receptor agonists and di-peptidyl peptidase-4 (DPP-4) inhibitors are rapidly evolving, despite limited recommendations on their use in current guidelines (American Diabetes Association: Standards Of Medical Care In Diabetes – 2008 [21].

The AACE (American Association of Clinical Endocrinologists) guidelines make it clear that incretins can be used early and are not limited to third- or fourth-line therapy for type 2 diabetes. The AACE recommends a DPP-4 inhi-

bitor as monotherapy for those who have an initial A1C level of 6% to 7% or as combination therapy for those with an initial A1C >7%, whereas a GLP-1 receptor agonist can be used if the A1C goal of ≤6.5% is not achieved. A DPP-4 inhibitor can be added to metformin or a thiazolidindione (TZD) for previously treated patients who are not at goal (A1C ≤6.5%). A GLP-1 receptor agonist can be added to sulfonylurea, metformin, or TZD monotherapy or to combination therapy with sulfonylurea / metformin or metformin/TZD for patients who are not at goal (AACE/ACE algorithm, December 2009) [15, 19, 26].

AIM AND OBJECTIVES

Authors are proposing to evaluate the efficacy and safety of sitagliptin in patients with type 2 diabetes mellitus not adequately controlled with metformin.

Primary endpoint: HbA1C change after 52 weeks; *Secondary endpoint*: fasting plasma glucose, postprandial glucose, body weight change, hypoglicemia, adverse events.

MATERIALS AND METHODS

The study was conducted on the Emergency Clinical County Hospital of Arad, Diabetes Mellitus Department, during June 2009-July 2010 and comprises 108 patients admitted in the hospital for diabetological disorders, treat with metformin 1500-2500 mg daily.

Inclusion criteria:

- type 2 diabetes as defined by the American Diabetes Association;
- baseline HbA1c beetween 7 and 9,5 %;
- patiens having received stable doses of metformmin before randomization (patients with type 2 diabetes on metformin for at least 3 mounths and have been on a stable dose of metformin of at least 1500 mg daily for a minimum of 4 weeks);
- fasting plasma glucose <220 mg/dl;
- agreement to maintain the same dose of metformin throughout the study;
- agreement to maintain prior diet and exercise habits during the full course of the study.

Exclusion criteria:

- a history of acute metabolic diabetic complications;
- evidence of significant diabetic complications;
- insulin treatment for longer than 10 days within the past 6 months;
- treatment with any oral anti-diabetic other than metformin.

Characteristic subjects:

- age (years): 35-76;
- sex: male- 43(40%) and female- 65 (60%);
- body mass index (kg/m2): 27-33;
- duration of diabetes mellitus (years): 3-12;
- HbA1c at screening: 7-9,5%;

Patients who had an A1c (hemoglobin glycate) >9% or fasting glucose value >220 mg/dl were not eligible to be randomized. The mean baseline Hb A1c was 8,2%.

HbA1c distribution at baseline, n 108 patiens:

- group 1-HbA1c >7 to <8% (7,4%)-40 patients (37%),
- group 2-HbA1c >8 to <8,5% (8,2%)-26 patients (24%),
- group 3-HbA1c >8,5 to <9,5% (9%) - 42 patients (39%).

HbA1c distribution at 52 weeks, 105 patiens:

- group 1-HbA1c 6,9%--40 patients (38,1%),
- group 2-HbA1c 7,4%--26 patients (24,8%),
- group 3-HbA1c 7,8%--39 patients (37,1%).

Results: Coadministration sitagliptin 100mg daily and metformin 1500-2500 mg daily provided significant improvements in HbA1c. HbA1c change from baseline was: -0,5% for the first group, -0,8% for the second group, respectively -1,3% (group 3).

Greater reduction in HbA1c was associated with higher baseline HbA1c (fig.1). From a mean baseline of 8,2%, changes from baseline were-0,84%. The proportion of patiens achieving an HbA1c<6,5% was 30% , but for 62% the addition of sitagliptin provided HbA1c lowering<7% (fig.2). Fasting plasma glucose changes from baseline (mean baseline 170mg/dl) were -19mg/dl; postprandial plasma glucose changes from baseline (mean baseline 245mg / dl) were -56mg/dl.

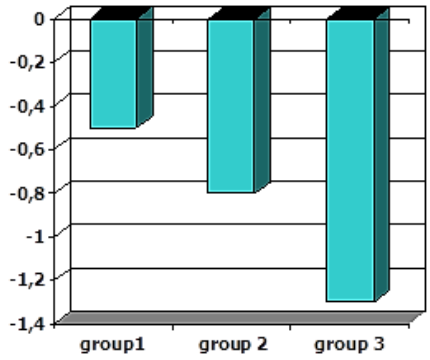


Fig.1 HbA1c reduction after 52 weeks

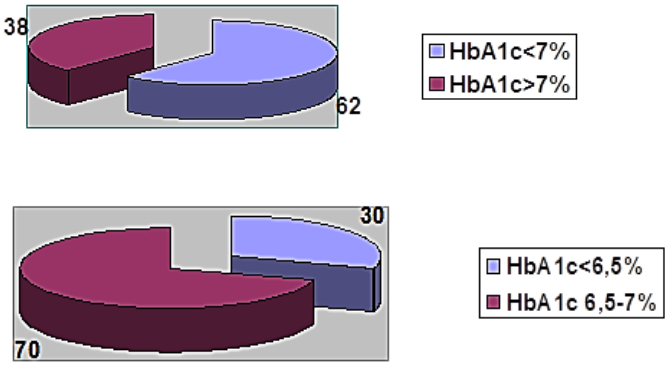


Fig.2 Greater proportions of patients achieved HbA1c target

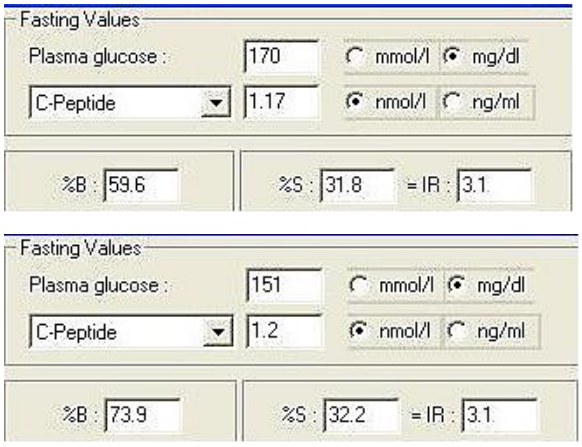


Fig.3 Homa beta was significantly improved

Finally, homeostasis model assessment-beta cell function (HOMA beta) was calculated in 10 patients with initial HbA1c > 8,5%, with HOMA Calculator Version 2.2.2. available at htpp: //www.dtu.ox.ac.uk./homa (was measured C-peptide level initial and after 52 weeks, respectively fasting plasma

glucose level). Homa beta was significantly improved: 14,3%. (fig.3).

3 patients reported gastrointestinal symptoms these symptoms (nausea, diarrhea or abdominal pain) were not related to study drug and resolved while patients continued in the study. There were 2 patients who repor-ted hypoglycemia symptoms, but none of them had fingerstik glucose values <70 mg/dl. At 52 weeks the mean of body weight was slowly reduced.

DISCUSSIONS

The addition of sitagliptin in patients on ongoing metformin therapy provided HbA1c lowering without risk of hypoglycemia or weight gain and was well tolerated.

The pathogenesis of type 2 diabetes was described recently by De Fronzo, "the ominous octet" [8]:

- impaired insulin secretion,
- increased hepatic glucose production,
- decreased muscle and hepatic glucose uptake,
- increased glucagons secretion,
- lipotoxicity,
- increased renal glucose reabsorbtion,
- neurotransmitter disfunction,
- decreased incretin effect.

The incretin defect in patients with type 2 diabetes has been considered a possible candidate for a primary deficiency in type 2 diabetes (the incretin effect was shown to be affected in subjects who had impaired glucose tolerance) [20]. On the other hand, in patients with chronic pancreatitis and secondary diabetes the incretin effect was found significantly reduced compared with patients with chronic pancreatitis and normoglycaemia, and compared with healthy subjects. These results suggest that this deficiency is most likely a consequence of the diabetic state, and not a primary pathogenic trait leading to type 2 diabetes [11].

The mechanism by which metformin exerts its antihyperglycemic effects is to decrease hepatic glucose output, primarily by decreasing gluconeogenesis, but it may also, as a lesser effect, increase glucose uptake by skeletal muscles. Metformin activates hepatic and muscle adenosine monophosphate-activated protein kinase (AMPK), an enzyme normally activated by adenosine monophosphate, the breakdown product of adenosine triphosphate and a cellular signal for increased energy requirements.

Activation of hepatic AMPK results in the phosphorylation and inhibition of acetyl-coenzyme A carboxylase, which catalyzes the rate-limiting step of lipogenesis. This block in fatty acid synthesis promotes fatty acid oxidation. In addition, activation of hepatic AMPK decreases expression of sterol – regulatory – element - binding protein - 1 (SREBP-1), a transcription factor implicated in the pathogenesis of insulin resistance, dyslipidemia and diabetes. Decreased SREBP-1 expression results in decreased gene expression of lipogenic enzymes, which further contributes to decreased triglyceride synthesis and hepatic steatosis. AMPK activation appears to be a critical step in the metformin-mediated reduction of hepatic glucose production and increase in skeletal muscle glucose uptake. Thus, AMPK is a major regulator of lipid and glucose metabolism and may be the key mediator of all the beneficial effects of metformin.

Coadministration of metformin and sitagliptin has complementary me-

chanism of action (Wajchenberg BL, Nov. 2009).

In his study has been shown that metformin increases total GLP1, but not GIP, by enhancing GLP1 release; sitagliptin stabilize GLP1 and GIP by inhibition of DPP-4, results in a more than additive effect on both preprandial and postprandial GLP1active concentrations.

CONCLUSIONS

Sitagliptin add to metformin provides a more comprehensive approach for addressing the key pathophysiologies of type 2 diabetes.

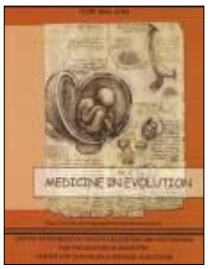
REFERENCES

1. Aaboe K, Krarup T, Madsbad S, Holst JJ: GLP-1 Phyziological Effects And Potential Therapeutic Application, Diabetes Obes Metab 2008: 10: 994-1003
2. Ahren B: Gut Peptides And Type 2 Diabetes Mellitus Treatment, Curr. Diab. Rep. 2003; 3: 365-72
3. Ajay D. Rao, Nitesh Kuhadiya, Kristi Reynolds, Vivian A. Fonseca: Is the Combination of Sulfonylureas and Metformin Associated With an Increased Risk of Cardiovascular Disease or All-Cause Mortality? A meta-analysis of observational studies, Diabetes Care, 31,2008:1672-1678
4. Amori RE, Lau J, Pittas AG: Efficacy and safety of incretin therapy in type 2 diabetes: systematic review and meta-analysis. JAMA 2007; 298: 194-206
5. Busko M. Glucose-lowering for the prevention of cardiovascular disease: positive, negative, or neutral? International Diabetes Federation 20th World Diabetes Congress; October 21, 2009; Montreal, QC.from heartwire
6. Deacon CF, Wamberg S, Bie P, Hughes TE, Holst JJ: Preservation Of Active Incretin Hormones By Inhibition Of Dipeptidyl Peptidase IV Suppresses Meal-Induced Incretin Secretion In Dogs, J. Endocrinol. 172, 2002: 355-362
7. Deacon CF, Nauck MA, Toft-Nielsen M, et al. Both sc and iv administered glucagon-like peptide-1 are rapidly degraded from the NH2-terminus in type II diabetic patients and healthy subjects. Diabetes 1995; 44: 1126-31
8. DeFronzo RA: From The Triumvirate To The Ominous Octet: A New Paradigm For The Treatment Of Type 2 Diabetes Mellitus, Diabetes 2009; 58: 773-95
9. Drucker DJ, Nauck MA: The Incretin System: Glucagon Like Peptide – 1 Receptor Agonists And Dipeptidyl Peptidase – 4 Inhibitors In Type 2 Diabetes, Lancet 2006; 368: 1696-705
10. Drucker DJ. The evidence for achieving glycemic control with incretin mimetics. Diabetes Educ.32 (Suppl. 2), 2006: 72S-81S
11. Filip K. Knop; Tina Vilsbøll; Patricia V. Højberg; Steen Larsen, et al: Reduced Incretin Effect in Type 2 Diabetes: Cause or Consequence of the Diabetic State? Diabetes. 2007; 56(8):1951-1959
12. Hancu N, Roman G, Veresiu IA: Farmacoterapia diabetului zaharat, ed. Echinox, Cluj-Napoca, 2008; 169-184
13. Herman GA, Stein PP, Thornberry NA, Wagner JA: Dipeptidyl peptidase-4 inhibitors for the treatment of Type 2 diabetes: focus on sitagliptin. Clin. Pharmacol. Ther.81 (5), 2007:761-767
14. Holst JJ, Deacon CF: Inhibition Of The Activity Of The Dipeptidyl Peptidase IV As Treatment For Type 2 Diabetes, Diabetes (47), 1998: 1663-16670
15. Jellinger PS, Davidson JA, Blonde L, et al. Road maps to achieve glycemic control in type 2 diabetes mellitus: ACE/AACE Diabetes Road Map Task Force. Endocr Pract 2007; 13(3): 260-268
16. Kahn SE, Haffner SM, Heise MA, et al: Glicemic Durability Of Rosiglitazone, Metformin Or Glyburide Monotherapy, N. Engl. J. Med., 2006; 355: 2427-2443
17. Karasik A, Aschner P, Katzeff H, Davies MJ, Stein PP: Sitagliptin, a DPP-4 inhibitor for the treatment of patients with Type 2 diabetes: a review of recent clinical trials. Curr. Med. Res. Opin.924), 2008:489-496
18. Karter AJ, Ahmed AT, Liu J, Moffet HH, Parker MM. Pioglitazone initiation and subsequent hospitalization for congestive heart failure. Diabet Med. 2005; 22:986-993
19. Kent DM, Hayward RA. Limitations of applying summary results of clinical trials to individual patients: the need for risk stratification. JAMA. 2007; 298:1209-1212.
20. Muscelli E, Mari A, Natali A, Astiarraga BD, et al: Impact of incretin hormones on beta-cell function in subjects with normal or impaired glucose tolerance. Am J Physiol Endocrinol Metab (291), 2006:E1144-E1150.
21. Nathan DM, Buse JB et al. Management of hyperglycemia in Type 2 diabetes: a consensus al-

gorithm for the initiation and adjustment of therapy. A consensus statement from the American diabetes association and the European Association for the Study of Diabetes. Diabetes Care (29), 2006:1963-1972

22. Nauck MA, Meininger G, Sheng D, Terranella L, Stein PP: Efficacy and safety of the dipeptidyl peptidase-4 inhibitor, sitagliptin, compared with the sulfonylurea, glipizide, in patients with Type 2 diabetes inadequately controlled on metformin alone: a randomized, double-blind, non-inferiority trial. Diabetes Obes. Metab.9 (2), 2007: 194-205
23. Nauck MA, Meier JJ, Creutzfeldt W. Incretins and their analogues as new antidiabetic drugs. Drug News Perspect. (16), 2003:413-422
24. Nichols GA, Koro CE,.Gullion CM, Ephross S, Brown JB: The Incidence Of Congestive Heart Failure Associated With Antidiabetic Therapies, Diabetes Metab. Res. Rev. 2005; 21 (1): 51-7
25. Nissen SE, Wolski: Effect Of Rosiglitazone On The Risk Of Myocardial Infarction And Death From Cardiovascular Causes, N. Engl. J. med., 2007; 356: 2457-2471
26. Rodbard HW, Jellinger PS, Davidson JA et al. Statement by an American Association of Clinical Endocrinologists/American College of Endocrinology consensus panel on type 2 diabetes mellitus: an algorithm for glycemic control. Endocr Pract. 2009; 15:540-559
27. Seck T, Nauck M, et al.: Safety and Efficacy of Treatment with Sitagliptin or Glipizide in Patients with Type 2 Diabetes Inadequately Controlled on Metformin: A 2-year Study Int J Clin Pract. 2010; 65(5):562-576
28. Thornberry NA, Weber AE: Discovery of Januvia (sitagliptin), a selective dipeptidyl peptidase IV inhibitor for the treatment of Type 2 diabetes. Curr. Top. Med. Chem.7 (6), 2007:557-568
29. Tzoulaki I, Molokia M, et al: Risk Of Cardiovascular Disease All-Cause Mortality Among Patients With Type 2 Diabetes Prescribed Oral Antidiadetes Drugs: Retrospective Cohort Study Using UK General Practice Research Database, BMJ, 2009; 339.

PROGNOSTIC MARKERS IN CHRONIC LYMPHOCYTIC LEUKEMIA



IOANA IONITA[1], LIVIU CHEVERESAN[1], MARIA CHEVERESAN[1], CLAUDIU IONITA[1], DESPINA CALAMAR[1], MIHAI IONITA[1], HORTENSIA IONITA[1]

1. **University of Medicine and Farmacy „Victor Babes", Timisoara**

ABSTRACT

Chronic lymphocytic leukemia (CLL) is one of the most common lymphoid malignancies characterized by a variable clinical course. The median age at diagnosis is 72 years but CLL is diagnosed with increasing frequency in younger patients. Many prognostic markers have been identified that predict outcomes for patients with CLL.

The majority of treatments carrying significant toxicities and knowing whom to treat and when becomes very important. The chemoimmunotherapy regimens increase the potential for deep remissions, but is important to worry about the long-term toxicities of treatments that might limit survival on patients.

In assessing prognostic markers, it is important to determine whether the information will aid in the care of the patient or will influence treatment decisions.

***Key words**: chronic lymphocytic leukemia (CLL), traditional prognostic factors, novel/molecular-based prognostic markers.*

Correspondence to:

Ioana Ioniță
Adress: County Emergency Hospital, Gh. Dima Street, nr. 3
Phone: 0723539800
E-mail address: mdioanaionita@yahoo.com

INTRODUCTION

Patients with CLL are being diagnosed earlier in the course of their disease and are living longer with their CLL diagnosis, resulting in longer time periods in which to determine when to start therapy. The relative survival for patients age 40 to 59 years is 69.9% versus 38.1% for patients greater than age 80 years [1]. There is great need to determine when to intervene, regardless of age.

An aspect to explore is whether variables can be identified that can reliably and accurately predict prognosis. The literature identifies over 35 different prognostic markers. These are classified as "traditional", and "novel." The traditional prognostic markers tend to be those obtainable from routine history, physical examination, and lab work. The novel prognostic factors tend to assess molecular aspects of the CLL cell themselves [2]. The other aspect is whether the prognostic marker will impact on clinical decision making.

Prognostic factors - basic considerations: CLL has a chronic relapsing course requiring multiple treatment episodes. Overall survival varies considerably between individual patients and is governed by: the pace of the disease, the effectiveness and toxicity of available treatments, and the propensity of the disease to undergo clonal evolution [2, 3].

A number of clinical and laboratory features of CLL correlate with prognosis, can be divided into baseline clinical features, response to therapy and laboratory tests or "prognostic biomarkers" [4,5,6] (Table 1).

Table 1 A very partial list of prognostic markers

clinical	chromosome/ genetic bassed	Cytokine/soluble molecule	Cell based	miscellaneous
stage	IgVH mutation	beta-2-microglobulin	CD 38	Bone marrow vessel density
lymphocyte doubling time	interphase FISH	VEGF	ZAP-70	direct antiglobulin test
pattern of bone marrow involvement	karyotype	bFGF	CD49d	relA
age	microRNA	IL-6	CD26	CLLUI
gender	V gene usage	IL-8	FCRL2	Circulating endothelial cells
percent smudge cells	IRF4 polymorphism	thrombospondin-1	p27	
circulating prolymphocytes	deletion 6q	plasma thrombopoietin	HIS	
MRD status	MDM2 SNP	soluble ICAM-I	p53 mutation	
treatment response	Bcl-2 polymorphism	soluble NK.G2D ligands		
duration of response	bcl-6 mutation	soluble CD23		
performance status	telomere length	soluble CD27		
ALC	nucleolar morphology	serum free light chains		
# *of nodal groups*		angiopoietin-2 circulating Ki-67 lipoprotein lipase serum thymidine kinase		

VEGF indicates vascular endothelial growth factor; bFGF, basic fibroblast growth factor; IL, interleukin; IRF4, interferon regulatory factor 4; MRD, minimal residual disease; SNP, single nucleotide polymorphism; ICAM-1, intercellular adhesion molecule 1 ; ZAP-70, zeta-associated protein-70.

Basic clinical features comprise those that reflect tumor burden at diagnosis (Rai and Binet staging); [7, 8] and those that reflect the pace of the disease at diagnosis (lymphocyte doubling time) [9]. Both have been used for many years to inform on prognosis and when to start treatment.

Another powerful predictor of outcome is therapeutic response. The depth of response obtained following treatment correlates with remission duration and survival. Remission duration appears to depend on the depth of remission obtained more than on the type of treatment employed [10].

Prognostic biomarkers include: serum factors β_2-microglobulin, phenotypic features such as CD38 and ZAP-70 expression and genetic features such as immunoglobulin gene mutation status and deletion/mutation of TP53 or ATM.

Traditional prognostic markers – The first prognostic marker was the Rai stage [7]. The Rai Clinical Staging System utilized lymphadenopathy, organomegaly, and cytopenias (anemia and thrombocytopenia) to established five prognostic groups with median survivals of: (1) stage 0, > 150 months; (2) stage I, 101 months; (3) stage II, 71 months; (4) stage III, 19 months; and (5) stage IV, 19 months. The Rai Clinical Staging System was modified to include three groups: stage 0 being low risk, stages I and II being intermediate risk stages III and IV being high risk.

The Binet Staging System, which relied on the number of involved nodal areas involved by CLL and cytopenias to create a three-group classification [8]. The Rai and Binet systems provide not only a prognosis for a patient, but also identify when a patient is appropriate for therapy.

Other basic laboratory features that have been found to provide prognostic information include: pattern of bone marrow involvement, [8, 11] number of prolymphocytes in blood or bone marrow, [12] age, [13] gender, [13] lymphocyte doubling time, [9] absolute lymphocyte count [14] and β_2-microglobulin [15].

Weirda et al [16] developed a nomogram that merged into a single model: age, β_2-microglobulin, absolute lymphocyte count, sex, Rai stage, and number of involved lymph node groups. This model has been validated using the Mayo Clinic database, with estimated median survival times of not reached for low risk, 10.1 years for intermediate risk, and 7.2 years for high risk [17].

Novel/molecular-based prognostic markers – The distinction between novel and traditional prognostic markers helps by designating those that are more likely to yield insights into the biology of CLL. The four novel prognostic markers that currently are in use in clinical practice are the following [18]:

1. immunoglobulin heavy-chain variable region (IGVH) mutational status
2. interphase fluorescence in-situ hybridization (FISH) abnormalities,
3. CD38, and
4. zeta-associated protein (ZAP)-70.

Immunoglobulin gene status – The initial reports about untreated patients with early-stage disease, showed that those patients with unmutated IGHV genes had a shorter overall survival and a shorter time to disease progression indicating more rapid clonal expansion [18, 19].

Once the germline immunoglobulin genes were characterized. it was possible to determine that 40% of CLL cases were unmutated and 60% of cases mutated. Two studies published about IGVH mutational status, with unmutated cases demonstrating a much more aggressive course and a median survival of 8 to 9 years versus > 20 years for

those CLL cases with mutated IGVH genes [20].

It has also been confirmed as a predictor of remission duration and/or survival in most clinical trials of first-line chemotherapy or immunochemotherapy-rituximab-fludarabine, cyclophosphamide (R-FC) [21]. It is universally poor at predicting which patients will achieve a complete response (CR). The prognostic value of IGHV status relative to other biomarkers such as CD38 and ZAP-70 expression remains controversial; a recent study suggested that ZAP-70 was the dominant biomarker, and that IGHV status was only informative in ZAP-70 negative cases [22].

Knowledge of IGVH status is of more difficult to interpret once treatment is required and is of no value in patients who are refractory to fludarabine. There is no evidence that knowledge of IGHV status, might improve outcome. IGHV status should not be used to influence the initiation of treatment, the choice of induction therapy or post-induction maintenance / consolidation strategies.

Another study [23] demonstrated that CD38 correlated with IGVH mutational status and predicted clinical outcome. Additional controversy has developed regarding whether CD38 expression can vary over the course of disease. The data from deuterium-labeling experiments suggest that CD38 is transiently expressed on cells that are proliferating, with deuterium-labeled cells becoming CD38 negative and vice-versa. CD38 represented an easily obtainable prognostic marker that correlated with IgVH mutational status and predicted prognosis independent of IgVH mutational status [18,23].

Fluorescence In-situ hybridisation defects – It has been known for some time that recurrent chromosomal abnormalities occur in CLL and are associated with prognosis. FISH has emerged as the method of choice for detecting chromosomal abnormalities in CLL.

The most well-characterised chromosomal abnormalities in CLL are del13q14 (13q-), trisomy 12 (+12), del11q22-23 (11q-) and del17p13 (17p-). The prognostic value of these abnormalities was clarified in a pivotal study in which patients untreated early-stage CLL could be separated into distinct prognostic groups according to the presence or absence of the genetic abnormalities [24].

Patients with more than one FISH abnormality were categorised in order of importance: 17p- > 11q- > +12 > 13q-. 17p- was associated with the worst outcome, followed by 11q- in the absence of 17p-. Patients with +12 but not 17p- or 11q- fared similarly to patients with no FISH defects, whereas those with 13q- as the sole abnormality had a better outcome [18, 24]. FISH has emerged as a robust and reliable test and may have a role in helping to select the appropriate treatment strategy once treated is indicated.

In this prognostic model, patients were grouped into one of five groups, with median survivals of the following: (1) del 13q as a sole abnormality (133 months); (2) del 11q (79 months); (3) trisomy 12 (114 months); (4) del 17p (32 months), and (5) normal (111 months). All groups, except for del 13q. included samples that had multiple abnormalities [25]. In 2001, Rosenwald et al, [26] using gene array profiling, demonstrated that all CLL patients share a common CLL-specific gene expression signature, regardless of IgVH mutational status. Among the genes that were differentially expressed between mutated and unmutated CLL cases, ZAP-70 was the most differentially expressed gene between the two subtypes, being expressed in unmutated CLL.

Other groups confirmed that ZAP-70 expression correlated with IgVH

mutational status and predicted outcome for CLL patients [27]. Rassentiet al [28] demonstrated that IgVH mutational status and ZAP-70 status were independent predictors of outcome, ZAP-70 proved to be a stronger predictor of outcome compared with IgVH mutational status.

17p- The real strength of FISH analysis lies in its ability to predict the outcome of patients who have already progressed to the point of requiring first-line therapy. Prospective clinical trials have shown that deletion of TP53 at 17p13 is strongly associated with resistance to a range of first-line chemotherapy and immunochemotherapy regimens including FC, F-R, R-FC and FCM, R-FCM (M-mytoxanthrone) [29, 30].

The chemoresistance of 17p- CLL can be explained by fact that TP53 deletion is usually associated with an inactivating mutation of the remaining TP53 allele resulting in required for the full cytotoxicity of DNA-damaging chemotherapy [31] including purine analogues [32]. Other drugs which are active in CLL do not rely on p53 for their action. The agent with the most evidence supporting its use in 17p- CLL is alemtuzumab [33, 34].

Other agents with activity in this setting include flavopiridol, [35] lenalidomide [36] and methylprednisolone [37]. 17p- status should be checked prior to each treatment episode if used as a basis for treatment decisions.

TP53 mutation **–**The minimum deleted region at 17p13 includes the TP53 gene, which encodes the p53 tumor suppressor protein. The p53 protein is a transcription factor which, when activated by cellular stresses such as DNA damage, accumulates in the nuleus and transactivates multiple genes that induce apoptosis or cell cycle arrest or effect DNA repair [38].

The p53 protein may also induce apoptosis directly in CLL cells by physically interacting with mitochondria [18]. P53 suppresses clonal expansion, [39] mediates the action of DNA-damaging chemotherapy, and maintains genomic stability [39].

P53 is frequently inactivated in neoplastic cells, the usual defect being deletion of one TP53 allele and mis-sense mutation of the other [40] or as p53 protein overexpression. TP53 mutation alone is a powerful predictor of adverse outcome, in patients with predominantly early-stage CLL.

11q- and ATM deletion – Deletion of ATM at 11q22-23 occurs in about 20-25% of patients with previously untreated CLL and has been associated with early disease progression and short progression-free survival following first-line chemotherapy.

ATM (ataxia telangiectasia mutated) is a serine/threonine kinase that activates a range of targets including p53 in response to double-strand DNA breaks such as those induced by chemotherapy and ionising radiation [41]. ATM is inactivated by mutation in 12% of patients with CLL and is associated with an impaired p53 response to DNA damage and reduced overall survival.

p53 protein over-expression and p53 functional analysis – Mutant p53 protein is often present at increased levels, and immunohistochemical detection of p53 protein in bone marrow samples has been shown to predict adverse outcome following frontline R-FC [42].

Application of Prognostic Factors to Clinical Practice – The most important is how the prognostic data can be used to guide clinical practice. The decisions that need to be made prior to treating a patient are when and with which therapy. Treatment is only indicated for those patients with documented active disease according to the International Working Group on CLL Treatment Guidelines [2]. The only prog-

nostic markers included in the criteria are Rai stage III or IV disease (progressive marrow failure) or a lymphocyte doubling time of less than 6 months.

A possible means for impacting on the poor prognosis of patients with high-risk disease might include reducing disease burden before CLL cells have time to acquire additional genetic changes that make them more resistant to therapy. Because the secondary genetic changes are typically the result of therapy, avoiding treatment for as long as possible is advantageous [2, 18].

Currently approved therapies for CLL include chlorambucil, fludarabine (or purine)-based chemotherapy, alemtuzumab, bendamustine, orofatumumab. Extensive data demonstrate that 17p deleted or p53 mutated cases of CLL respond very poorly to purine analog or alkylator-based therapy [43].

Alemtuzumab appears to work via a p53 independent pathway, and has demonstrated efficacy in 17p deleted or p53 mutated CLL [44]. In the fludarabine and alemtuzumab refractory CLL patients treated as part of the pivotal ofatumumab study, deletion of 17p did not predict for lack of response.

Bendamustine does demonstrate some responses in fludarabine refractory CLL patients [45]. In the setting of a CLL patient demonstrating p53 dysfunction, avoiding alkylator and purine analog-based therapies in favor of alemtuzumab, and arguably ofatumumab [46] would allow avoiding toxicides associated with therapies that have a low expectation of efficacy.

Another debate regarding the treatment of CLL patients is whether FR or FCR should be the choice for initial therapy. Data from the German CLL Study Group CLL4 trial demonstrated improved response rates and progression-free survival, but no improvement in overall survival, for FC as compared to F as initial therapy for patients with CLL [46]. Deletion 11q was the only negative prognostic marker that remained predictive of improved progression free survival for FC, compared with F chemotherapy.

CONCLUSIONS

A great deal of work has led to the identification of a large number of prognostic markers for CLL patients. Although prognostic information will provide many insights into the biology of CLL, the utility of this information in clinical practice is a separate question.

The only genetic marker of prognosis in CLL that provides information of sufficient reliability and clinical importance to influence treatment decisions in CLL is TP53 deletion and, to a lesser extent, TP53 mutation. Other genetic markers of prognosis, such as 11q- and IGHV status, provide reliable prognostic information that may help patients to plan their lives. These biomarkers do not provide information of sufficient impact in the context of modern immunochemotherapy to influence treatment decisions in routine clinical practice.

REFERENCES

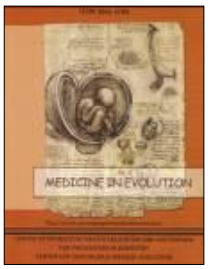
1. Jemal A, Siegel R. Ward E, Hao Y. Xu J, Thun MJ. Cancer statistics, 2009. CA Cancer J Clin. 2009; 59: 225-249.
2. Furman RR. Prognostic markers and stratification of Chronic Lymphocytic Leukemia. Hematology 2010: 77-81.
3. Zenz T, Mertens D, Küppers R, Döhner H, Stilgenbauer S. From pathogenesis to treatment of

chronic lymphocytic leukaemia. Nat Rev Cancer 2010; 10: 37-50.
4. Hallek M; German CLL Study Group. Prognostic factors in chronic lymphocytic leukemia. Ann Oncol 2008; 19: 51-3.
5. Moreno C, Montserrat E. New prognostic markers in chronic lymphocytic leukemia. Blood Rev 2008; 22: 211-9.
6. Montserrat E, Moreno C. Genetic lesions in chronic lymphocytic leukemia: clinical implications. Curr Opin Oncol 2009; 21: 609-14.
7. Rai KR, Sawitsky A, Cronkite EP, Chanana AD, Levy RN, Pasternack BS. Clinical staging of chronic lymphocytic leukemia. Blood 1975; 46: 219-34.
8. Binet JL, Lepoprier M, Dighiero G, Charron D, d'Athis P, Vaugier G et al. A clinical staging system for chronic lymphocytic leukemia: prognostic significance. Cancer 1977; 40: 855-64.
9. Montserrat E, Sanchez-Bisono J, Viñolas N, Rozman C. Lymphocyte doubling time in chronic lymphocytic leukaemia: analysis of its prognostic significance. Br J Haematol 1986; 62: 567-75.
10. Boettcher S, Fischer K, Stilgenbauer S, Busch R, Fingerle- Rowson G, Fink A-M et al. Quantitative MRD assessments predict progression free survival in CLL patients treated with fludarabine and cyclophosphamide with or without rituximab — a prospective analysis in 471 patients from the randomized GCLLSG CLL8 Trial. Blood (ASH Annual Meeting Abstracts) 2008; 112: 3139.
11. Han T, Barcos M. Emrich L. et al. Bone marrow infiltration patterns and their prognostic significance in chronic lymphocytic leukemia: correlations with clinical, immunologic, phenotypic, and cytogenetic data. J Clin Oncol. 1984; 2: 562-570.
12. Meló JV, Catovsky D. Gregory WM, Galton DA. The relationship between chronic lymphocytic leukaemia and prolymphocytic leukaemia. IV. Analysis of survival and prognostic features. Br J Haematol. 1987;65:23-29.
13. Catovsky D. Fooks J, Richards S. Prognostic factors in chroni clymphocytic leukaemia: the importance of age. sex and response to treatment in survival. A report from the MRC CLL 1 trial. MRC Working Party on Leukaemia in Adults. Br J Haematol. 1989; 72: 141-149.
14. Bergmann MA. Eichhorst BF. Busch R. et al. Prospective evaluation of prognostic parameters in early stage chronic lymphocytic leukemia (CLL): results of the CLL 1-protocol of the German CLL Study Group (GCLLSG). Blood. 2007; 110.
15. Hallek M, Wanders L. Ostwald M, et al. Serum beta(2)-microglobulin and serum thymidine kinase are independent predictors of progression-free survival in chronic lymphocytic leukemia and immunocytoma. Leuk Lymphoma. 1996; 22: 439-447.
16. Wierda WG. O'Brien S. Wang X, et al. Prognostic nomogram and index for overall survival in previously untreated patients with chronic lymphocytic leukemia. Blood. 2007; 109: 4679-4685.
17. Shanafelt TD. Jenkins G. Call TG. et al. Validation of a new prognostic index for patients with chronic lymphocytic leukemia. Cancer 2009: 115: 363-372.
18. Pettitt AR. Genetic markers of prognosis in chronic lymphocytic leukemia. EHA 2010; 4: 85-92.
19. Damle RN, Wasil T, Fais F, Ghiotto F, Valetto A, Allen SL et al. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood 1999; 94: 1840-7.
20. Hamblin TJ. Davis Z, Gardiner A. Oscier DG. Stevenson FK. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood. 1999: 94: 1848-1854.
21. Lin KI, Tam CS, Keating MJ, Wierda WG; O'Brien S, Lerner S et al. Relevance of the immunoglobulin VH somatic mutation status in patients with chronic lymphocytic leukemia treated with fludarabine, cyclophosphamide, and rituximab (FCR) or related chemoimmunotherapy regimens. Blood 2009; 113: 3168-71.
22. Rassenti LZ, Jain S, Keating MJ, Wierda WG, Grever MR, Byrd JC et al. Relative value of ZAP-70, CD38, and immunoglobulin mutation status in predicting aggressive disease in chronic lymphocytic leukemia. Blood 2008; 112: 1923-30.
23. Damle RN. Wasil T. Fais F. et al. lg V Gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood. 1999: 94: 1840-1847.
24. Pittman S, Catovsky D. Prognostic significance of chromosome abnormalities in chronic lymphocytic leukaemia. Br J Haematol 1984; 58: 649-60.
25. Döhner H, Stilgenbauer S, Benner A, Leupolt E, Kröber A, Bullinger L et aL Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med 2000; 343: 1910-6.
26. Rosenwald A. Alizadeh AA, Widhopf G. et al. Relation of gene expression phenotype to immunoglobulin mutation genotype in B cell chronic lymphocytic leukemia. J Exp Med. 2001: 194: 1639-1647.
27. Del Principe MI, Del Poeta G. Buccisano F. et al. Clinical significance of ZAP-70 protein expression in B-cell chronic lymphocytic leukemia. Blood. 2006: 108: 853-861.
28. Rassenti LZ. Huynh L, Toy TL. et al. ZAP-70 compared with immunoglobulin heavy-chain gene mutation status as a predictor of disease

progression in chronic lymphocytic leukemia. N Engl J Med. 2004:351:893-901.

29. Byrd JC, Gribben JG, Peterson BL, Grever MR, Lozanski G, Lucas DM et al. Select high-risk genetic features predict earlier progression following chemoimmunotherapy with fludarabine and rituximab in chronic lymphocytic leukemia: justification for risk-adapted therapy. J Clin Oncol 2006; 24: 437-43.
30. Hallek M, Fingerle-Rowson G, Fink A-M, Busch R, Mayer J, Hensel M et al. First-line treatment with fludarabine (F), cyclophosphamide (C), and rituximab (R) (FCR) improves overall survival (OS) in previously untreated patients (pts) with advanced chronic lymphocytic leukemia (CLL): Results of a randomized phase III trial on behalf of an international group of investigators and the German CLL Study Group. Blood (ASH Annual Meeting Abstracts) 2009; 114: 535.
31. Lowe SW, Ruley HE, Jacks T, Housman DE. p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. Cell 1993; 74: 957-67.
32. Pettitt AR. Mechanism of action of purine analogues in chronic lymphocytic leukaemia. Br J Haematol 2003; 121: 692-702.
33. Lozanski G. Heerema NA. Flinn IW, et al. Alemtuzumab is an effective therapy for chronic lymphocytic leukemia with p53 mutations and deletions. Blood. 2004: 103: 3278-3281.
34. Osuji NC, Del Giudice I, Matutes E, Wotherspoon AC, Dearden C, Catovsky D. The efficacy of alemtuzumab for refractory chronic lymphocytic leukemia in relation to cytogenetic abnormalities of p53. Haematologica 2005; 90: 1435-6.
35. Lin TS, Ruppert AS, Johnson AJ, Fischer B, Heerema NA, Andritsos LA et al. Phase II study of flavopiridol in relapsed chronic lymphocytic leukemia demonstrating high response rates in genetically high-risk disease. J Clin Oncol 2009; 27: 6012-8.
36. Ferrajoli A, Lee BN, Schlette EJ, O'Brien SM, Gao H, Wen S et al. Lenalidomide induces complete and partial remissions in patients with relapsed and refractory chronic lymphocytic leukemia. Blood 2008; 111: 5291-7.
37. Thornton PD, Matutes E, Bosanquet AG, Lakhani AK, Grech H, Ropner JE et al. High dose methylprednisolone can induce remissions in CLL patients with p53 abnormalities. Ann Hematol 2003; 82: 759-65.
38. Junttila MR, Evan GI. p53 - a Jack of all trades but master of none. Nat Rev Cancer 2009; 9: 821-9.
39. Chen PL, Chen YM, Bookstein R, Lee WH. Genetic mechanisms of tumor suppression by the human p53 gene. Science 1990; 250: 1576-80.
40. Vogelstein B, Kinzler KW. p53 function and dysfunction. Cell 1992; 70: 523-6.
41. Lavin, MF Ataxia-telangiectasia: from a rare disorder to a paradigm for cell signalling and cancer. Nature Rev Mol Cell Biol 2008; 9: 759-69.
42. Zenz T, Stilgenbauer S. Therapy with the FCR regimen does not overcome chronic lymphocytic leukemia biology: aberrant p53 expression predicts response and survival. Leuk ymphoma 2009; 50: 1559-61.
43. Catovsky D, Richards S, Matutes E, Oscier D, Dyer MJ, Bezares RF et al. Assessment of fludarabine plus cyclophosphamide for patients with chronic lymphocytic leukaemia (the LRF CLL4 Trial): a randomised controlled trial. Lancet. 2007; 370: 230-9.
44. Hillmen P. Skotnicki AB. Robak T, et al. Alemtuzumab compared with chlorambucil as first-line therapy for chronic lymphocytic leukemia. J Clin Oncol. 2007; 25: 5616-5623.
45. Knauf WU, Lissichkov T, Aldaoud A, et al. Phase III randomized study of bendamustine compared with chlorambucil in previously untreated patients with chronic lymphocytic leukemia. J Clin Oncol. 2009; 27: 4378-4384.
46. Wierda WG. Kipps TJ. Mayer J. et al. Ofatumumab as singleagent CD20 immunotherapy in fludarabine-refractory chronic lymphocytic leukemia. J Clin Oncol. 2010; 28: 1749-1755.

IMPORTANCE OF THE CYTOGENETIC EXAMINATION IN MONITORING THE TREATMENT WITH TYROSINE-KINASE INHIBITORS IN CHRONIC MYELOGENOUS LEUKEMIA PATIENTS. CASE REPORT



MARIA MIHAELA DELAMARIAN[1], IOANA IONITA[1], DACIAN OROS[1], CRISTINA GUG[1], HORTENSIA IONITA[1]

1. **University of Medicine and Farmacy „Victor Babes", Timisoara**

ABSTRACT

Chronic myelogenous leukemia (CML) is a clonal disease of the hematopoietic, pluripotent stem cell characterized by the presence of the Philadelphia chromosome (Ph1) and/or the BCR/ABL rearrangement. The common clinical evolution is triphasic: chronic phase (CP), accelerated phase (AP) and blastic phase (BP).

Treatment with tyrosine-kinase inhibitors (TKI), Imatinib, Dasatinib, Nilotinib, in CML patients fundamentally changed the prognosis of this disease. The monitoring of the TKI treatment is done at hematological, cytogenetical and molecular level. The complete cytogenetic response has become the golden standard for therapeutic response.

***Key words**: tyrosine-kinase inhibitors, cytogenetic response, Imatinib, Dasatinib.*

Correspondence to:

Ioana Ioniță
Adress: County Emergency Hospital, Gh. Dima Street, nr. 3
Phone: 0723539800
E-mail address: mdioanaionita@yahoo.com

INTRODUCTION

CML was the first neoplasic disease for which genotype knowledge led to targeted treatment. Due to a good knowledge of physiopathology, clear diagnostic criteria, well established prognosis factors and etiologic disease treatment, CML serves as a model for other types of cancer [1].

CML is a hematopoietic disease which typically appears as benign during the chronic phase and progresses, usually through an accelerated phase, to the blastic, fatal phase during a 3-5 years period. CML is defined by the presence of BCR-ABL fusion genes resulting from translocation of the long arms of the 9 and 22 chromosomes leading to the formation of the Ph1 chromosome.

A decade has passed since the first TKI, Imatinib Mesilat (Glivec) was introduced into clinical practice. Before Imatinib, the therapy of Philadelphia positive CML (Ph+) included hydroxiurea, alpha interferon, and allogenic transplantation of hematopoietic stem cells. The introduction of Imatinib which directly targets the tyrosine-kinase activity of the oncogenic proteins encoded BCR/ABL, rapidly and dramatically changed the CML treatment and led to important disease management changes [2].

Concomitantly, other TKI developed. Some of these have been tested by clinical investigations, and two of them, Dasatinib (Sprycel) and Nilotinib (Tasigna) were registered throughout the world for the treatment of patients with Imatinib intolerance or resistance [3, 4].

Precise definitions of the response to TKI treatment may vary between studies, which is why the European Leukemia Net has developed a standard terminology to allow high accuracy comparisons between studies and a standard approach of the treatment. The complete haematologic response (CHR) is defined by a number of platelets <450 x 109/l, leukocytes <10 x 109/l, absence of immature granulocytes, basophiles <5% and non-palpable spleen. Cytogenetic response is defined as the proportion of Ph+ cells in the bone marrow: complete cytogenetic response (CCyR): Ph+ 0%; major cytogenetic response (MCyR) Ph+ 1% 35%; minor cytogenetic response Ph+ 36% 65%; minimal cytogenetic response Ph+ 66% 95%; no cytogenetic response Ph+ >95%.

The molecular response depends on the level of BCR-ABL mRNA residual transcript as a proportion between BCR-ABL and the blood level of the control gene transcript (complete molecular response [CMR]: undetectable transcript level; major molecular response [MMR]: transcript level ≤ 0.10%) [5, 6].

CASE REPORT

Patient R.I., male gender, aged 53 years, is admitted in the Haematology Clinic of the County Hospital in Timisoara, in April 2008, for the following symptoms: asthenia, fatigue, weight loss, sweating, muscle pain, early satiety sensation. Objective clinical examination: pale teguments and mucous membranes, hepatomegaly (+2 cm), splenomegaly (+12 cm). Blood count: leukocytosis with formula expanded to myeloblast, basophilia (26%), moderate anemia. Bone marrow kariotype: by standard cytogenetic method the t (9; 22) (q34; q11) translocation resulting in the Philadelphia 1 chromosome, the cytogenetic marker of chronic myelogenous leukemia. Molecular biology:

transcript Bcr-Abl p210 present, transcript type – b3a2 = 34%.

Based upon the performed investigations, the diagnosis of CML-AP is formulated and treatment with hydroxiurea for two months is initiated without achieving haematologic remission. In June 2008, treatment with Imatinib 600 mg/day is initiated. Haematologic, cytogenetic and molecular monitoring of the treatment:

- At 3 months: CHR;
- At 6 months: CHR, CCyR (Ph+ clone = 0%), without MMR (Bcr-Abl transcript = 0.25%);
- At 12 months: CHR, CCyR (Ph+ clone = 0%), without MMR (Bcr-Abl transcript = 0.25%);
- At 18 months: CHR, MCyR (Ph+ clone = 28%), without MMR (Bcr-Abl transcript = 0.92%). Kariotype (Figure 1) – by standard cytogenetic method three cellular clones are revealed: one normal 46xy (58%), one pathological 47, xy + 8 (14%), one pathological 46, xy, Ph1t (9; 22) (q34; q11) (28%). A new pathological cellular clone must be mentioned, i.e. the total 8 trisomy, which is a typical abnormality for the accelerated phase of the disease. Considering that previously, under Imatinib therapy, the patient reached CCyR which is presently lost with additional aquisition of a new pathological cellular clone (+8), two problems may be brought into discussion: the patient did not strictly adhere to the Imatinib treatment or there is a risk for the development of resistance to Imatinib.

The patient correctly followed the Imatinib treatment, nevertheless, upon the 18 months assessment he loses the CCyR and no MMR was achieved, for which reason the initiation of second generation TKI treatment is decided. Since July 2010, treatment with dasatinib 100 mg/day is initiated which is well tolerated. Haematologic, cytogenetic and molecular monitoring of the treatment reveals:

- At 3 months: CHR, CCyR (Ph+ clone = 0%), MMR (Bcr-Abl transcript = 0.0024%);
- At 6 months: CHR, CCyR (Ph+ clone = 0%), CMR (Bcr-Abl transcript - negative);
- At 12 months: CHR, CCyR (Ph+ clone = 0%), CMR (Bcr-Abl transcript - negative).

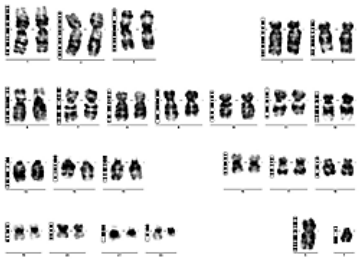


Fig. 1

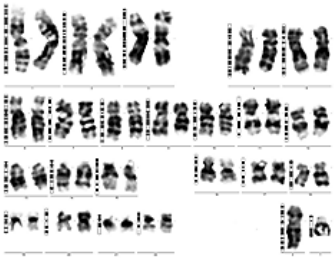


Fig. 3

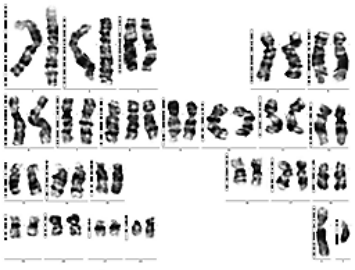


Fig. 3

CONCLUSIONS

CCy response has become the golden standard for the favourable response in CML due to its association to a survival advantage.

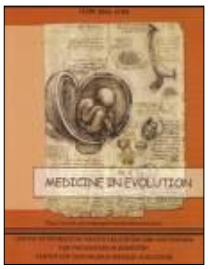
The achievement of CCyR remains the objective of TKI treatment in CML even though the additional achievement of MMR may offer added protection against the increased risk of

disease progression. Dasatinib induces complete cytogenetic response and is well tolerated by patients with Imatinib resistance of intolerance.

REFERENCES

1. Rudiger Hehlmann, Andreas Hochhaus, Michele Bacarani, on behalf of European LeukemiaNet - Chronic Myeloid Leukemia. Lancet, 2007, vol. 370, p. 342-350;
2. Michele Bacarani, Jorge Cortes, Hagop Kantarjian, Timithy Huges, et all - Chronic Myeloid Leukemia: An Update of Concepts and Management Recommendations of European Leukemia Net. Journal of Clinical Oncology, 2009.
3. Talpaz M., Shah P.S., Kantarjian H. et all – Dasatinib in Imatinib resistant Philadelphia chromosome positive leukemia. N Engl. J of Med. 354: 2531-2541, 2006.
4. Hochhaus A., Baccarani M., Deininger M. et al – Dasatinib induces durable cytogenetic responses in patients with chronic myelogenous leukemia in chronic phase with resistance or intolerance to Imatinib. Leukemia 22: 1200-1206, 2008.
5. Baccarani M., Saglio G., Goldman J., Hochhaus A., Simonsson B., Appelbaum F., et al – Evolving concept in the management of chronic myeloid leukemia; Recommendations from an expert pannel of behalf of the European Leukemia Net. Blood, 2006; 108: 1809-20.
6. Timothy Hughes and Andreas Hochhaus – Clinical Strategies to Achieve an Early and Successful Response to Tyrosine Kinase Inhibitor Therapy. Seminars in Hematology, Vol. 46, No. 2, Suppl. 3, 2009, p S11-S15.

THE IMPORTANCE OF THE CAROTID INTIMA-MEDIA THICKNESS IN THE PREDICTION OF CARDIOVASCULAR EVENTSIN PATIENTS WITH VASCULAR DISEASE



ADRIAN GRUICI[1], ELENA ARDELEANU[2], DANIELA GURGUS[2], ROXANA ALAMAN[3], RODICA MIHAESCU[4]

1. **MD, Phd Student, Family Medicine Office,Timiş County**
2. **Family Medicine Department, University of Medicine and Farmacy "Victor Babes" Timisoara**
3. **Cardiology Department, Cardiovascular Institute, Timisoara, Romania**
4. **Semiology Department, University of Medicine and Farmacy "Victor Babes" Timisoara**

ABSTRACT

***Background and objectives**: The carotid intima-media thickness (IMT) is an independent predictor of the cardiovascular events in the general population and an important marker regarding the evaluation of subclinical vascular damage. Currently, little is known about the relationship between the IMT and new cardiovascular events in patients with manifest arterial disease. We investigated whether IMT is associated with cardiovascular risk in patients who already have vascular disease and atherosclerotic risk factors.*

***Materials and Methods**: The study was performed between 2008-2011 and included 283 patients with manifest arterial disease or atherosclerotic risk factors, aged >50 years, mean age 63±5 years, 68% men and 32% females, registered in family medicine offices from Timiş County. IMT was measured at baseline in both of the carotid arteries by high resolution ultrasound as agreed in Mannheim consensus. Increased IMT (>0.9 mm) or the presence of the carotid atherosclerotic plaques are evidences of subclinical vascular damage.*

***Results** – Major vascular events were vascular death, ischaemic coronary events, or stroke. Adjusted for age and sex, an increase in the carotid IMT of 1 SD (~0.32 mm) was associated with an increased risk of any vascular event (Hazard Ratio-HR 1.17; 95% CI 1.04-1.30). Increasing IMT was most strongly related to ischaemic stroke incidence (HR 1.37; 95% CI 1.16-1.58), than to myocardial infarction incidence (HR 1.21; 95% CI, 1.04 to 1.38).*

***Conclusions** – IMT is associated with the occurrence of new vascular events, mostly for ischaemic stroke, in patients with manifest arterial disease or atherosclerotic risk factors.*

***Keywords**: Intima-media thickness, cardiovascular events, atherosclerotic risk factors, stroke.*

Correspondence to:

Adrian Gruici
Adress: Family Medicine Department, University of Medicine and Farmacy "Victor Babes" Timisoara
Phone: 0745149849
E-mail address: adriangruici@yahoo.com

INTRODUCTION

The carotid arteries are among the vessels that are prone to developing overt atherosclerotic lesions in the presence of the risk factors such as cigarette smoking and hypertension.

The carotid intima-media thickness (IMT) is an independent predictor of the cardiovascular events in the general population and an important marker regarding the evaluation of the subclinical vascular damage [1, 2]. Its use relies on its ability to predict future clinical cardiovascular end points [3, 4].

It has been shown that with high resolution two - dimensional ultrasound, the vessel wall characteristics of the carotid arteries can be assessed in an effective and accurate way at large-scale. This technique facilitates the evaluation of the lumen diameter, the intima-media thickness, the presence and extent of the plaques of the carotid arteries [5, 6]. The increased IMT reflects the atherosclerotic vessel wall disease in other arteries that are at high risk of atherosclerosis, such as the coronary arteries, the abdominal aorta, and the arteries of lower extremities [7, 8, 9].

The widespread clinical application of the B-mode ultrasound measurement of the IMT for stratifying the cardiovascular risk in the primary prevention is due to the epidemiological evidence that the increased IMT is a worthwhile predictor of subsequent coronary heart disease (CHD) and stroke, the two leading causes of the cardiovascular death [8, 9]. A growing number of studies and clinical trials used the IMT as an early marker of systemic atherosclerosis [4]. Good-quality images of the common carotid arteries are easy to obtain and the IMT can be reliably measured in nearly all subjects.

AIM AND OBJECTIVES

Our main objective was to investigate the association of the carotid IMT with the occurrence of new vascular events as myocardial infarction, stroke, peripheral arterial disease and cardiovascular death in adult patients at high risk, presenting clinically manifest arterial disease.

We aimed to study the association between the carotid IMT, ankle-brachial index (ABI) and lower extremity arterial atherosclerosis.

MATERIAL AND METHODS

The study enrolled between 2008-2011 patients from 6 family medicine offices of Timiş County. The average follow-up time was 34±6 months.

The initial study population consisted of 658 patients, aged >50 years, who were investigated by medical history, physical examination, vital signs, ECG, biochemistry, echocardiography, IMT and ABI measurement. Written and informed consent was obtained from all participants. Of these, only 283 patients presented manifest arterial disease, which included cerebral, coronary, peripheral arterial disease, renal artery stenosis, abdominal aortic aneurysm, and were validated for the study.

Cardiovascular Risk Factors and Events

The patients were evaluated in terms of cardiovascular risk factors and

cardiovascular events, current medications, demographic and anthropometric data (weight, height, body mass index-BMI, body surface area-BSA). The assessment was made considering age, sex, smoking habits, glucose tolerance, systolic and diastolic blood pressure, total, high- and low-density lipoprotein cholesterol. The body mass index was calculated as weight to height squared. The obesity was defined as BMI≥30 kg/m^2. The abdominal obesity was defined according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) guide as abdominal circumference > 88 cm in women and > 102 cm in men.

All participants underwent a complete vascular screening including laboratory analysis, a 12-lead electrocardiogram and echocardiography at the time of enrolment to verify the study inclusion criteria. The using of current medication, alcohol intake, actually or past cigarette smoking behaviour were reported by the patients in the medical history. The blood pressure was measured in supine position at the right brachial artery using a random-zero sphygmomanometer.

The average of two measurements, separated by a count of the pulse rate, obtained at two different visits, was used in the study. Hypertension was defined as a systolic blood pressure > 140 mmHg or a diastolic blood pressure > 90 mmHg or the current use of antihypertensive drugs for the indication of hypertension.

For the blood chemistry we used a CardioChek-PA analyzer with PTS-Panels test strips. The blood samples were collected after an overnight fast (fasting was defined as a self-reported interval of >12 hours since the last intake of food) and were drawn for glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides and ketones. Total cholesterol > 200 mg/dl and triglyceride levels > 150 mg/dl were considered as dyslipidemia. Diabetes mellitus was considered to be present when a subject was currently receiving oral antidiabetic drugs or insulin treatment or had a fasting blood glucose level > 126 mg/dl. The echocardiographic studies were performed in lateral decubitus position with a Sonoscape SSI-8000 Ultrasound system. The LV internal dimension, septal and posterior wall thickness were measured according to the American Society of Echocardiography. The LV ejection fraction was calculated according to the Simpson method. The LV mass was calculated according to the Devereux formula and was indexed to the body surface area. The LV hypertrophy was considered present when LV mass index (LVMI) was >125 g/m2 in men or >110 g/m2 in women. The relative wall thickness (RWT) was calculated as: (septal wall thickness + posterior wall thickness)/LV internal dimension in diastole. Increased RWT was present when this ratio was >0.43.

Ultrasonography of the Carotid Arteries

The intima-media thickness (IMT) was measured at baseline in both carotid arteries, as agreed in the Mannheim consensus (10), with a Sonoscape SSI-8000 high-resolution ultrasonography system, equipped with a 10-Mhz linear array transducer. The transducer aperture was 46 mm. The carotid arterial scanning was performed by a certified sonographer. The subjects were examined in a supine position with the head turned 45° from the side being scanned (Fig.1).

On a longitudinal two-dimensional ultrasound image of the carotid artery, the near and the far walls of the carotid artery are displayed as two bright white lines separated by a hypoechogenic space [5, 10]. The distance from the

leading edge of the first bright line of the far wall (lumen-intima inter-face) to the leading edge of the second bright line (media-adventitia interface) indicates the intima-media thickness [6, 10]. The studies have indicated that the posterior (far) wall intima-media thickness as seen with ultrasound truly reflects the anatomic intima-media layer [10, 12].

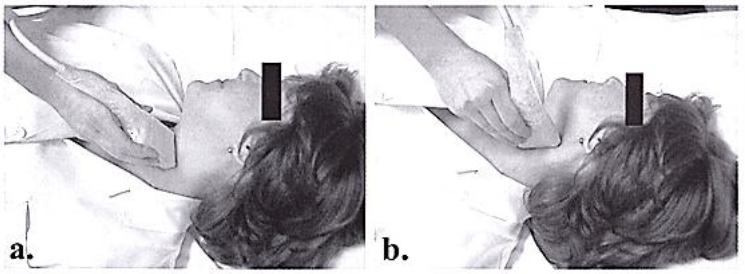


Fig.1. Angles used for intima-media thickness measurement:
a. transversal; b. longitudinal

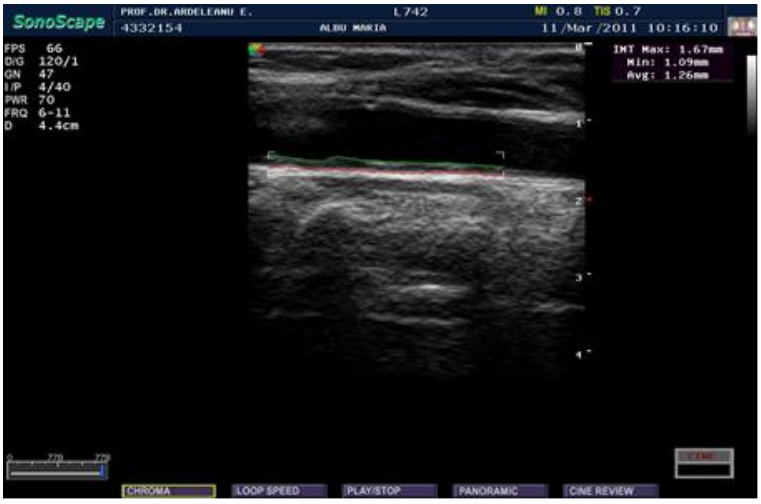


Fig.2. Ultrasound measurement technique of the intima media thickness (IMT)

When an optimal longitudinal image was obtained, it was frozen on the R wave of the ECG and stored on the system hard-disk. This procedure was repeated three times for both sides. The intima-media thickness was calculated online by built-in software of the ultrasound system (Fig.2). The mean IMT was calculated for the 6 measurements sites in each patient.

Indicators of Atherosclerosis

The presence of atherosclerosis of the lower extremities arteries was evaluated by measuring the systolic blood pressure level of the posterior tibial artery and the pedal artery (Fig.3) at both left and right sides with an 8 MHz continuous-wave Doppler probe and a sphygmomanometer [15]. The same test was performed at the brachial arteries.

For the calculation of the ankle-brachial index, we used the highest values obtained. The ratio of the systolic blood pressure at the ankle to the systolic blood pressure at the arm (ankle-brachial index-ABI) was calculated for each leg, being used the lowest ABI of either leg. In patients with leg pain du-

ring walking and having a normal resting ABI, we used an exercise test to settle the diagnosis. The lower extremity arterial disease was considered present when the resting ABI was <0.90 or when post exercise the ABI decreased at least in one leg with >20% (15).

The carotid color Doppler scanning was performed to detect the hemodynamic severity of the carotid artery stenosis. An artery lumen stenosis of more than 50% of the cross-sectional diameter was considered significant [4, 11]. The abdominal aortic aneurysm, measured with ultrasound, was defined as a distal anteroposterior diameter > 3 cm. The renal artery stenosis, measured with angiography, was defined as a diameter reduction of > 50% in at least one side [15]. The events of interest during the follow-up period were ischemic stroke, coronary ischemic events and vascular death. Hospitalization or outpatient discharge letters and results of relevant laboratory and radiology examinations were collected from the patients who recorded different events.

Statistical analysis

The data analysis included:

- Cox proportional hazards, performed to estimate the hazard ratios (HRs), with 95% confidence intervals (CIs), for the occurrence of new vascular events associated with an increase of 1 SD of IMT (~0.32 mm). The association of IMT and vascular events was adjusted for age and sex.
- The association between the carotid IMT and the ABI was evaluated by linear regression analysis, adjusted for age and sex.
- The student t test was used for comparing two groups; P values < 0.05 were considered statistically significant.
- The calculations were performed using the statistical software package Statcalc Epi-Info Version 6.

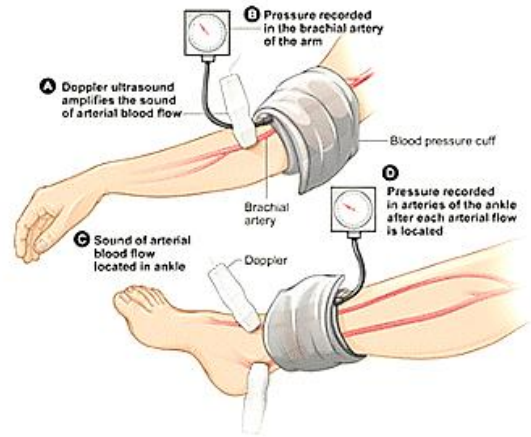


Fig.3. Technique for measuring blood pressure with Doppler probe.

RESULTS

Of the 625 patients with complete clinical and laboratory data, only 283 (45%) presented the study inclusion criteria and were considered eligible for the study. The mean age of the study group was 63±5 years. A number of 192 (68%) were men and a number of 91 (32%) were females. Baseline characteristics of the study group are presented in Table I and Table II.

Tabel 1 Characteristics of the study patients depending on the ongoing disease:

Characteristics	Study group (n=283)	With IHD (n=145)	Stroke or TIA (n=94)	PAD (n=91)	CAS (n=15)	AAA (n=24)	RAS (n=5)	Concomitent event (n=55)
SBP (mmHg)	150±22	145±21	155±23	151±19	149±20	153±21	157±21	160±21
DBP (mmHg)	91±12	86±10	96±11	88±9	90±11	93±12	97±10	98±11
Gender (male %)	68	74	67	63	65	80	66	75
Age (years)	63±5	60±8	65±3	63±5	65±3	66±2	60±4	65±5
BMI (kg/m²)	27.5±4.5	28.2±5.1	27.4±4.4	26.6±4	27.2±5	27±4.2	26.5±4	28.5±4
Total Cholesterol (mg/dl)	205±21	195±24	220±22	225±27	215±19	212±22	190±21	231±22
HDL-Cholesterol (mg/dl)	43±8	42±7	40±6	41±7	44±6	46±5	52±7	41±4
LDL-Cholesterol (mg/dl)	137±14	128±11	144±13	145±12	139±10	135±11	125±12	151±14
Triglycerides (mg/dl)	150±18	165±21	137±18	172±20	138±17	155±21	175±22	173±21
DM (%)	21	15	29	27	21	9	31	28
Smokers (%)	39	29	35	57	33	51	45	47
Ex-smokers (%)	76	74	75	86	72	33	71	88
Mean IMT (mm)	0.98±0.35	0.93	1.05	1.03	1.05	1.06	0.98	1.11
ABI (mean)	0.93±0.24	1.17	0.84	0.76	0.85	0.83	0.95	0.74

IHD= ischemic heart disease; TIA= transient ischemic attack; PAD= peripheral arterial disease; CAS= carotid artery stenosis; AAA= abdominal aortic aneurysm; RAS= renal artery stenosis; SBP= systolic blood pressure; DBP= diastolic blood pressure; BMI= body mass index; DM= diabetes mellitus; IMT= intima-media thickness; ABI= ankle-brachial index.

Tabel 2 Differences between the study groups with normal and increased IMT

Characteristics	Total study group (n=283)	IMT>0.9 (n=166)	IMT<0.9 (n=117)	P value
SBP (mmHg)	150±22	155±21	145±23	0.001
DBP (mmHg)	91±12	96±10	86±11	0.001
Gender (male %)	68	74	67	<0.001
Age (years)	63±5	65±8	60±3	<0.001
BMI (kg/m²)	27.5±4.5	28.2±5.1	27.4±4.4	0.01
Total Cholesterol (mg/dl)	205±21	209±24	199±22	0.01
HDL-Cholesterol (mg/dl)	43±8	40±7	42±6	0.01
LDL-Cholesterol (mg/dl)	137±14	141±15	133±12	0.01
Triglycerides (mg/dl)	150±18	155±21	147±18	0.02
Diabetes Mellitus (%)	21	25	17	0.001
Smokers (%)	39	51	32	<0.001
Ex-smokers (%)	76	88	63	<0.001
ABI (mean)	0.93±0.24	0.84	1.05	<0.001

Tabel 3 Cardiovascular diseases present at the study population

Nr.crt.	Vascular disease	Nr. of patients	Percentage (%)
1.	Ischemic heart disease	145	51.2
2.	Stroke or TIA	94	33.2
3.	Peripheral artery disease	91	32.1
4.	Abdominal aortic aneurysm	24	8.5
5.	Carotid artery stenosis	15	5.3
6.	Renal artery stenosis	5	1.8
7.	Concomitent diseases	55	19.4

The vascular diseases of the study group are presented in Table III. It can be seen that most of the patients had an ischemic heart disease (51.2%), stroke

or TIA (33.2%) and peripheral arterial disease (32.1%). A number of 55 (19.4%) patients presented two or more concomitant cardiovascular diseases.

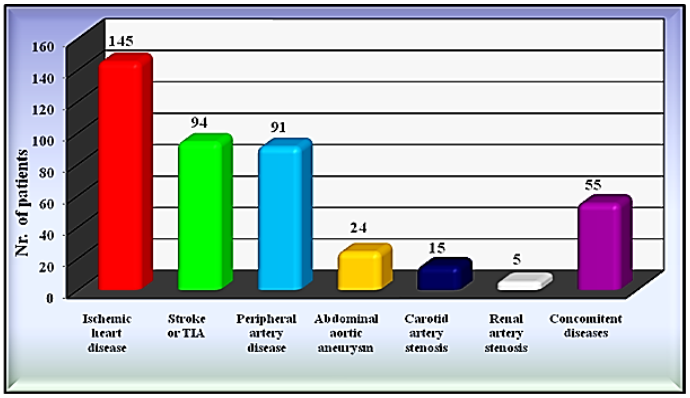


Fig.4. Cardiovascular diseases present at the study population

The values obtained from the measuring of IMT ranged from 0.41 to 3.89 mm, with a mean of 0.98 mm (SD 0.32 mm). The IMT was considered increased when it exceeded 0.9 mm. A value of IMT>2 mm, measured at one of the six measurement sites, was considered to reflect a plaque and it was incorporated in the IMT value.

Of the 283 patients who presented the study inclusion criteria, 166 (58.7%) of them showed increased IMT values while 117 (41.3%) had normal values (Fig. 5).

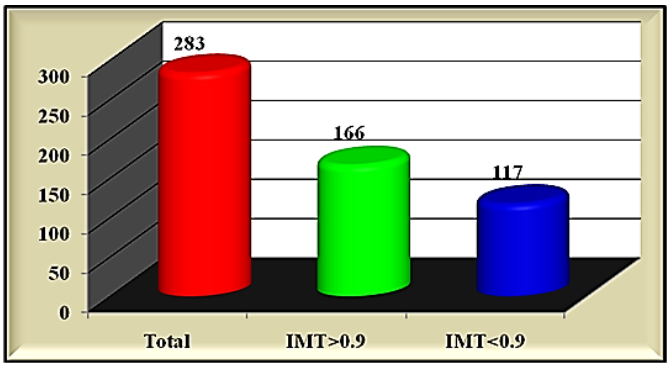


Fig.5. Distribution of the study population according to the IMT values

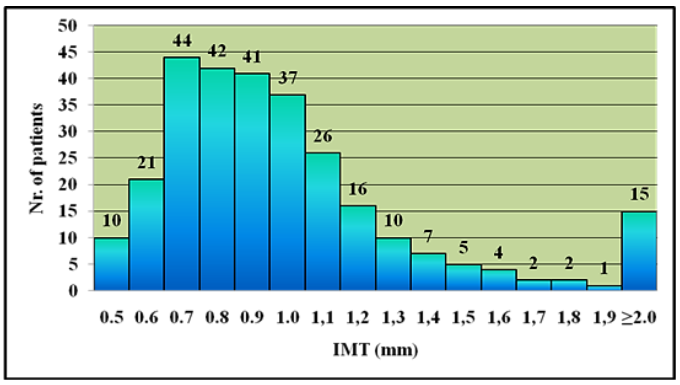


Fig.6. Values of IMT at the study population

Incidence of Stroke and Myocardial Infarction in the study group

During a median follow-up of 34±6 months, 42 patients experienced a new vascular event, of whom, 24 (57%) presented a myocardial infarction and 12 (28.5%) had a stroke; 15 (35.7%) patients died of a vascular cause.

The Hazard Ratios of the different vascular events are given for 1 SD increase in the IMT (Table IV). Adjusted for age and sex, an increase in the carotid IMT of 1 SD (~0.32 mm) was associated with an increased risk of any vascular event (Hazard Ratio-HR 1. 17; 95% CI 1. 04 - 1. 30).

When the vascular events were separated into ischemic stroke and ischemic coronary events, the increasing IMT was most strongly related to ischemic stroke incidence (HR 1. 37; 95% CI 1. 16-1. 58), than to myocardial infarction incidence (HR 1. 21; 95% CI, 1. 04 to 1. 38).

Because of the limited number of patients with an abdominal aortic aneurysm, renal artery stenosis or carotid artery stenosis, no separate analyses were done for them.

Tabel 4 Relation between IMT and cardiovascular events

	Nr. of events (%)	IMT > 0.9	IMT < 0.9	Hazard Ratio*	Confidence Interval (95%)
Total events	42 (100%)	26	16	1.17	1.04-1.30
Vascular death	15 (35.7%)	9	6	1.11	0.93-1.29
Stroke or TIA	12 (28.5%)	8	4	1.37	1.16-1.58
Myocardial infarction	24 (57%)	15	9	1.21	1.04-1.38

*Hazard Ratio adjusted for age and sex.

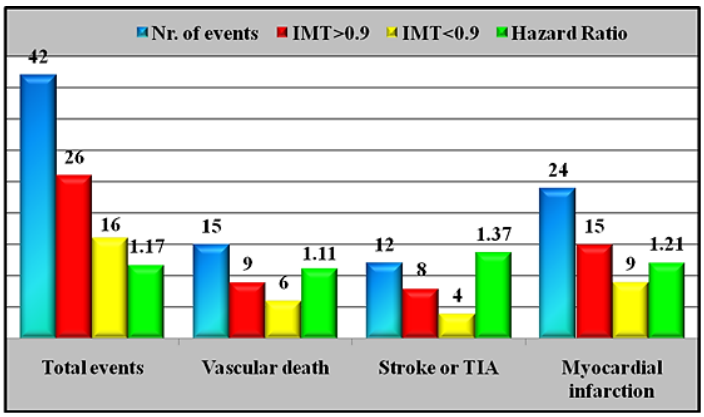


Fig.6. Values of IMT at the study population

The relation between Intima-Media Thickness and Ankle-Brachial Index

The values obtained from measuring the AB index ranged from 0. 42 to 1. 53, with a mean of 0. 93. The linear regression analysis showed a significant inverse association between IMT and ABI (Table V).

The age- and sex-adjusted results indicated that an increase of 0.1 mm in the IMT was associated with a mean reduction of the AB index of 0.039 (95% CI: 0.028 to 0.051), slightly more pronounced in men than in women. A gradual decrease of the AB index was observed with increasing levels of the IMT (Fig.8).

Tabel 5 Relations between IMT, ABI and PAD

Intima-media thickness	Ankle-brachial index	Peripheral artery disease	
		N=97	%
0.61-0.70	1.1	6	6.2
0.71-0.80	1.08	4	4.1
0.81-0.90	1.05	6	6.2
0.91-1	1.01	19	19.6
1.01-1.10	0.95	17	17.5
1.11-1.20	0.88	19	19.6
1.21-1.30	0.82	12	12.4
1.31-1.40	0.75	8	8.2
1.41-1.50	0.68	6	6.2

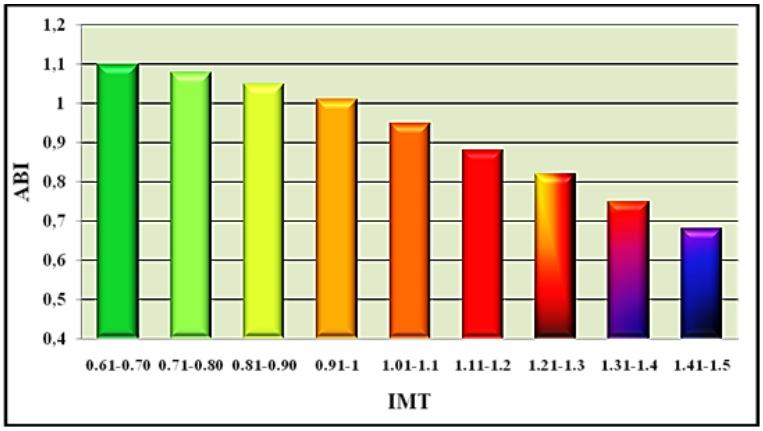


Fig.8. Relation between intima-media thickness and ankle-brachial index

DISCUSSIONS

Our study shows that the carotid IMT is a marker of the increased cardiovascular risk in the patients who already have manifestations of vascular disease. The increased IMT was associated with increased cardiovascular and total mortality risk. The use of this parameter may improve the identification of the patients at high cardiovascular risk.

The patients have been followed for cardiovascular events since the moment they entered the study and although the number of events (vascular death, myocardial infarction, or stroke) currently was small, it showed a significantly increased risk of cardiovascular events [11, 12, 13]. Our findings indicate that the association of the IMT with the cardiovascular risk also applies to high-risk populations. We found a stronger relation of the IMT with stroke (HR 1. 37; 95% CI 1. 16 - 1. 58), than with myocardial infarction (HR 1. 21; 95% CI, 1. 04 to 1. 38) [14].

Our results in an elderly subjects based population indicate that a gradual increase in the carotid IMT is associated with a steady reduction of the AB index, in particular in men and less pronounced in women and demonstrate that the IMT may reflect generalized atherosclerosis [2, 14]. The results from the present study suggest that a carotid IMT >0.90 mm may be a good predictor of the lower extremity arterial disease [2, 15]. A relation of the IMT with new vascular events in patients with manifest arterial disease justifies its use as an endpoint also in this population [3]. O-

ther criteria, notably the potential for regression or stabilization of the IMT by treatment, need to be further documented.

CONCLUSIONS

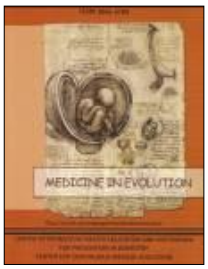
The carotid IMT has proved to be an important epidemiologic marker of atherosclerosis and a strong predictor of future cardiovascular events in patients at high risk and justifies its use as an endpoint in this population. In our study a positive history of major cardiovascular events (stroke, angina pectoris, myocardial infarction, or intermittent claudication) was significantly associated with an increased carotid IMT. The increased IMT was more strongly related to ischemic stroke incidence (HR 1. 37; 95% CI 1. 16-1. 58), than to myocardial infarction incidence (HR 1. 21; 95% CI, 1. 04 to 1. 38). The study showed a significant inverse association between the carotid IMT and the AB index. Our results demonstrate that an increased IMT is a good predictor of lower extremity arterial disease and of general atherosclerosis with the advantages of a quick, cheap, and noninvasive assessment.

REFERENCES

1. Hodis HN, Mack WJ, LaBree L, Selzer RH, Liu CR, Liu CH, Azen SP. The role of carotid arterial intima-media thickness in predicting clinical coronary events. Ann Intern Med. 1998; 128: 262–269
2. Bots ML, Hofman A, de Jong PTVM, Grobbee DE. Common carotid intima-media thickness as an indicator of atherosclerosis at other sites of the carotid artery: the Rotterdam Study. Ann Epidemiol. 1996; 6: 147–153.
3. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction. Circulation. 1997; 96: 1432–1437.
4. Grobbee DE, Bots ML. Carotid artery intima-media thickness as an indicator of generalized atherosclerosis. J Intern Med. 1994; 236: 567–553.
5. Kanters SDJM, Elgersma OEH, Banga JD, van Leeuwen MS, Algra A. Reproducibility of measurements of intima-media thickness and distensibility in the common carotid artery. Eur J Vasc Endovasc Surg. 1998; 16: 28 –35.
6. Riley WA, Evans GW, Sharrett AR, Burke GL, Barnes RW. Variation of common carotid artery elasticity with intima-medial thickness: the ARIC study. Ultrasound Med Biol. 1997;23:157–164.
7. Bots ML, Hoes AW, Hofman A, Witteman JC, Grobbee DE. Crosssectionally assessed carotid intima-media thickness relates to long-term risk of stroke, coronary heart disease and death as estimated by available risk functions. J Intern Med. 1999; 245: 269 –276.
8. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK Jr. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. N Engl J Med. 1999; 340: 14 – 22.
9. Iglesias del Sol A, Bots ML, Grobbee DE, Hofman A, Witteman JC. Carotid intima-media thickness at different sites: relation to incident myocardial infarction; the Rotterdam Study. Eur Heart J. 2002; 23: 934–940.
10. Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N, Csiba L, Desvarieux M, Ebrahim S, Fatar M, Mannheim Carotid Intima-Media Thickness Consensus (2004 – 2006). An Update on Behalf of the Advisory Board of the 3rd and 4th Watching the Risk Symposium 13th and 15th European Stroke Conferences, Mannheim, Germany, 2004, and Brussels, Belgium, 2006. Cerebrovasc Dis. 2006; 23: 75– 80.
11. Chambless LE, Heiss G, Folsom AR, Rosamond W, Szklo M, Sharrett AR, Clegg LX. Association of coronary heart disease incidence with carotid arterial wall thickness and major risk factors: the Atherosclerosis Risk in Communities (ARIC) Study, 1987–1993. Am J Epidemiol 1997; 146: 483–494.
12. Hollander M, Hak AE, Koudstaal PJ, Bots ML, Grobbee DE, Hofman A, Witteman JC, Breteler MM. Comparison between measures of atherosclerosis and risk of stroke: the Rotterdam Study. Stroke 2003; 34: 2367–2372.

13. Rosvall M, Janzon L, Berglund G, Engstrom G, Hedblad B. Incident coronary events and case fatality in relation to common carotid intima-media thickness. J Intern Med 2005; 257: 430–437.
14. Cupini LM, Pasqualetti P, Diomedi M, Vernieri F, Silvestrini M, Rizzato B, Ferrante F, Bernardi G. Carotid artery intima-media thickness and lacunar vs nonlacunar infarcts. Stroke 2002; 33: 689–694.
15. Hirsch AT, Haskal ZJ, Hertzer NR, Bakal CW, Creager MA, Halperin JL, et al. ACC/AHA 2005 Practice Guidelines for the management of patients with peripheral arterial disease (lower extremity, renal, mesenteric, and abdominal aortic). Circulation 2006; 113: e463–e654.

COLOR DOPPLER ULTRASONOGRAPHIC EXAM IN MALIGN VERSUS REACTIVE CERVICAL LYMPHADENOPATHIES DIFFERENTIATION



RIVIS MIRCEA[1], BRAD SILVIU[2], IONITA HORTENSIA[3], LAZAR ELENA[4], BITA ROMINA[2], VALEANU ADINA NORA[1]

1. **Department of Oral and Maxilo-Facial Surgery, University of Medicine and Pharmacy "Victor Babes" Timisoara, Romania.**
2. **Department of General and Oral Radiology, University of Medicine and Pharmacy "Victor Babes" Timisoara, Romania.**
3. **Department of Hematolology, University of Medicine and Pharmacy "Victor Babes" Timisoara, Romania.**
4. **Department of Morphopathogy, University of Medicine and Pharmacy "Victor Babes" Timisoara, Romania.**

ABSTRACT

The aim of this paper is to point out the value of color ultrasonographical cervical lymphadenopathy examination Doppler in the differentiation of malignity from benign reactivity.

We used a statistic retrospective method to evaluate the results obtained after the examination of 64 patients diagnosed with oral cavity pathology and lymphadenopathy with Doppler color ultrasonography methods.

All the color Doppler ultrasonographic examined lymph nodes showed the specific reactive or malignant criteria which made the assessment of oral cavity pathology possible.

The color Doppler ultrasonographic examination is the method of choice in daily practice for differentiation of malignity from benign reactivity in cervical lymphadenopathies.

***Key words**: oral cavity pathology, cervical lymphadenopathy, color Doppler ultrasonography, malign characteristics, inflammatory characteristics.*

Correspondence to:

Rivis Mircea MD
Adress: Street: Department of Cranio-Maxilo-Facial Surgery, University of Medicine and Pharmacy "Victor Babes" Timisoara, Romania
E-mail address: mrivis@yahoo.com

INTRODUCTION

As a general rule, by infectious lymphatic spread or by granulomatous or tumoral infiltration, cervical lymphadenopathies are common marks, in daily practice, for diagnosing, evaluation and staging of oral cavity pathology [1, 2].

The ultrasonographic examination of superficial lymphadenopathies can detect the morphologic and volume changes from the local limph nodes as a common result of any related anatomic pathologies [2, 3].

The sonomorphologic data: diameters, shape, contour, the presence or absence of an echogenic center allowed the diagnosis of a lymphadenopathy, but can not differentiate the malignancy from reactive benignity [2, 3].

The color Doppler ultrasonography identifies the intranodal angioarchitecture and by the accurate detection of malignant modifications can asses the etiology of local lymphadenopathies and as a result the pathology of related anatomic structures [4].

There where described 4 criterions of intranodal angioarchitectural malignant modification: focal absence of perfusion, aberrant course of central vessels, displacement of intranodal vessels, subcapsular vessels [4].

AIM

The aim of this paper is to point out the value of color Doppler ultrasonographic cervical lymphadenopathy examination in the differentiation of malignity from benign reactivity and to underline the ability of this method, in daily practice for diagnosing, evaluateon and staging of oral cavity pathology.

MATERIALS AND METHODS

Color Doppler ultrasonography was performed in cervical lymphadenopathies of 64 patients with oral cavity pathology clinically and biologically assessed and in cases of malignant diseases even with histopathological attestation. Each lymph node was completely assessed with 7.5 MHz linear-array transducer in order to detect initially the sonomorphologic data: diameters, shape, contour, the presence or absence of an echogenic center and then to identify by color Doppler the intranodal angioarchitectural criteria of malignancy: focal absence of perfusion, aberrant course of central vessels, displacement of intranodal vessels, subcapsular vessels.

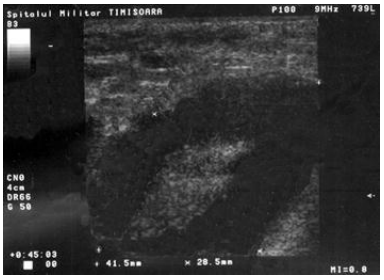


Fig.1 Sonomophological data of a cervical reactive lymphadenopathy

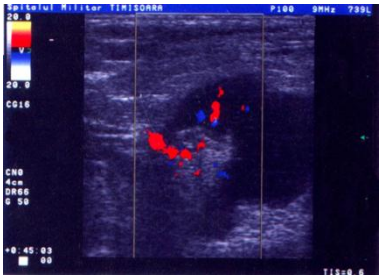


Fig. 2 The color Doppler exam of the same reactive cervical lymphadenopathy assessed an reactive type of angioarchitecture with no malignancy criteria detected

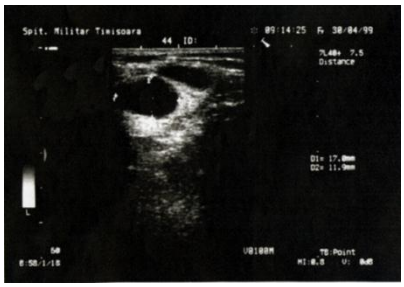


Fig.3 Onomorphological data in a cervical malignant lymphadenopathy with the characteristic absence of the ecogenic center.

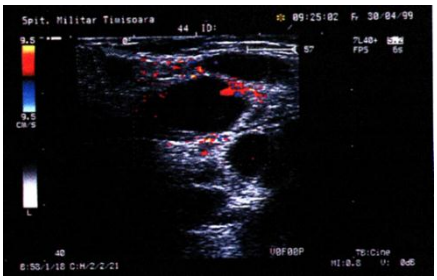


Fig.4 The color Doppler exam of the same lymph node show the displacement of intranodal vessels, an accurate criterion og malignancy

RESULTS

The color Doppler ultrasonographic cervical lymphadenopathy exam de-tected 22 cases of malignant lymphadenopathies and 42 cases of reactive lymphadenopathies

Table 1 The oral cavity pathology associated with cervical lymphadenopathy

Oral cavity pathology	Number of cases
Malignant lymphadenopathies:	**22**
- carcinoma of the oropharynx	8
- carcinoma of the floor the mouth	4
- carcinoma of the salivary glands	3
- carcinoma of the tongue	2
- malignant Hodgkin limfoma	
- mixt celularity	2
- nodular sclerosis	2
- limphocitic depletion	1
Reactive lymphadenopathies:	**42**
- viral parotidites	8
- tonsilites	14
- vestibular abcesses	8
- acute pharingites	10
- citomegalic inclusion disease	2

The sonomorphologic data identifies 46 lymph nodes with measures between 10-20 mm and only 18 lymph nodes with measures between 20-30 mm. The oval shape was found in 46 cervical lymph nodes and the round shape in 18 lymph nodes. The lymph nodes contour was sharp in every cervical limph nodes examed and the ecogenic center was present in 42 cases and in the rest of 22 cases was absent.

The color Doppler ultrasonography points out that all the malignant limph nodes showed at least one criterion of malignancy in the assessment of the intranodal angioarchitecture.

Focal absence of perfusion and subcapsular vessels were the criteria more frequently seen – 12 cases – and which were correlated with the absence of ecogenic center and with the presence of histopathologic alteration like necrosis or arteriovenous shunts induced by central malignant lymph node infiltration. Displacement of intranodal vessels was present in 7 cases and in only 3 cases it was identified aberrant course of central vessels.

Table 2 Sonomorphologic data of cervical lymphadenopathies

Sonomorfological parameters	Number of cases
Length: < 10 mm.	0
10-20 mm.	46
20-30 mm.	18
Width: < 10 mm.	2
10-20 mm.	44
20-30 mm.	18
Thickness: < 10 mm.	2
10-20 mm.	44
20-30 mm.	18
Shape: round	18
oval	46
Contour: sharp	64
blur	0
Echogenic center: present	42
absent	22

Table 3 Color Doppler intranodal angioarchitectural criteria of malignancy

Malignancy criteria	Number of cases
Focal absence of perfusion	12
Aberrant course of central vessels	3
Displacement of intranodal vessels	7
Subcapsular vessels	12

There were, in this way, identified 2 simultaneous criteria of malignancy in 12 cases, 3 simultaneous criteria of malignancy in 7 cases and only one criterion in 3 cases.

There weren't identified 4 simultaneous criteria of malignancy in any cases.

None of the cervical lymph node e-xamed from the 42 cases of non-malignant oral cavity pathology identified any of the malignancy criteria. This reactive lymphadenopathy had an unmodified angioarchitecture with sharp and straight vessels and no subcapsular vessels.

Table 4 Number of color Doppler ultrasonographic malignancy criteria

Number of present criteria	Number of cases
One criterion	3
Two criteria	12
Three criteria	7
Four criteria	0

CONCLUSIONS

Since the imaging methods criteria for cervical lymphadenopathy diagnosis are size and architecture, color Doppler ultrasonography introduce intranodal angioarchitectural criteria with great importance not only in de-

tection but also in mlign versus reactive differentiation.

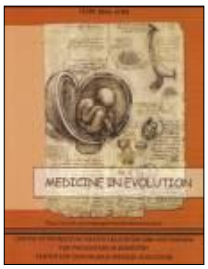
The color Doppler ultrasonography intranodal angioarchitectural criteria helps diagnose and differentiate cervical lymphadenopathies with small size not only those detected by size imaging criteria. Due to possible differentiation of malignity from benign reactivity The color Doppler ultrasonographic cervical lymphadenopathy exam is the method of choice in daily practice for diagnosing, evaluation and staging of oral cavity pathology.

All these facts reveal the ability of ultrasonographic cervical lymph node examination in the assessment of oral cavity health.

REFERENCES

1. Vasile L., Safta M., Urtilă E., Raica M., Lazăr E.; Probleme de diagnostic în formațiunile tumorale latero-cervicale; Editura Mirton; Timişoara 1999
2. Watt I.; Disorders of the lymphoreticular system and other haemopoietic disorders în SUTTON D.; A textbook of radiology and imaging Fifth edition; Churchill Livingstone; Edinburgh, London, Madrid, Melbourne, New York and Tokyo 1993; part 1 Bones and joints; pag. 189-218
3. Fultz P.J., Feins R.H., Strang J.G., Wandtke J.C., Johnstone D.W., Watson T.J., Gottlieb R.H., Voci S.L., Rubens D.J.; Detection and diagnosis of nonpalpable supraclavicular lymph nodes in lung cancer at CT and US în Radiology Volume 222, Number 1; January 2002; pag. 245-251
4. Tschammler A., Ott G., Schang T., Seelbach-Goebel B., Schwager K., Hahn D.; Lymphadenopathy: differentiation of benign from malignant disease-color Doppler US assessment of intranodal angioarchitecture în Radiology Volume 208, Number 1; July 1998; pag. 117-123

BISPHOSPHONATE - RELATED OSTEONECROSIS OF THE JAWS – A CASE SERIES REVIEW



CRISTIAN VLADAN[1], OCTAVIAN DINCA[1] , MIHAI BOGDAN BUCUR[2], TIBERIU NITA[1], ALEXANDRU BUCUR[1]

1. **Carol Davila University of Medicine and Pharmacy Bucharest, School of Dentistry, Department of Oro-Maxillo-Facial Surgery**
2. **Carol Davila University of Medicine and Pharmacy Bucharest, School of Dentistry, Department of Oral Implantology**

ABSTRACT

Oral bisphosphonates are known to have potentially profound effects on oral health.

A review of the evidence supporting answers to key clinical questions is necessary to assist surgeons in the care of their patients who are receiving oral bisphosphonates.

Emerging evidence after reviewing of 12 consecutive patients supports clinical decisions in favor of the oral and maxillofacial surgery patient taking oral bisphosphonates.

***Keywords**: bisphosphonates, osteonecrosis of the jaws*

Correspondence to:

Bucur Alexandru MD
Adress: Department of Oro-Maxillo-Facial Surgery, Calea Plevnei 17-21, Sector 1, Bucharest:
E-mail address: alex_bucur2002@yahoo.com

INTRODUCTION

In late 90`s, alendronate (Fosamax; Merck & Co) was proposed for the prevention and treatment of postmenopausal osteoporosis and for treatment of corticosteroid-induced osteoporosis in men and women and for osteoporosis in men [1]. The efficacy of bisphosphonates in controlling bone fractures in osteoporotic patients coupled with a relatively low level of toxicity and adverse events resulted in a widespread use of these medications in endocrinology [2]. Furthermore, intravenous bisphosphonates were used by specialists to those patients who could not tolerate the oral preparations, especially in refractory osteoporotic disease [3].

The first cases of bisphosphonate related osteonecrosis of the jaws (BRONJ) associated with the use of these nitrogen-containing oral bisphosphonates were presented by Ruggiero et al in 20034. Only a small number from their original series of cases of osteonecrosis were associated with treatment for osteoporosis with oral bisphosphonates, observation confirmed by many subsequent studies [5]. Although the association between medication use and osteonecrosis is established, the extent to which osteonecrosis is attributable to the use of oral bisphosphonates is by no means as clear as it is with i.v. bisphosphonates [6]. With the background level of osteonecrosis in the nonbisphosphonate population, the epidemiologic assessment of risk in the oral bisphosphonate population remains an important but incomplete task.

We will now report our findings in 12 consecutive patients not previously reported. We will examine one case in detail.

We will then conclude with recommendations for evaluation and treatment based upon our experience.

MATERIALS AND METHODS

The patient population consisted of 12 consecutive patients evaluated in the Oro-Maxillo-Facial Surgery Department. The data were collected from a chart review, and measures were taken to preserve patient anonymity. A suspected diagnosis of BRONJ was based on symptoms noted in the chart. Treatment intervention was that which was recorded in the chart.

RESULTS

This series involved twelve women. The average age of the 12 patients was 63 years (range: 53-72 years).

The average duration of symptoms prior to diagnosis was 9 months (range: 2-15 months). Three patients responded surgical treatment, but were not seen in follow-up to confirm lasting positive response. Of those nine patients seen at follow-up, three were healed and have no clinical evidence of exposed bone (one of them is described later), two have exposed bone, are asymptomatic and have no evidence of infection, two have exposed bone, with pain and clinical evidence of infection, one have exposed necrotic bone extending beyond the region of alveolar bone, with pain and infection, and one have pathologic mandible fracture and

extraoral fistula and was considered a treatment failure. Seven patients were treated with alendronate (10 mg daily). Three were treated with risedronate (35 mg weekly), two with ibandronate (150 mg monthly).

CASE REPORT

In August 2009, a 59-year-old woman was referred for oro-maxillo-facial examination by her treating dentist. The patient`s chief complaint was the presence of an open wound and soreness at the extraction site performed 10 weeks ago by her dentist. Two weeks ago, she called up the family doctor for persistent diffuse pain that followed the tooth extraction. She started a treatment with Amoxicilin with clavulanic acid. The patient's medical history revealed that she was being treated for moderate osteoporosis, confirmed by endocrinologist by DEXA scanning.

She also had chronic obstructive bronchopneumopathy, premature atrial beats, hypertension and duodenal ulcer. The patient had no known drug allergies. Her medications included alendronate sodium (10 mg daily) (since august 2005), vitamin D3 and calcium, theophylline and metoprolol.

Clinical examination that revealed subtotal edentulism with no prosthodontic replacement, chronic marginal parodontopathy and a poor oral hygiene level. Inspection revealed denuded osseous tissue on the left mandible, where the tooth extraction was performed 8 weeks ago (Figure 1).

Serous discharge and erythema was noticed. X-ray showed no fractured tooth root debris or alveolar bone at extraction site and no signs of other osseous abnormalities. Aswab from alveolar wound was sent to bacteriology testing which revealed the majority of bacteria was Arcanobacterium haemolyticum.

The peripheral blood test showed slight leucocytosis and accelerated blood sedimentation rate value.

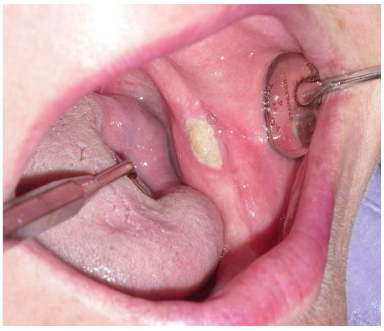


Fig.1 Mandibular bone exposure in patient undergoing treatment with alendronate

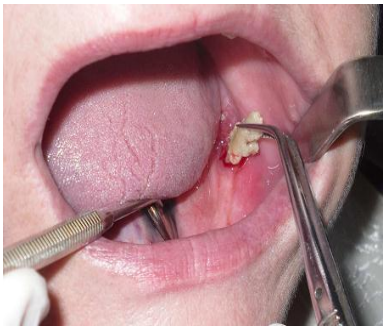


Fig.2 Remove of bone sequestrum

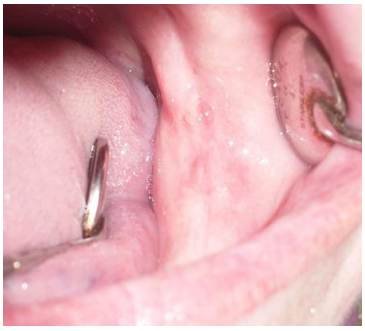


Fig.3 Clinical aspect 16 months after sequestrectomy

Simultaneously, the directed antibiotic therapy was applied according to the antibiogram against Arcanobacterium haemolyticum - Clindamycin 300 mg every 12 hours. The patient was released from the hospital with a recommendation to perform good oral hygiene and every day control visits at the out-patient service.

She returned daily during the following ten days for check-up. By the end of that period, we allowed the patient to monitor the site and return for intermittent follow-up appointments. We saw her two times during the following four-week period and performed gentle debridement procedures at each visit.

In late October 2009, we recovered a 2 × 0.5 × 0.3 centimeters piece of bone sequestrum from the tooth extraction site while debriding the area (Figure 2) and submitted the excised necrotic tissue for histopathologic analysis. Microscopy revealed a decalcified section of nonviable bone with lacunae devoid of osteocytes, inflammatory cells and bacteria. These findings sustained the microscopic diagnosis of osteonecrosis.

The postoperative healing proceeded uneventfully after sequestrectomy and in february 2011 there had been no recurrence of the lesion (Figure 3).

DISCUSSIONS

Our current series of 12 new patients have resulted in the following findings. Exposed devitalized bone is the hallmark of osteonecrosis of the jaws. Most cases of bisphosphonate osteo-necrosis of the jaws occurs after woun-ding of the bone, such as tooth removal; however, spontaneous cases of bisphosphonate-related osteo-necrosis have been recognized and documented [7]. The process is more common in the mandible, but the maxilla can be affected. Inflammation and infection are noted in advanced cases and are the more significant reasons for the symptomatic features of BRONJ. Pathologic fracture, oral or cutaneous fistula formation can occur in advanced cases of BRONJ.

The risk of developing BRONJ associated with oral bisphosphonates increased when the duration of therapy exceeded 3 years [8]. No information is available to suggest that monthly dosing of oral bisphosphonates (ibandronate or risedronate) is associated with either an elevated or reduced risk of BRONJ compared with weekly dosing regimens.

The treatment objectives for patients with BRONJ are to eliminate pain, control infection of the soft and hard tissue, and minimize the progression or occurrence of bone necrosis [9, 10]. These patients respond less predictably to the established surgical treatment algorithms for osteomyelitis or osteoradionecrosis.

Surgical debridement has been variably effective in eradicating the necrotic bone. It could be difficult to obtain a surgical margin with viable bleeding bone, therefore, surgical treatment should be delayed if possible and reserved for those cases with well-defined sequestrum. Areas of necrotic bone that are a constant source of soft-tissue irritation should be removed or recontoured without exposure of additional bone.

Patients benefit from the use of oral antimicrobial rinses combined with antibiotic therapy. The antibiotic regimen should be adjusted accordingly to the isolated microbes on cultures. Noted that microbial cultures have not consistently identified specific pathogens related to bisphosphonate-related osteonecrosis of the jaws [11, 12].

Discontinuation of oral bisphosphonate therapy in patients with BRONJ can result in either spontaneous sequestration or resolution after debridement surgery [13]. If systemic conditions per-

mit, it was suggested that modification of oral bisphosphonate therapy should be done in consultation with the treating endocrinologist and the patient [14]. However, long-term, prospective studies are also required to establish the efficacy of drug holidays in reducing the risk of BRONJ for these patients. The efficacy of using a systemic marker of bone turn-over to assess the risk of developing jaw necrosis in patients at risk requires additional research [15].

CONCLUSIONS

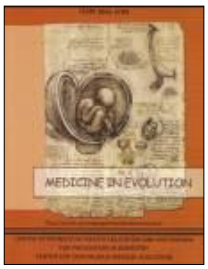
The earliest manifestation of BRONJ can be difficult to recognize clinically and radiographically. The efficacy of using bone marker turnover assays to assess potential risk for BRONJ are currently lacking.

The recognition of this entity needs to be emphasized as this helps in establishing diagnosis, to assess prognosis and to aid in selection of a rational approach to therapy. Many of the causes of BRONJ could be avoided by providing good health facilities, the failure of which ultimately lead to the tremendous cost as these patients need long-time treatment.

REFERENCES

1. FDA approves Actonel (risedronate) for osteoporosis. Available at: http: //www .pslgroup.com/dg/1cd17e.htm. Accessed March 5,2009
2. McClung MR: Bisphosphonates. Endocrinol Metab Clin North Am 32:253, 2003 3. Grbic J, Landesberg L, Lin S, et al: Incidence of osteonecrosis of the jaw in women with postmenopausal osteoporosis in the Health Outcomes and Reduced Incidence With Zoledronic Acid Once Yearly Pivotal Fracture Trial. J Am Dent Assoc 139:32, 2008
3. Ruggiero SL, Mehrotra B, Rosenberg TJ, et al: Osteonecrosis of the jaws associated with the use of bisphosphonates: A review of 63 cases. J Oral Maxillofac Surg 62:527, 2004
4. Murad OM, Arora S, Farag AF, Guber HA: Bisphosphonates and osteonecrosis of the jaw: A retrospective study. Endocr Pract 13:232, 2007
5. AAOMS: American Association of Oral and Maxillofacial Surgeons position paper on bisphosphonate-related osteonecrosis of the jaws. J Oral Maxillofac Surg 65:369, 2007
6. Pazianas M, Miller P, Blumentals WA, et al: A review of the literature on osteonecrosis of the jaw in patients with osteoporosis treated with oral bisphosphonates: Prevalence, risk factors, and clinical characteristics. Clin Ther 29:1548, 2007
7. Hamada S: Osteonecrosis of jaw associated with oral application of bisphosphonates. J Oral Maxillofac Surg 65:43, 2007
8. Montebugnoli L, Felicetti L, Gissi DB, et al: Biphosphonate-associated osteonecrosis can be controlled by nonsurgical management. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 104:473, 2007
9. Yarom N, Yahalom R, Shoshani Y, et al: Osteonecrosis of the jaw induced by orally administered bisphosphonates: Incidence, clinical features, predisposing factors and treatment outcome. Osteoporos Int 18:1363, 2007
10. Hansen T, Kunkel M, Springer E, et al: Actinomycosis of the jaws - Histopathological study of 45 patients shows significant involvement in bisphosphonate - associated osteonecrosis and infected osteoradionecrosis. Virchows Arch 451: 1009, 2007
11. Etminan M, Aminzadeh K, Matthew IR, et al: Use of oral bisphosphonates and the risk of aseptic osteonecrosis: A nested case-control study. J Rheumatol 35:1, 2008
12. Black DM, Schwartz AV, Ensrud KE, et al: Effects of continuing or stopping alendronate after 5 years of treatment: The Fracture Intervention Trial Long-Term Extension (FLEX): A randomized trial. JAMA 296:2927, 2006
13. Somerman MJ, McCauley LK: Bisphosphonates: Sacrificing the jaw to save the skeleton? Bonekey Osteovision 3:12, 2006
14. Marx RE, Cillo JE, Ulloa JJ: Oral bisphosphonate-induced osteonecrosis: Risk factors, prediction of risk using serum CTX testing, prevention, and treatment. J Oral Maxillofac Surg 65: 2397, 2007

MINIMALLY INVASIVE TREATMENT OF THE SALIVARY GLANDS OBSTRUCTIVE DISEASES



ALEXANDRU BUCUR[1], CRISTIAN VLADAN[1], OCTAVIAN DINCA[1]

1. **Carol Davila University of Medicine and Pharmacy Bucharest, School of Dentistry, Department of Oro-Maxillo-Facial Surgery**

ABSTRACT

The endoscopic technique opens new horizons in the field of salivary gland diseases.
Salivary gland stones and sialadenitis no longer are absolute indications for open surgery.
Owing to growing experience and surgical skills, new endoscopic techniques are in clinical use, and there is constant improvement in endoscopic treatment success rates.

***Keywords**: obstructive diseases of the major salivary, minimally invasive techniques*

Correspondence to:

Bucur Alexandru MD
Adress: Department of Oro-Maxillo-Facial Surgery, Calea Plevnei 17-21, Sector 1, Bucharest
E-mail address: alex_bucur2002@yahoo.com

INTRODUCTION

Treatment of salivary lithiasis has seen radical changes in the last 20 years, following the introduction into practice of modern, minimally invasive treatment - extracorporeal lithotripsy and sialendoscopy.

In the University Clinic of Oro-Maxillo-Facial Surgery in Bucharest, since 2010 the minimally invasive procedures being adopted for that have become first-line therapy of salivary lithiasis.

EXTRACORPOREAL SHOCK WAVE LITHOTRIPSY

Is a non-invasive therapeutic method of calculation allows fragmentation and natural way of removing fragments by salivary secretion. Extracorporeal lithotripsy principle is fragmentation by shock waves produced by a generator and targeted directly to the obstacle. Fragmentation of stones under the action of shock waves can occur through direct force, erosion or cavitation. Extracorporeal lithotripsy does not require general anesthesia, resulting in minimal discomfort for the patient. Extracorporeal lithotripsy success depends upon a number of factors including: number and size of calculus, obstacle location, its chemical composition, anatomical characteristics of salivary tree. Procedure indications procedure refers mainly to stones located in Stensen`s duct or in the parotyd gland. If submandibular lithiasis, the method addresses to 2-10 mm diameter stones located in the proximal portion of the basin or Wharton's duct; those located intraglandular are object of submaxillectomy and those positioned in the distal segment of the duct require wall incision. In general, the larger stones or those with complex shapes usually requires several sessions to fragmentation, especially in calcium oxalate monohydrate stones.

The method has broad addressability and can be done at any age. Note that the procedure is not recommended in pregnancy, while some systemic diseases uncompensated as the presence of coagulopathy / bleeding disorders and in uncooperative patients too.

Complications of extracorporeal lithotripsy are less severe and require least-invasive treatment procedures (sialendoscopy).

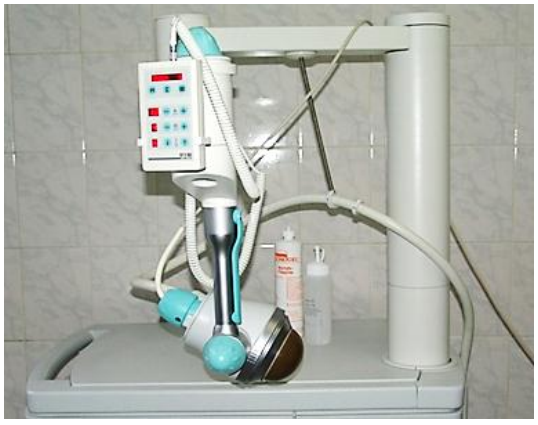


Fig. 1 Extracorporeal shock wave lithotripter

SIALENDOSCOPY

Represents a stone fragmentation method using a device that made contact with the calculation, by endoscopic devices. The method consists in procedure consists in salivary canalicular system catheterization under ultrasound guidance and management of the endoscope working channel with different accessories that allow the removal of stone fragments under echo-video control. Fragmentation of stones can be achieved mechanically, ultrasonic, electrohydraulic or by laser effect. Fragments are removed by suction with flexible forceps or basket probes.

Sialendoscopy allow treating stones located in the main duct and hilum, when the obstacle size does not exceed 5-6 mm. The method serves to identify prospective screening for residual calculi after incision of the duct and diagnosis of idiopathic enlargement of major salivary glands. Besides the general limitations similar to those of extracorporeal lithotripsy, sialendoscopy is contraindicated in the following situations:

- distal obstruction (stenosis, another stone, etc.)
- sialodochitis
- stones more than 8 mm in size
- intraglandular stone
- anatomical abnormalities that prevent instrumentation.

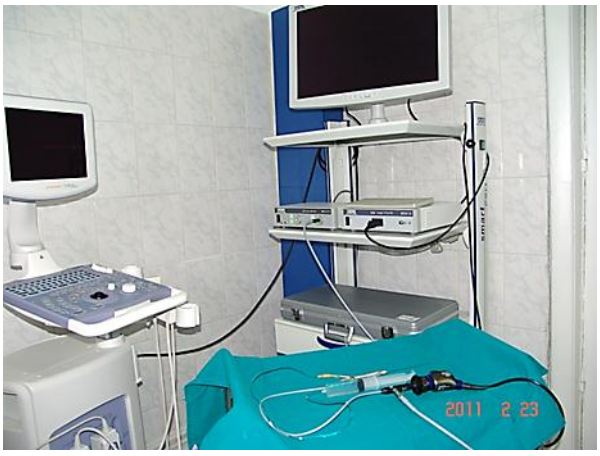


Fig. 2 Endoscope for salivary ducts

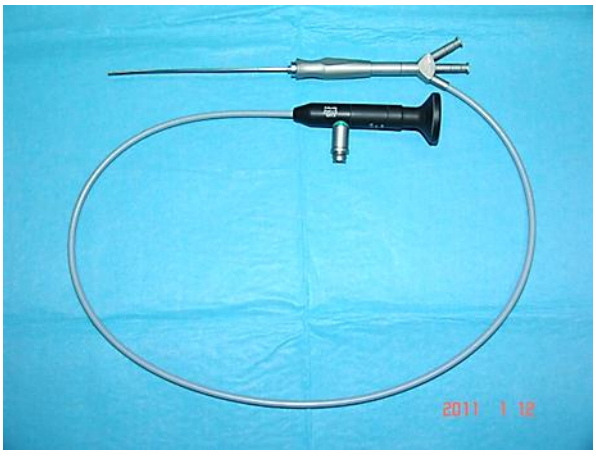


Fig. 3 "All-in-one" interventional sialendoscope

In addition it provides comfort of the patient, significantly reducing treatment duration, the average being 45-60 minutes compared to an average of 75-90 minutes for open surgery. Also, the average duration of postoperative hospitalization is reduced than the period that follows open surgery (1-2 days versus 3-5 days). In our clinic we use the "all – in - one" rigid endoscope (Karl Storz 11 576), for salivary system explore, performed under continuous irrigation with a saline solution mixed with lidocaine 2%, instilled under pressure. Stone removal method is selected depending on the size, position and its mobility. If stones of 4-8 mm, inclavated, is required extracorporeal lithotripsy. In the presence of stenosis, handling is difficult, requiring permeability, if the stenosis is short and wide, through the wells with balloon dilation.

Residual stones benefits of endoscopic removal in the same session or in a subsequent one. In situations that require timing (perforations, false paths, etc.) it is recommended to place a stent 2 mm diameter, which avoids

postoperative retraction and facilitate small stones evacuation.

A particular situation is the inadvertent retrograde migration of stones by incorrect dosing of irrigation fluid pressure, pulse transmitted by lithotripsy or through gravitational migration. If it is not possible to address, overcome or endoscopic stones fragmentation, due to underlying stenosis or edema, open surgery conversion is recommended. A similar attitude is necessary if intraductal probe basket blockages occurs and in salivary duct wall complete breaks with migration outside of the stone.

CONCLUSIONS

Extracorporeal lithotripsy is the first line of treatment for most of the salivary stones, associated with a low rate of complications. Although more invasive and accompanied by a higher morbidity, sialendoscopy is an effective method of diagnosis and treatment of salivary glands pathology.

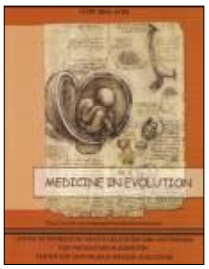
Both minimally invasive procedues are safe, with wide range of indications.

Limits are given on the one hand by the need of experience in procedures performing, especially in sialendoscopy, and by the expensive costs of acquisition and maintenance.

REFERENCES

1. Baurmash HD. Submandibular salivary stones: current management modalities.J Oral Maxillofac Surg. 2004 Mar; 62(3): 369-78.
2. Bowen MA, Tauzin M, Kluka EA, Nuss DW, DiLeo M, McWhorter AJ, Schaitkin B, Walvekar RR. Diagnostic and interventional sialendoscopy: a preliminary experience. Laryngoscope. 2011 Feb; 121(2): 299-303.
3. Fritsch M. Sialendoscopy and Lithotripsy, an Issue of Otolaryngologic Clinics. Saunders, 2010.
4. Fritsch MH. Sialendoscopy and lithotripsy: literature review. Otolaryngol Clin North Am. 2009 Dec; 42(6): 915-26
5. Gillespie MB, Koch M, Iro H, Zenk J. Endoscopic-assisted gland-preserving therapy for chronic sialadenitis: a German and US comparison. ArchOtolaryngol Head Neck Surg. 2011 Sep; 137(9): 903-8.
6. Koch M, Zenk J, Iro H. Algorithms for treatment of salivary gland obstructions. Otolaryngol Clin North Am. 2009 Dec; 42(6): 1173-92
7. Nahlieli O, Nakar LH, Nazarian Y, Turner MD. Sialoendoscopy: A new approach to salivary gland obstructive pathology. J Am Dent Assoc. 2006 Oct; 137(10): 1394-400.
8. Ziegler CM, Steveling H, Seubert M, Mühling J. Endoscopy: a minimally invasive procedure for diagnosis and treatment of diseases of the salivary glands. Six years of practical experience. Br J Oral Maxillofac Surg. 2004 Feb; 42(1):1-7.

EFFECTS OF PIROXICAM, TENOXICAM AND MELOXICAM ON LIPID PEROXIDATION AND OUTCOME MESURES IN PATIENTS WITH KNEE OSTEOARTHRITIS



AMZOIU DENISA-CONSTANTINA[1], PISOSCHI CATALINA[2], BEZNA MARINELA[3], POPESCU FLORICA[4]

1. **University of Medicine and Farmacy „Victor Babes", Timisoara**

ABSTRACT

***Aim**: To compare the effects of three non-steroidal anti-inflammatory drugs (NSAIDs): piroxicam and tenoxicam, nonselective inhibitors of cyclooxygenase (COX), and meloxicam, a selective COX-2 inhibitor, on malondialdehyde (MDA) as a lipid peroxidation marker in patients with knee osteoarthritis and evaluate patients' outcome measures.*

***Material and methods**: Twenty-eight adult patients diagnosed with knee osteoarthritis were enrolled and MDA serum levels were assessed at baseline and after 20 days of treatment with piroxicam (20 mg po daily), tenoxicam (20 mg po daily) and meloxicam (15 mg po daily). Western Ontario and McMaster Universities (WOMAC) LK3.1 Osteoarthritis Index and pain using a visual analog scale (VAS) were performed at baseline and at the end of treatment.*

***Results**: After the treatment MDA levels were not significantly changed compared to baseline, but significant improvement was found in pain-VAS and WOMAC pain, stiffness and physical function scores in all treated groups.*

***Conclusions**: Piroxicam, tenoxicam and meloxicam showed no significant influence on lipid peroxidation.*

***Keywords**: lipid peroxidation, malondialdehyde, osteoarthritis, piroxicam, tenoxicam, meloxicam.*

Correspondence to:

Denisa-Constantina Amzoiu
Adress: Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Medicine and Pharmacy of Craiova, 2–4 Petru Rareş Street, 200349 Craiova, Romania
Phone: 0251–523929
E-mail address: damzoiu@yahoo.com

INTRODUCTION

Osteoarthritis (OA) is defined as a heterogeneous group of diseases that cause joint manifestations associated with alterations in the integrity of cartilage, subchondral bone and periarticular structures. OA incidence is increasing mainly due to higher life expectancy and ranks second among chronic conditions after the cardiovascular diseases (Rosu&Vreju, 2007).

OA articular cartilage suffers modifications of the normal turnover derived from an imbalance between anabolic and catabolic processes (Herrero-Beaumont et al., 2009). Reactive oxygen species (ROS), such as superoxide anion radical, singlet oxygen, hydroxyl and perhydroxyl radical are implicated in OA pathogenesis. Maneesh et al. found higher levels of oxidative stress markers in patients with OA compared with healthy patients (Maneesh et al., 2005).

Lipid peroxidation is initiated by many reactive species among which superoxide and hydroxyl radical. Lipid peroxides act as damaging substances by altering cellular, lysosomal and mitochondrial membrane properties leading to alterations of osmotic, electric and chemical gradients (Dejica, 2000). MDA is one of the end products of lipid peroxidation and a marker of ROS mediated damage and oxidative stress. MDA can react with DNA bases resulting adducts which affect DNA structure and functions (Kasperska-Zajac et al., 2008) Increased oxidative stress accelerates chondrocyte senescence and initiates apoptosis, reducing chondrocytes' ability to maintain and repair cartilage (Starodubtseva, 2011).

According with Osteoarthritis Research Society International (OARSI) recommendations, cyclooxygenase non - selective and selective inhibitors are included among pharmacological treatment strategies for OA (Zhang et al., 2008). NSAIDs influence prostaglandin synthesis through inhibition of COX isoforms, COX-1 and COX-2. NSAIDs affect oxidative stress: some attenuate whereas others ehhance ROS generation.

AIM AND OBJECTIVES

Our purpose was to compare the in vivo effect of meloxicam, a selective COX-2 inhibitor, with piroxicam and tenoxicam, two non-selective COX inhibitors on MDA level as a marker of lipid peroxidation in patients with knee osteoarthritis and to asses their influence on the patient-centered self-reported measures.

MATERIAL AND METHODS

Patients clinically and radiographically diagnosed with knee osteoarthritis enrolled in this study were recruited from the Medical Clinic No. 1, Emergency County Hospital of Craiova. Distribution of patients in groups was made according to a study protocol approved by the Ethics Committee of the University of Medicine and Pharmacy of Craiova. Twenty-eight patients with OA of the knee were selected to assess blood MDA levels at baseline and after 20 days of treatment with piroxicam, tenoxicam or meloxicam. Ten patients (2 men, 8 women) were treated with piroxicam 20 mg po daily and had a me-

an age of 52.8 ± 4.23 yr (range 46-61). Ten patients (2 men, 8 women) were treated with tenoxicam 20 mg po daily and had a mean age of 54.2 ± 2.15 yr (range 50-57). Eight patients (2 men, 6 women) were treated with meloxicam 15 mg po daily and had a mean age of 61 ± 10.02 yr (range 51-73).

Inclusion criteria. Patients aged ≥18 years with primary OA of the knee, who met American College of Rheumatology diagnostic criteria were recruited for the study. All the patients voluntarily participated in the study and gave their informed consent.

Exclusion criteria. Patients who had the following conditions were not included in the study: current drug usage for concomitant disease or chronic condition(s) that might interfere with the assessment of clinical findings of OA (inflammatory arthritis, gout or Paget's disease) and serum oxidative stress markers (atherosclerosis, diabetes or other metabolic diseases, hypertension, allergies, asthma, Parkinson's disease, hematopoietic disorders, inflammatory bowel disease); a history of gastro-intestinal ulcers and bleeding; diagnosis of chronic pain syndrome (e.g., fibromyalgia, chronic fatigue syndrome); smoking, excessive alcohol consumption and regular aerobic exercise program; intramuscular, intravenous or soft tissue corticosteroids within 1 month prior to the study; use of intra-articular corticosteroids within 2 months prior to the study; history of clinically significant intolerance to oxicams. Patients who had infectious disease, renal or hepatic dysfunctions were excluded. Treatment with other NSAIDs, statins, enzyme-inducing drugs or enzyme- inhibiting drugs was not allowed. No supplementary therapies, special diets or aerobic exercise programs were allowed during the study period.

Biochemical assay. Venous blood samples were collected à jeun at baseline and at the end of the clinical study period for analysis of MDA level. The blood was centrifuged to obtain plasma and processed for MDA measurement based on the reaction with thiobarbituric acid: lipid peroxides were degraded by acid hydrolysis at high temperature to form MDA as the main product; than, MDA reacted with thiobarbituric acid to form a colored adduct. Briefly, 0.5 ml plasma, 2.5 ml of 20% trichloroacetic acid and 1 ml of 0.7% thiobarbituric acid were incubated at 100oC for 20 minutes. After cooling, samples were extracted in n-butanol: pyridine mixture and centrifuged at 2500 rpm for 10 minutes. Supernatant absorbance was read at 532 nm against a blank of reagents without biological material. Measurements were made with a Beckman DU-65 UV-VIS spectrophotometer. The results were expressed as nmol/ml.

Outcome measures. Western Ontario and McMaster Universities (WOMAC) LK3.1 Osteoarthritis Index and pain using a visual analog scale (VAS) were performed at baseline and at the end of treatment. The WOMAC Osteoarthritis Index LK3.1 is a validated multidimensional questionnaire and consist in 24 questions (5 on pain, 2 on stiffness, 17 on physical function) each scored on a 5-point Likert scale (0 to 4, 0 representing none, 4 representing extreme). Pain intensity was assessed using a visual analog scale (VAS; 0-100 mm, 0 representing no pain, 100 representing worst pain imaginable).

Statistical analysis. Statistics Package for Social Sciences was used for the statistical analysis and results were expressed as mean ± SD (standard deviation). Differences between the three groups at baseline were assessed by independent sample t test. Changes observed before and after treatment were assessed by the paired sample t test. A p value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSIONS

All patients completed the study. The baseline MDA levels in tenoxicam and meloxicam groups did not have significant difference when compared with each other.

In the piroxicam group, MDA baseline levels were slightly higher then in the other two groups (Table 1). The variations of MDA values for each patient at baseline and at the end of treatment with the studied oxicams are presented in Figure 1-3.

Results showed that MDA levels were slightly decreased in all three goups of patients (Figure 4) but none of the three NSAIDs belonging to the oxicam group had a statistically significant influence on MDA.

Tabel 1 Plasma malondialdehyde (MDA) levels in the groups of study

Oxicam-treated group	Baseline MDA (nmol/ml) (mean ±SD)	Final MDA (nmol/ml) (mean ±SD)	p value
Piroxicam	1.169 ± 0.663	1.085 ± 0.591	0.19
Tenoxicam	0.933 ± 0.388	0.834 ± 0.191	0.512
Meloxicam	0.986 ± 0.495	0.889 ± 0.401	0.477

p values represent baseline versus final mesurements in each group (paired t test)

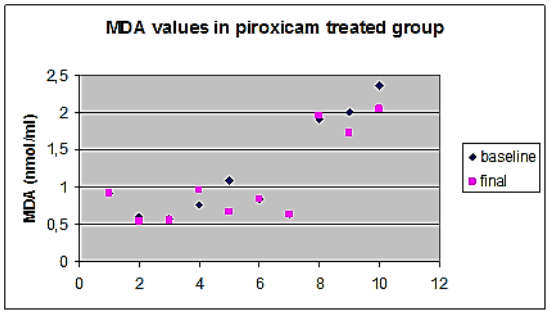


Fig. 1 MDA variations in patients treated with piroxicam

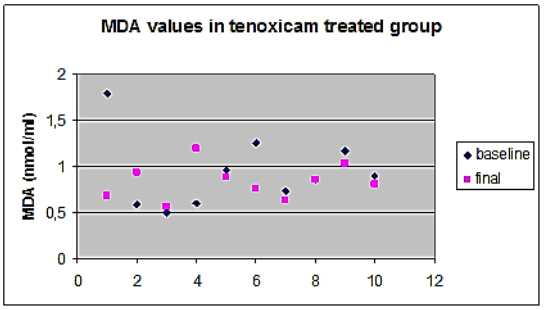


Fig. 2 MDA variations in patients treated with tenoxicam

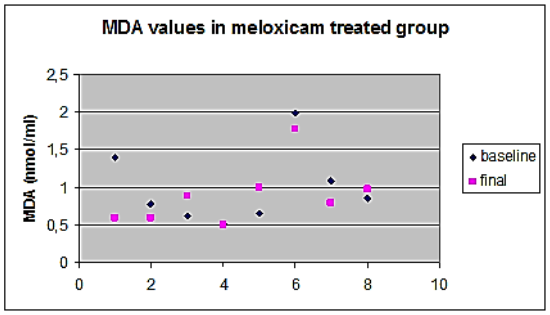


Fig. 3 MDA variations in patients treated with meloxicam

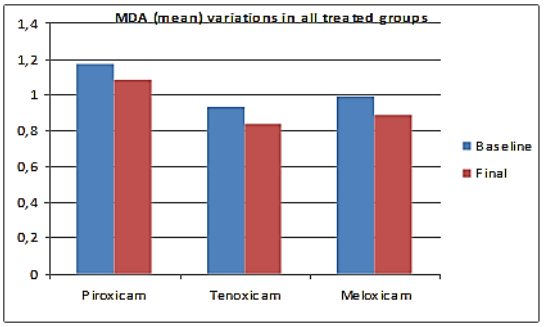


Fig. 4 Variations of MDA (mean) in piroxicam, tenoxicam, and meloxicam treated groups

Significant improvement was observed in the pain-VAS and WOMAC pain, stiffness and physical function scores in all treated groups ($p<0.01$) (Table 2).

NSAIDs effects on oxidative stress markers in OA patients was assessed in relatively few studies. Van Antwerpen and Nève investigated in vitro antioxidant effects of piroxicam, meloxicam, tenoxicam, lornoxicam, ibuprofen and nimesulide and observed that oxicams are more reactive against ROS than nimesulide and ibuprofen (Van Antwerpen & Nève, 2004). Bartosiewicz et al. observed that serum levels of MDA and serum oxidative capacity decreased in patients with OA treated with piroxicam (Bartosiewicz et al., 1993), Ozgocmen et al. showed that tenoxicam and celecoxib had no statistically significant influence on MDA levels in OA patients serum (Ozgocmen et al., 2005), and Tuzun et al. discovered that tiaprofenic acid and flurbiprofen influence serum levels of NO, MDA and the activity of superoxide dismutase (Tuzun et al., 2005). Our findings – slightly but not significantly decreased in MDA levels in OA patients treated with meloxicam (a selective COX-2 inhibitor) and with piroxicam and tenoxicam (non-selective COX inhibitors) – are in agreement with these experimental studies. Further research is required to asses the NSAIDs effects on free radicals production since those mechanisms might provide new therapeutic approaches for prevention of cartilage damage and OA progression.

Tabel 2 Outcome measures in patients with OA of the knee

Parameters	**Piroxicam-treated group**	**Tenoxicam-treated group**	**Meloxicam-treated group**
WOMAC LK3.1 OA Index			
Pain			
Baseline, mean ±SD	15 ± 2.494	13.7 ± 3.198	12.1 ± 2.948
Final, mean ±SD	8.5 ± 2.46	7.4 ± 3.339	5.3 ± 2.445
p value	<0.01	<0.01	<0.01
Stiffness			
Baseline, mean ±SD	5 ± 1.563	4.5 ± 1.957	4.3 ± 0.916
Final, mean ±SD	2.5 ± 1.178	2.6 ± 1.429	2 ± 1.069
p value	<0.01	<0.01	<0.01
Physical function			
Baseline, mean ±SD	52.2 ± 8.243	47.4 ± 6.931	44.3 ± 10.848
Final, mean ±SD	33.7 ± 6.992	32.1 ± 6.822	25.2 ± 6.902
p value	<0.01	<0.01	<0.01
Pain-VAS (mm)			
Baseline, mean ±SD	85 ± 11.785	84 ± 11.737	85 ± 11.952
Final, mean ±SD	66.5 ± 8.181	65 ± 8.498	56.2 ± 11.877
p value	<0.01	<0.01	<0.01

CONCLUSIONS

After the treatment with the three members of the oxicam group, plasma MDA levels slightly decreased compared to baseline, but none of NSAIDs caused statistically significant changes.

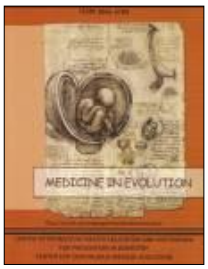
Significant improvement was found in pain-VAS and WOMAC pain, stiffness, and physical function scores in all treated groups, with better scores for meloxicam on pain-VAS scores.

Our study revealed that these NSAIDs belonging to the oxicam group have no significant influence on lipid peroxidation.

REFERENCES

1. Bartosiewicz G, Mezes M, Nemet J, Changes in lipid, peroxide and anti-oxidant blood levels during piroxicam (Hotemin) treatment, Ther Hung, 1993, 41, 67-71.
2. Dejica D, Comes L, Duma L, Mureşan A, Pop S, Porr P J, Szanto P, Tache S – Stresul oxidative în bolile interne, Ed. Casa Cărţii de Ştiinţă, Cluj-Napoca, 2000.
3. Herrero-Beaumont G, Roman-Blas JA, Castaneda S, Jimenez SA, Primary osteoarthritis no longer primary: tree subsets with distinct etiological, clinical, and therapeutic caracteristics, Semin Arthritis Rheum, 2009, 39,2, 71-80.
4. Kasperska-Zajac A, Brzoza Z, Rogala B, Polaniak R, Birkner E, Antioxidant enzyme activity and malondialdehyde concentration in the plasma and erythrocytes of patients with urticaria induced by nonsteroidal anti-inflammatory drugs, J Investig Allergol Clin Immunol, 2008, 18, 5, 372-375.
5. Maneesh M, Jayalekshmi H, Suma T, Chatterjee S, Chakrabarti A, Singh TA, Evidence for oxidative stress in osteoarthritis, Indian J Clin Biochem, 2005, 20, 1, 129-130.
6. Ozgocmen S, Ardicoglu O, Erdogan H, Fadillioglu E, Gudul H, In vivo effect of celecoxib and tenoxicam on oxidant/anti-oxidant status of patients with knee osteoarthritis, Ann Clin Lab Sci, 2005, 35, 2, 137-143.
7. Roşu A, Vreju F, Artroza. In Reumatologie, pp. 387-414. Edited by P. Ciurea, Ed. Medicală Universitară, Craiova, 2007.
8. Starodubtseva MN, Mechanical properties of cells and ageing, Ageing Res Rev., 2011, 10, 1, 16-25.
9. Tuzun S, Uzun H, Aydin S, Dinc A, Sipahi S, Topcuoglu MA, Yucel, R; Belce A.: Effects of flurbiprofen and tiaprofenic acid on oxidative stress markers in osteoarthritis: A prospective, randomized, open-label, active- and placebo-controlled trial, Curr Ther Res, 2005, 66, 4, 335-344.
10. Van Antwerpen P, Jean Nève J, In vitro comparative assessment of the scavenging activity against three reactive oxygen species of non-steroidal anti-inflammatory drugs from the oxicam and sulfoanilide families, Eur J Pharmacol, 2004, 496, 55-61.
11. Zhang W, Moskowitz RW, Nuki G, Abramson S, Altman RD et al., OARSI recommendations for the management of hip and knee osteoarthritis, Part II: OARSI evidence-based, expert consensus guidelines, Osteoarthritis Cartilage, 2008, 16, 137-162.

SCREENING METHODS FOR METHADONE IDENTIFICATION



ANNE MARIE CIOBANU[1], DANIELA BACONI[1], MARIA BARCA[1], CLAUDIA NICOLA[1], DAN BALALAU[1]

1. **University of Medicine and Pharmacy „Carol Davila" Bucharest, Faculty of Pharmacy**

ABSTRACT

Aim and objectives: The aim of the paper is to apply the screening tests (color tests and Thin Layer Chromatography, TLC) for identification of methadone, as the main therapeutic option in heroin addiction and as a substance abuse.

Material and methods: The comparative color tests (using alkaloid reagents) for methadone, codeine, dihydrocodeine, naloxone, pentazocine and tramadol have been applied. For TLC studies, methadone, the main metabolite EDDP, some benzodiazepines (alprazolam, diazepam, oxazepam), and clonidine have been selected.

Results: In contrast to other opioids, the identification of methadone was possible only when sulfovanadate reagent was used. From the five solvent systems used in the TLC studies, the best results have been obtained with the methanol: strong ammonia (100:1.5) system.

Conclusions: The color tests and TLC method can be used as rapid screening methods for methadone identification mainly in the delict corps analysis.

***Keywords**: methadone, color tests, TLC.*

Correspondence to:

Anne Marie Ciobanu
Adress: University of Medicine and Pharmacy „Carol Davila" Bucharest, Faculty of Pharmacy
E-mail address: annemarieciobanu@yahoo.com

INTRODUCTION

Opioids, particularly heroin, continue to be the main drug abuse problem in the world (as shown by the statistics of the treatment demands) [1].

Opioids (opiates, morphinomimetics) are a class of drugs with an extremely high potential to induce dependence, with very rapid development of tolerance and withdrawal syndrome. Opioid addiction requires long-term treatment and care because it is a complex disorder. Addiction treatment is an important strategy in improving quality of life and social functioning of opiate addict persons. It also reduces the health and social consequences (including prevention of HIV infection) [2]. Treatment of opiate addiction is a complex treatment, including psycho-therapy and drug therapy. The main treatment option for opiate addiction is methadone [5, 6]. However, methadone is also an abuse substance.

Besides methadone, symptomatic treatment like benzodiazepines is necessary to reduce manifestations of abstinence syndrome.

Clonidine is also used to treat heroin addiction, in detoxification stage, alone or associated with methadone [3].

AIM AND OBJECTIVES

Given the concern for analytical diagnosis of abuse substance, the objectives of this work is focused on analytical toxicology studies of methadone alone or in combination with other opiates or benzodiazepines. Hence, we used screening methods of identification such as color tests and thin layer chromatography (TLC). Color tests are especially useful as a rapid screening method in toxicological analysis. In case of drugs and substances of abuse, color tests are especially useful as a preliminary step in the analysis of material evidence, giving some indication of the presence of certain substances in the sample [4].

Therefore in this study, we proposed the color tests using usual color reagents for this class of drugs.

MATERIAL AND METHODS

For color tests the following were used:

- Methadone hydrochloride, codeine phosphate, dihydrocodeine, naloxone, tramadol, pentazocine, analytical grade;
- Chloroform;
- Ethanol;
- Mandelin reagent (ammonium vanadate in concentrated sulfuric acid);
- Marquis reagent (concentrated sulfuric acid and formaldehyde);
- Fröedhe reagent (sodium molybdate in concentrated sulfuric acid);
- Lafon reagent (sodium selenite in concentrated sulfuric acid);
- 0.1% solution of methadone hydrochloride in chloroform;
- 0.1% solution of codeine phosphate in chloroform;
- 0.1% solution of dihydrocodeine in ethanol;
- 0.1% solution of naloxone in chloroform;
- 0.1% solution of tramadol in ethanol;
- 0.1% solution of pentazocine in chloroform;

– Sartorius balance.

Color tests were performed on residues obtained by evaporation of solutions of chloroform / ethanol; 1-2 drops of reagent were added to the residue.

For TLC study the following were used: methadone hydrochloride, methadone's main metabolite 2-ethyliden-1,5-dimethyl-3 ,3-diphenyl-pyrrolidine (EDDP), alprazolam, diazepam, oxazepam, clonidine, analytical grade TLC Merck glass plates (silica gel 60 GF 254);

Developing tanks;

– 0.1% solution of methadone hydrochloride in chloroform;
– 0.1% solution of EDDP in chloroform;
– 0.1% solution of alprazolam in chloroform;
– 0.1% solution of diazepam in chloroform;
– 0.1% solution of oxazepam in chloroform;
– 0.1% solution of clonidine in ethanol;
– Solvent systems;
– System Camag TLC Scanner 3 with darkroom, UV lamps and coupled to the computer (soft WinCATS)
– Semi-automatic system Camag Linomat 5 for sample application on the chromatographic plate;
– Photo-camera for capturing images;
– Sartorius balance.

The analyte solutions were applied to chromatographic plates using semi-automatic Linomat 5 system equipped with a Hamilton microsyringe. The solvent front is plotted at a distance of 10 cm from the starting line.

The development was done in the following solvent systems:

– S1 methanol: strong ammonia 100: 1.5
– S2 ethyl acetate: methanol: strong ammonia 85 : 10 : 5
– S3 methanol
– S4 ethyl acetate
– S5 chloroform: methanol 90 : 10

The revelation technique used is the ascending one. The plates were examined under UV lamp (l = 254 nm). The chromatograms were acquired and processed with the TLC Scanner 3.

RESULTS

Color-test results are summarized in Table I.

Tabel 1 Color-test results

Coloration	**Reagent**			
	Mandelin	Lafon	Marquis	Fröedhe
Methadone	blue	-	-	-
Codeine	green	greenish blue	purple	blue-brown
Dihydrocodeine	greenish grey	blue	purple	brown
Naloxone	brown	greenish yellow	light purple	indigo
Tramadol	brown-green	orange	blue	purple
Pentazocine	brown	orange	-	blue

The results obtained in the TLC study (Rf values) are summarized in Table II. The chromatographic image obtained using solvent system S1 is shown in Figure 1.

Tabel 2 Evaluation of methadone and other drug substances by TLC (Rf values)

Solvent systems		R_f					
		M	**E**	**A**	**D**	**O**	**C**
S_1	methanol: strong ammonia 100 : 1.5	0.62	0.24	0.86	0.92	0.84	-
S_2	ethyl acetate: methanol: strong ammonia 85 : 10 : 5	0.90	0.94	0.57	0.87	0.54	-
S_3	methanol	0.22	0.07	0.74	0.86	0.78	-
S_4	ethyl acetate	0	0	0.05	0.73	0.56	-
S_5	chloroform : methanol 90 : 10	0.17	0.27	0.50	0.90	0.56	-

*A = alprazolam; C = clonidine; D = diazepam; E = EDDP; M = methadone; O = oxazepam

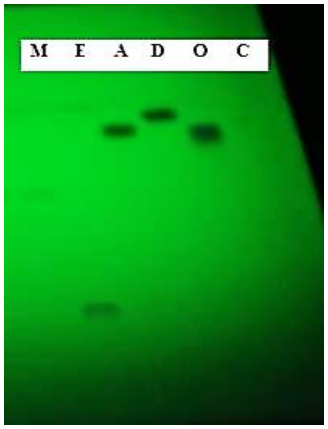


Fig.1 Chromatographic plate obtained with methanol: strong ammonia 100: 1.5 (S1)

DISCUSSIONS

Color tests have shown that methadone gives negative results for most test reagents. The only reaction that led to a positive result was with Mandelin reagent (sulfovanadate reagent), when a blue color was achieved.

Note that all substances tested which had their core morphine structure intact (such as codeine, dihydrocodeine, naloxone) gave a purplish color with Marquis reagent. Methadone and pentazocine however did not react with this reagent, while tramadol was clearly differentiated from the rest of the analytes, being the only compound which gave a blue color.

As shown in table I, naloxone and pentazocine can not be distinguished by the test with Mandelin reagent, both compounds leading to a brown color. Similarly, tramadol and pentazocine can not be distinguished by the test with Lafon reagent, both compounds leading to an orange color.

TLC study showed that in most solvent systems tested, alprazolam and oxazepamul both migrated relatively in close range. However these two types of bezodiazepine clearly separated from methadone and its main metabolite EDDP. The only solvent system that allowed a clear separation of alprazolam and oxazepam was system S4. The third benzodiazepine studied, diazepam, migrated similarly and simultaneously with alprazolam and oxazepamul

when using S1 and S3 systems as mobile phase.

S1 and S3 systems allowed a proper separation of the methadone from its metabolite, EDDP, but also from the rest of the substances tested. The S3 system separated the methadone and EDDP from benzodiazepines; however the Rf value was reduced for EDDP. Decreasing polarity of S3 system by adding chloroform (system S5) slightly increased EDDP's Rf, yet it decreased the distance of migration of alprazolam and oxazepam. Increasing the alkalinity of S3 system by adding ammonia (S1 system) increased methadone's Rf, but the methadone - benzodiazepines separation became less obvious. With the S4 system methadone, EDDP and alprazolam are characteri-zed by low Rf values. Increasing the polarity and the alkalinity of the system (S2 system) resulted in an increase of the migration distances and of the net separation of alprazolam and oxazepam. In this system however, methadone and its metabolite are not clearly separated from each other, or from diazepam.

CONCLUSIONS

Using selected color tests, opioids can be identified and differentiated between them. In contrast to other opioids, the identification of methadone was possible only when sulfovanadate reagent was used. It should be noted that color tests in general have a probability character, giving some indication of the presence of test substance in the sample.

In interpreting the results should be taken into account the possible interference with other substances. For these reasons, color tests must be supplemented with greater certainty methods (like TLC) or confirmatory methods (performance instrumental methods like GC / MS).

TLC method can be used with good results for identification of methadone in the presence of benzodiazepines. Best results are obtained with systems containing mainly apolar solvents or solvents with medium polarity. Alkalinization of the solvent systems (by adding ammonia) or decreasing polarity leads to increased migration distances of methadone.

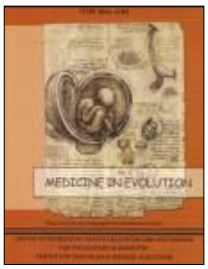
From the five solvent systems used in the TLC studies, the best results have been obtained with the methanol: strong ammonia (100:1.5) system (S1).

The color tests and TLC method can be used as rapid screening methods for methadone identification mainly in the delict corps analysis.

REFERENCES

1. *** - World Drug Report 2005. http: // www. unodc.org/unodc/en/world_drug_report.html
2. Baconi D., Bălălău D., Abraham P., Abuzul şi Toxicodependența – Mecanisme, manifestări, tratament, legislație, Editura Medicală, Bucureşti, 2008; 98
3. Kleber HD - Pharmacologic treatments for heroin and cocaine dependence. Am J Addict., 2003; 12 Suppl 2:S5-S18.
4. Moffat AC, Osselton MD, Widdop B (eds) – Clarke's analysis of drugs and poisons, third edition, Pharmaceutical Press, 2004.
5. Rawson RA, McCann MJ, Hasson AJ, Ling W- Addiction pharmacotherapy 2000: new options, new challenges. J Psychoactive Drugs, 2000; 32(4):371-8.
6. Stotts AL, Dodrill CL, Kosten TR - Opioid dependence treatment: options in pharmacotherapy. Expert Opin Pharmacother., 2009, 10 (11): 1727-40.

EVALUATION OF QUALITY OF LIFE IN HEALTHCARE



LIANA SUCIU[1], CARMEN CRISTESCU[1], MIRELA TOMESCU[2], MIRELA VOICU[1], MARIA SUCIU[1], RALUCA GRADINARU[1], BOGDAN BUMBACILA[1]

1. **University of Medicine and Pharmacy Timisoara, Faculty of Pharmacy, Department of Pharmacology-Clinical Pharmacy**
2. **University of Medicine and Pharmacy Timisoara, Faculty of Medicine, Department of Internal Medicine**

ABSTRACT

As defined by WHO (1998), the quality of life is given by the perceptions of the individuals regarding their social circumstances, within the cultural value systems they live in and dependency on their needs, standards and aspirations.

Studies on quality of life are particularly useful for medical practice for the assessment of physical, mental and social effects of diseases and medical treatments on daily life, in analyzing the effects of treatment or disease, by the patient's point of view and in determining the patient's needs for mental, physical and social support during the illness. They are also useful for medical staff because they have to take a correct therapeutic decision with minimum on the economic impact.

In this article, we reviewed the modalities of assessing quality of life and their applicability in clinical practice.

***Keywords**: quality of life, patients, instruments of evaluation, practicality*

Correspondence to:

Liana Suciu
Adress: 132 A, Bujorilor, 300431, Timisoara, Romania,
Phone: +4072315523
E-mail address: lianads@yahoo.com

INTRODUCTION

Health is the result of a complex combination of individual and social factors and is influenced on one hand by the individual genes, by the values related to health and lifestyle, by individual characteristics such as age, education, occupation, and on the other hand there is also a net influence by social point of view: the quality of medical services, the quality of the environment, etc.

The people can define their own state of health which is an important dimension for the quality of life. The 2010 report regarding the assessment of quality of life in Romania shows that the assessment of health has been deteriorated since the 90s, so that today only 46% of the respondents assessed their health as good, while 28% consider it poor or very poor.

These evaluations show that people are able to rate their own health, and they appreciate the healthcare system and care received in negative terms most of the time and the efficiency of the treatment and the reduced frequency of the preventive care lead in time to pathologies that worsen quicker than normal [1, 8].

THE CONCEPT OF QUALITY OF LIFE

As defined by WHO (1998), the quality of life is given by the perceptions of the individuals regarding their social circumstances, within the cultural value systems they live in and dependency on their needs, standards and aspirations [3, 4, 7].

According to Revicki & Kaplan (1993), quality of life reflects the preferences for certain health conditions that allow improvements in morbidity and mortality, which is expressed by a single weighted index – "standardized years of life" dependent on the quality of life.

II.a. The importance of evaluation of quality of life: Studies on quality of life are particularly useful for medical practice for the assessment of physical, mental and social effects of diseases and medical treatments on daily life, in analyzing the effects of treatment or disease, by the patient's point of view and in determining the patient's needs for mental, physical and social support during the illness. Medical determinations such as the clinical or laboratory ones are important for medical evaluations, but they are not so relevant for the patients, because the patient's only interest is to get better and feel good. The medical indicators regarding the improvement of the patient's condition of health often correlate less or do not correlate at all with the functional condition or the wellbeing, much easily assimilated by the patients.

Another element that requires determination of the quality of life is the observation according to which two patients from the same functional class under laboratory determinations have diametrically distinct clinical facets, for example, patients with rheumatologic conditions and the same medical evaluation as for the limitation of movement may experience conditions of totally different wellness tally different, which means that some patients may continue their work whilst others with the same pathology and symptoms experience the disease in another manner plus the depressive mood which will lead to early retirement [1, 4, 12]. The use of tools to assess the quality of patient's li-

fe helps the medical staff to choose between different alternative treatments, to inform patients about the possible effects of various medical procedures, to monitor progress of the treatment applied by the patient's perspective and allow medical staff to design effective and efficient medical care packages.

II.b. Means to assess the quality of life: The tools used for the measurement of the quality of life vary depending on who will use those tools (doctors, patients, caregivers) and depending on the context of administration (self administration, interview, telephone interview). Generic tools are most often used to assess a broad registry of applicable domains to a larger number of disease or health conditions. It doesn't characterize a particular type of pathology or subgroups of patients and are useful for global comparisons between different types of disease.

Specific instruments focus on relevant areas for a specific disease under useful analysis in clinical studies for evaluation of specific therapeutic interventions.

Modular instruments combine the generic approach to that specific to certain pathology and may be applied sequentially with a certain common group of items and other optional items depending on the particular context.

In the context of healthcare, it is necessary to find operational criteria for measuring quality of life (Rumboldt, 1997). Among existing models, we can note: the module of the 14 basic needs of the patient, systematized by Virginia Henderson (Henderson, 1996, 1977) and the 12 daily essential activities for a patient Nancy Roper (1990) [3, 4, 6].

Based on these essential items that assess the welfare of patients with various pathologies both the generic as well as the specific instruments were developed, instruments that may give clues about the degree to which the disease affects the patient's condition and also how the patient's overall condition is to improve after determining medication.

II. c. Types of measuring instruments of the quality of life

II.1. Global instruments: Flanagan Quality of Life Scale (Flanagan's life assessment scale 1978), measure personal satisfaction in 15 different areas of life such as: financial security, health, relationships with relatives and friends, learning abilities, work, creativity, socialization, reading and personal independence. The 15 items of the instrument are grouped on five scales: physical and material well-being, personal relationships, social, community and civic activities, personal development and fulfillment and leisure time. Each item is listed on a Likert scale from delighted to terrible. Higher scores indicate a superior quality of life.

II.2. Generic instruments: Generic instruments are applied to several groups of subjects to assess all types of pathologies or conditions, applicable to any medical facility or even on the general population. There are several types of assessment tools including the MOS - SF-36 which is the most complex instrument and we will detail it below. MOS-SF-36 – Medical Outcome Study-Short Form 36, main author John Ware Jr., 1992 comprises eight domains. Currently, it is the most utilized tool for assessing the quality of life using the latest version, v2. It includes the following domains:

1. The 10 items physical functioning scale. The items reflect the levels and types of limitations of physical activity extremes. The items include assessing both the current situation

as well as the long-term limitation of the physical function using a continuum of the response structured on three levels. Low scores signify important limitations in the performing of physical activities while high scores reflect a slight limitation or no limitation at all.

2. The 4 items scale of problems caused by physical conditions: reflects physical role limitations, including limiting the type of work or other usual activities, reducing the number of hours spent at work, difficulties in performance of the type of activity and fulfilling less than usual. Low values reflect restricting the conduct of various types of activities in good conditions.
3. The 2 items social functioning scale: the items of this scale assess the effects of health on social activities in terms of quality and quantity with emphasis on the physical and emotional impact on the social function. The degree the physical and / or emotional problems interfere with the normal social activities increases with decreasing scores. Getting the lowest scores mean frequent interference with normal social activities due to physical and emotional problems.
4. The 2 items bodily pain scale evaluates the intensity of the pain and its interference with normal activities due to pain. Low scores indicate a high level of pain which affects daily normal activities whilst high scores indicate that this symptom in manifested very little or has no influence at all.
5. The 5 items mental health scale: the items are classified under the fourth major dimensions of mental health: anxiety, depression, loss of behavioral-emotional control, the psychological well-being. Lower scores are an indicator of depression and anxiety while higher scores most of the time indicate happiness, peace and calm.
6. The 3 items scale of problems caused by emotional stress. The items evaluate the mental health which interferes with limitations from the point of view of the time spent at work or for other normal activities. Low scores indicate work problems or for performing other activities as result of emotional distress.
7. The 4 items vitality scale - was developed to highlight the differences in the welfare of patients. Low scores indicate fatigue and high scores indicate vitality, energy and energy for work.
8. The 5 items general health scale. One of the items assesses the general health from excellent to very poor and the other four items evaluate the patient's vision and expectation in terms of health. Low scores assess general health as poor and likely to worsen. High scores indicate a future assessment of health status as good.

The analysis and correlation between the 8 domains of each version (v1 and v2) of the SF-36 have identified two factors: the physical and psychical / mental component of health. Three scales: PF, RP, BP have a better correlation with the physical component and contribute to the assessment of average score of physical component and the mental component correlates much better with the MH, RE, SF, which help to the assessment of the average mental component score.

Three scales have correlations with both components: VT, SF and GH.

Unlike the first version of the questionnaire, within the latter version two global measures were introduced: PCS and MCS which measure the quality of life on those two directions: the mental

and physical one. The evaluation of these two measurements facilitates the interpretation of the dimension of the disease and the treatment's effects before the specific evaluation [6, 10, 11, 12].

II.3. Specific instruments: Specific instruments focus on some aspects of health which are specific for a certain area of interest. The purpose of this approach is related to the potential growth of the rate of response that aims a specific pathology. The instrument can be specific to a particular disease such as hypertension or asthma, or specific for a population of patients such as elderly, children, and pregnant women or for a particular function: sleep, anxiety or symptoms such as pain.

For example, for the cardiovascular domain there are instruments such as MACNEW – Mac Master-Newcastle related Quality of Life Qestionnaire, written by Neil R. Oldridge, 1998 and the Romanian version, Oldridge and all in 2003, with 27 items and 3 scales or SAQ – Seattle Angina Questionnaire, author John Spertus, 1995 with 19 items and 5 domains: limitation of physical activities, the stability of chest pain, satisfaction regarding the treatment and the subjective perception of the disease [2, 5, 9].

For the cancer domain there are Rotterdam Symptom Checklist author Johanna de Haes, 1990, including 39 items and 3 subscales: physical suffering scale, psychical suffering scale and daily activities scale. Assessment is made on a scale from 1 (very bad) to 7 (excellent) [4, 6].

For pediatric patients it has been used PedsQL-CM-Pediatric Quality of Life-Cancer Module, author John Varna, 1999 and KINDL, the German questionnaire to assess life of children and adolescents conducted by the Ravens-Sieberer, Ulrike, Bullinger, 1998 [4].

GENERAL CRITERIA FOR THE MANAGEMENT OF THE ASSESSMENT QUESTIONNAIRE

Respondents must meet the eligibility criteria in order to complete the questionnaire: age, reading ability, the ability to understand the questionnaire for example, psychotic patients may exaggerate the severity of the disease or symptoms or on the contrary to minimize them or patients with severe pain may exacerbate its influence on physical and mental issues due to different threshold of pain acceptance and affordability, the time of presentation of the questionnaire (before the initial consultation carried out by a doctor and before being set a therapeutic scheme and then after an interval of time from initiation of therapy) [10, 11].

CONCLUSIONS

1. The questionnaires differ by presenting a more subjective than objective side and by involvement in certain areas of quality of life. Measurement and evaluation of quality of life should cover all subjective and objective components important for the group under study and likely to be modified by therapeutic interventions. For example, in patients with cardiovascular pathology therapeutic intervention can be evaluated in two directions:

The impact on the severe end-points of the disease: the survival;

Subjective influence on daily life of the patients: mobility, symptoms, need

for hospital readmission, repeated controls, side effects of drugs, sleep, sexual activity, which means that the drug or interventional therapy may be effective clinically, but with a major negative impact on the quality of life.

2. Choosing a certain type of quality of life measurement is based on the purpose of the study:

a) Generic determinations are useful: for policy makers, in studies which document the range of disabilities in a population or group of patients; for the evaluation of specific domains which can benefit from therapy and if a specific instrument is used, it may not be detected; when there is evidence on the efficiency of the therapy and the use of a generic instrument will better appreciate the favorable effect on a global level.

b) Specific instruments are useful for the patient and the general practitioner; it focuses on certain aspects of a health condition which are specific to the area of interest and evaluate certain aspects of quality of life which are modified due to the pathology and the impact the medication has on the changes in quality of life; they are brief and easier to fill in by the patients.

3. Use of quality of life assessment in different types of studies:

a) In non - randomized studies: that highlights certain predictors of quality of life (medical treatment, psychotherapy for some form of depression), it requires a large number of patients for each study group, approximately 500 patients, and research results should be applicable to a broad range of patients;

b) In randomized studies that allow assessment on a small numbers of patients;

c) For cost-efficiency studies and cost-benefit studies which estimate the increase of the cost for implementing a new therapeutic procedure with maximum of health benefit using predictive models (such as Markov model) and the QALY-Quality Adjusted Life Years.

4. The responsiveness and validity of the method used should be tested, either the short version or the original one.

5. The translation of the original questionnaire is also important because a simple translation is usually not enough, the instrument can be easily distorted in another language taking the cultural differences, and this is why the validation process is important [12, 16, 17].

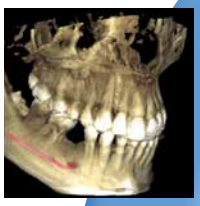
REFERENCES

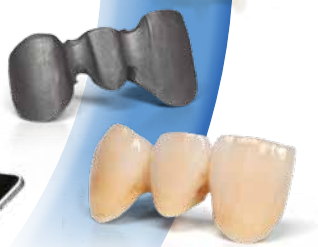
1. Carr Alison. J., Higginson Irene. J., Measuring quality of life: Are quality of life measures patient centered?, „British Medical Journal", 2001, 322, 1357–1360;
2. Dixon Tracy, Lim Lynette L.Y., Oldrige N.B.: "The MacNew heart disease health related quality of life instrument: Reference data for users. "Quality of Life Research, 2002, 11, (2), 173-183.
3. Fryback, D. G., Dunham, N. C., Palta, M., Hanmer, J., Buechner, J., Cherepanov, D., et al. (2007). US norms for six generic health –related quality-of-life indexes from the national health measurement study. Medical Care,2007, 45, 1162 - 1170;
4. Lupu I., Calitatea vieţii în sănătate, Calitatea vieţii, 2006, XVII, nr.1-2,73-91;
5. Li W, Liu L, Puente JG, Li Y, Jiang X, Jin S, et al. Hypertension and health related quality of life: an epidemiological study in patients attending hospital clinics in China. J Hypertens. 2005; 23 (9): 1667-76;
6. Quality of life instruments. Pharmacoeconomics. 2000; 17 (1): 13-35;
7. Marginean I. si colab., Calitatea vietii in Romania, raport de cercetare, 2010;
8. Petrescu V., Petrescu Mariana, "Conceptul de calitate a vietii in practica medicala curenta", Farmacist.ro, 2011 nr.142,5;
9. Roca-Cusachs A, Dalfo A, Badia X, Aristegui I, Roset M.: "Relation between clinical and therapeutic variables and quality of life in hypertension",J. Hypertens. 2001; 19 (10): 1913-9;
10. Ware J.E., et al., SF-36v2 Health Survey: Administration guide for clinical trial investigators, Lincoln, RI: Quality Metric Incorporated, Copyright 2008, 2010;

11. Ware, J. E., Jr., & Kosinski, M., Bayliss, M. S., Mchorney, C. A., Rogers, W. H., & Raczek, A. (1995). Comparison of methods for the scoring and statistical analysis of SF-36 health profile and summary measures: Summaryof result-sfrom the Medical Outcomes Study. Medical care, 33 (Suppl. 4), AS264-AS279;
12. Ware, J. E., Jr., & Kosinski, M. (2001a). Interpreting SF-36 summary health measures: A response. Quality of Life Research, 10,405-41.

ProMax 3D • PlanScan • ProFace

Unique 3D combination for open CAD/CAM





Digital perfection

Planmeca sets new standards with world's first dental unit integrated intraoral scanner for open connectivity to various CAD/CAM systems.

We would like to invite you to explore the dentistry in new dimensions – see the perfect combination of digital intraoral scan, CBVT and 3D facial photo datasets in one 3D image. This digital perfection enables you to study patient's complete anatomy in detail, plan and utilise open interface with modern CAD/CAM systems according to your needs. Now you can be one of the pioneering specialists, whether you are an implantologist, endodontist, periodontist, orthodontist or maxillofacial surgeon. The new era of dentistry is reality. It's your decision.

Planmeca Oy, Asentajankatu 6, 00880 Helsinki, Finland
tel. +358 20 7795 500, fax +358 20 7795 555, sales@planmeca.com, www.planmeca.com

PLANMECA

1971 - 2011

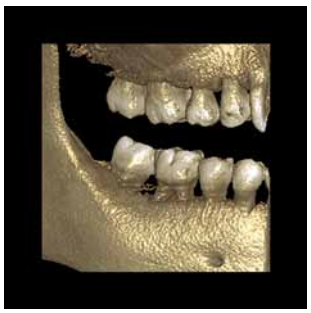
Planmeca ProMax 3D

All volume sizes

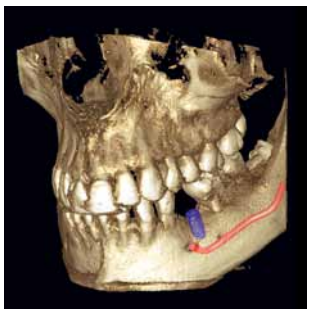
The Planmeca ProMax concept offers a full range of imaging volumes providing detailed information on patient anatomy. The comprehensive Planmeca ProMax platform complies with every need in dental radiology, offering digital panoramic, cephalometric, and 3D imaging as well as 3D face photo together with advanced imaging software.

At the heart of the concept is the robotic SCARA technology: the unique robotic arm enables any movement pattern required by existing or future program, eliminating all imaging restrictions. With the Planmeca ProMax concept superior maxillofacial radiography can be performed with a single platform, today and in the decades to come.

All volume sizes

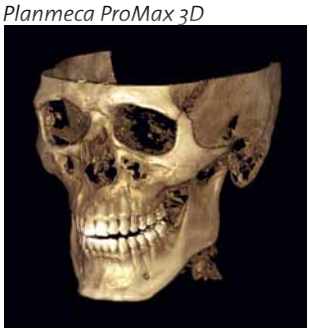


Planmeca ProMax 3D s
Ø42 x 42 mm–90 x 60 x 130 mm



Ø34 x 42 mm–140 x 105 x 130 mm

Planmeca ProMax 3D



Planmeca ProMax 3D Mid
Ø34 x 42 mm–Ø160 x 160 mm

Planmeca ProMax 3D Max
Ø42 x 50 mm–Ø230 x 260 mm

Planmeca Oy, Asentajankatu 6, 00880 Helsinki, Finland
tel. +358 20 7795 500, fax +358 20 7795 555
sales@planmeca.com, www.planmeca.com

PLANMECA

Planmeca Romexis

Software refined

Available on the App Store

Planmeca Romexis is the software of choice for all dental imaging purposes. All patient's digital images – intraoral and extraoral X-ray images, 3D volumes, and photographs – are processed and stored in one easy-to-use system. Planmeca Romexis offers a complete set of tools for image viewing, enhancement, measurement, and implant planning, and fully integrates digital imaging with the patient's other clinical data.

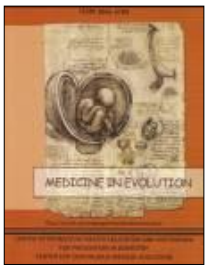
Thanks to its powerful printing features, stunning printouts can be produced. Planmeca Romexis provides direct image capture from Planmeca X-ray units, interfaces with 3rd party devices via TWAIN, and is fully DICOM-compatible. Planmeca Romexis is a JAVA software that runs on Windows, Mac OS, and Linux operating systems, and embraces modern IT standards.

Planmeca Oy, Asentajankatu 6, 00880 Helsinki, Finland
tel. +358 20 7795 500, fax +358 20 7795 555
sales@planmeca.com, www.planmeca.com

PLANMECA

1971 - 2011

A NEW APPROACH IN INTERCEPTIVE TREATMENT IN PSEUDO-CLASS III MALOCCLUSION. A CASE REPORT



MOISE MARCEL[1], ANGELA CODRUTA PODARIU[1], DANIELA JUMANCA[1]

1. **Department of Preventive, Community Dentistry and Oral Health, Faculty of Dentistry,**
2. **University of Medicine and Pharmacy "Victor Babeş" Timişoara**

ABSTRACT

The diagnosis of pseudo Class III malocclusion differs from that of skeletal Class III malocclusion because it is defined as functional forward displacement of the mandible as a result of retroclined maxillary incisors. Because the major underlying cause of pseudo Class III malocclusion is the inclination of the maxillary incisors, the treatment objectives aim to change the inclination of those incisors.

In general terms, the goal of interceptive orthodontics is to prevent an existing problem from getting worse. Specifically for pseudo Class III, the goals of early treatment are to correct the anterior displacement of the mandible before the eruption of the canines and premolars so that they can be guided into Class I in the proper mandibular position, to provide space for the eruption of the buccal segments as a result of proclination of the upper incisor, and to provide a normal environment for the growth of the maxilla, eliminating the anterior crossbite.

***Keywords**: class III malocclusion, interceptive treatment, ankyloglossia.*

Correspondence to:

Moise Marcel PhD student
Address: Department of Preventive, Community Dentistry and Oral Health, Faculty of Dentistry - University of Medicine and Pharmacy "Victor Babes" Timisoara, Spl.T. Vladimirescu no.14A
Phone: 0040-256-204950
E-mail adress: drzuba79@yahoo.com

INTRODUCTION

The etiology of malocclusions has been the subject of a long standing debate in the orthodontic literature. The role of both genetic and soft tissue factors has been well established.

Patients with a skeletal crossbite require aggressive management, and hence identification of the structural etiology is important. A combination of cephalometric, facial and occlusal analysis may be the most reliable method of determining structural etiology in the primary dentition. Early treatment of Class III malocclusion has been advocated to reduce the need of treatment in the permanent dentition, when camouflage orthodontic treatment or surgery become the only options.

CASE REPORT

A boy, age 9 years 5 months, presented with an anterior crossbite from the upper right deciduous canine to the upper left lateral incisor.

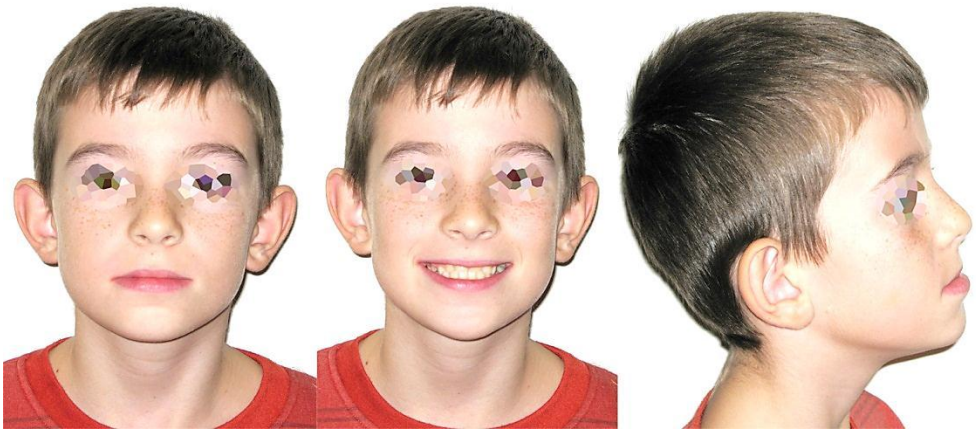


Fig.1 Initial photographs taken on December 2011. Frontal, smile and lateral views of the face.

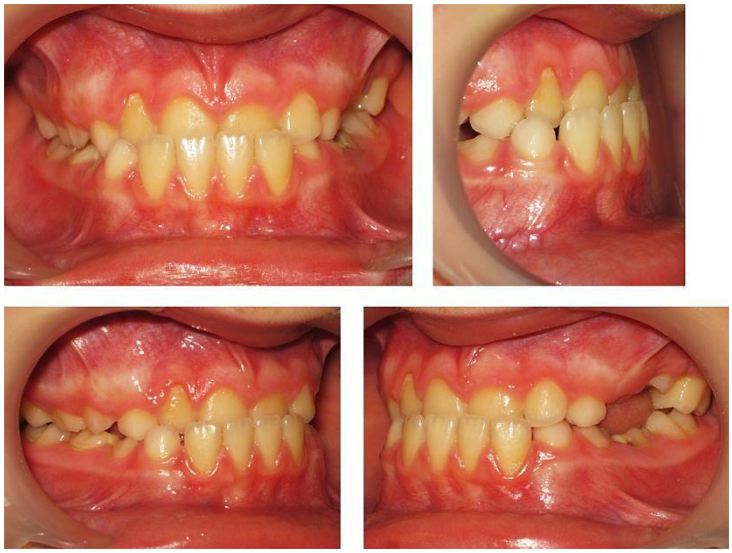


Fig.2 Initial photographs taken on December 2011. Anterior crossbite.

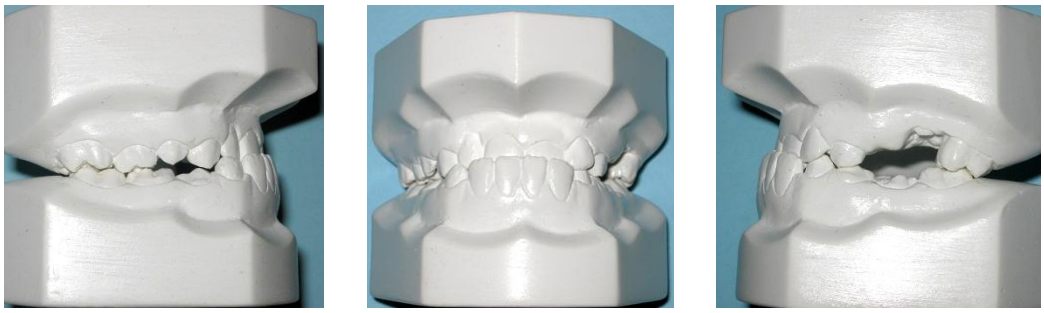
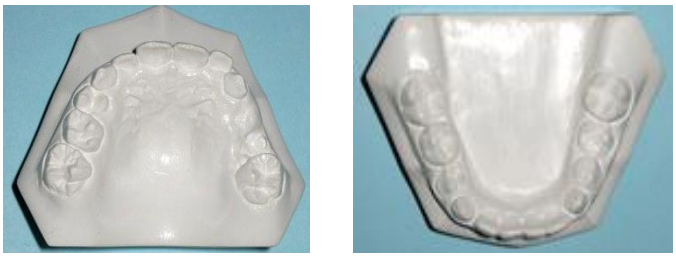


Fig.3 Initial dental casts.



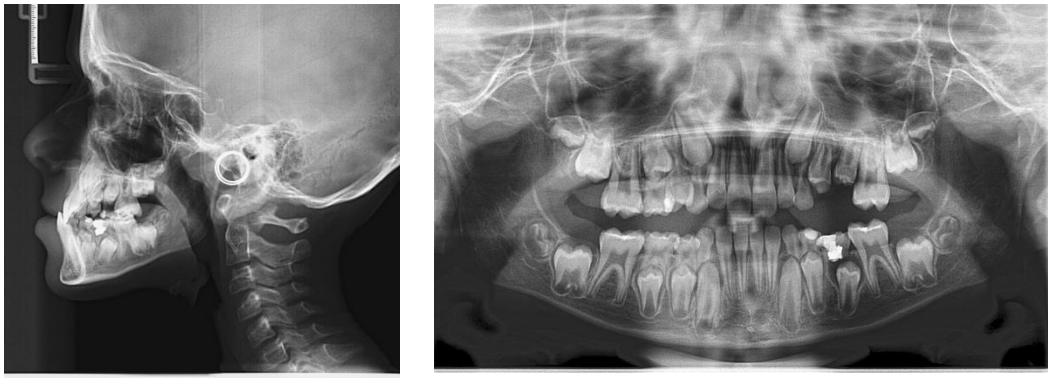


Fig.4. Initial cephalometric radiograph. Panoramic radiograph confirming presence of all permanent teeth.

The midline was not affected. The upper anterior teeth were retroclined while the lower anterior teeth were protrusive. The molars were in a Class I relationship.

Facial evaluation showed lack of development of the middle third. The patient had a poor profile and the lower lip appeared protruded. The initial panoramic radiographs revealed that all permanent teeth were present.

The patient had a short mucous membrane under the tongue (ankyloglossia), which was limiting the mobility of the tongue. The constant forward tongue position was contributing to the establishment of a Class III malocclusion.

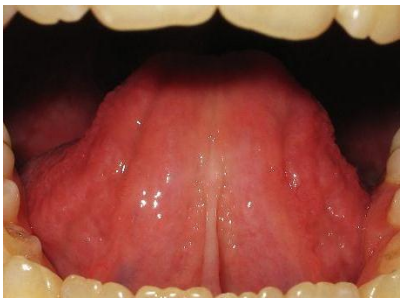


Fig.5. Shorten tongue frenum. The pacient cannot touch the palate with his tongue.

Treatment – the patient was at a mixed dentition stage, with great potential of growth, so the main goal of the treatment was to correct the anterior crossbite, while also correcting the functional forward deviation of the mandible, and allowing the maxilla to be in a forward position in relation to the mandible, thus affording a normal development.

The treatment was initiated with a fixed lingual arch which had incorporated anterior bite plane to allow the upper jaw advancement and the correction of the incisors axis.

After 1 month of wearing the appliance the anterior crossbite was corrected also the profile was changed significantly. Because of the frenoplasty the speech has improved.

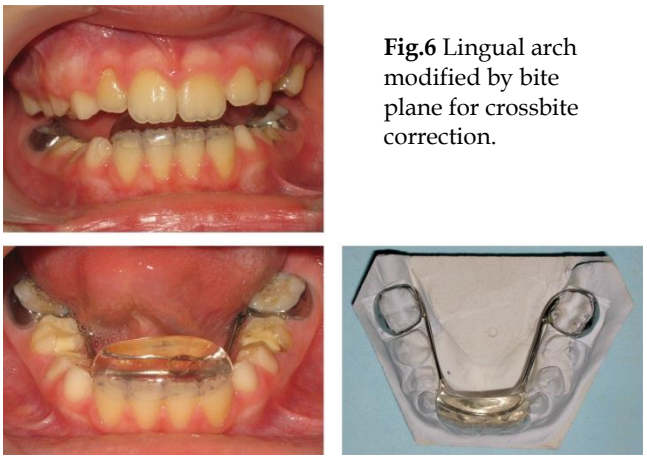


Fig.6 Lingual arch modified by bite plane for crossbite correction.

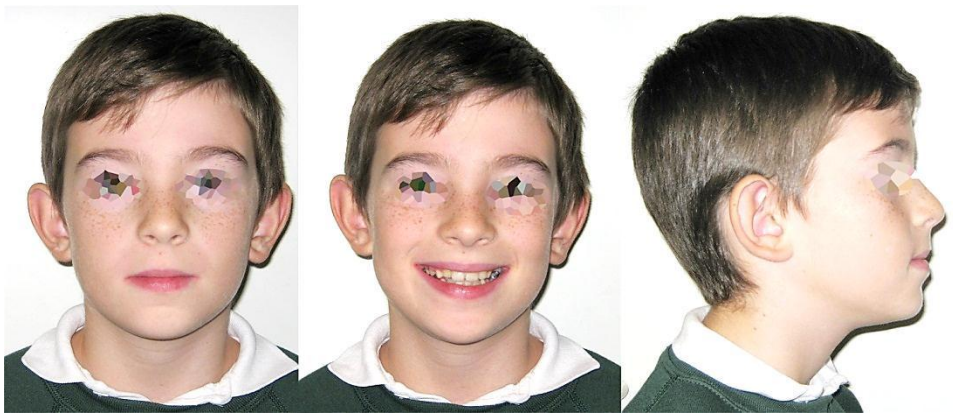


Fig.7 Photographs taken on January 2012. Frontal, smile and lateral views of the face.

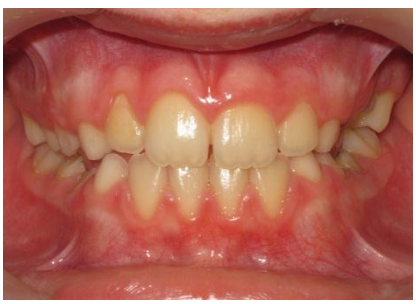


Fig.8 Clinical aspect after 1 month.

DISCUSSIONS

To obtain the best results in the treatment of patients with Angle Class III malocclusion, the etiologies of the malocclusion should first be clarified, and then an appropriate treatment modality should be decided [1, 3, 5].

Because a malocclusion may be regarded as an aesthetic problem, parents often inquire whether or not therapy might be required. The optimum period for the treatment suggested being between the ages 6–9 years [6, 8].

White has suggested intervention in cases of pseudo- Class III malocclusion in the mixed dentition when the maxillary and mandibular incisors have erupted [1]. This allows the permanent teeth to erupt into a better position and improves the dental aesthetics.

CONCLUSIONS

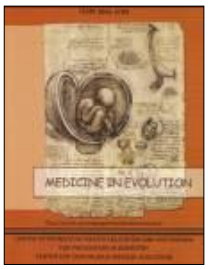
This case report shows that that the stability of the correction of a functional Class III malocclusion with minor skeletal involvement is related to both the correct diagnosis and the early intervention. This treatment allowed proper facial growth and development, preventing worsening of the malocclusion, with more severe consequences.

Benefits to early treatment may include: potential for a greater orthopedic change in a shorter period of time, earlier esthetic improvements in the smile and facial profile, prevention of periodontal recession and dental wear, gaining space for eruption of canines (lack of space could be caused by retroinclination of upper incisors frequently found in pseudo or Class III malocclusion), earlier functional improvement eliminating an anterior functional shift of the mandible, and avoiding or decreasing the chances of later orthognathic surgery.

REFERENCES

1. Gianelly AA, Bednar J, Cociani S, Giancotti F, Maino G, Richter O. Bidimensional technique: Theory and practice. Islandia, NY: GAC International, 2000:128–141.
2. Bench RW, Gugino CF, Hilgers JJ. Bioprogressive therapy part 8: Bioprogressive mixed dentition treatment. J Clin Orthod 1978;12:279–298.
3. Fränkel R, Fränkel C. Orofacial orthopedics with the function regulator. Basel: Karger, 1989: 36–38, 190–211.
4. Guyer EC, Ellis EW, McNamara JA Jr, Behrents RG. Components of Class III malocclusion in juveniles and adolescents. Angle Orthod 1986; 56:7–31.
5. McNamara JA Jr, Brudon WL. Tratamiento ortodóncico y ortopédico en la dentición. Ann Arbor: Needham, 1995:121–133.
6. Langlade M. Diagnostic orthodontique. Paris: Maloine, 1981:245–301, 696–714.
7. McNamara JA Jr. A method of cephalometric evaluation. Am J Orthod 1984;86:449–469.
8. Ricketts RM. Provocations and perceptions in craniofacial orthopedics. Glendora, CA: RMO, 1989: 735, 817–818.

THE ASSESSMENT OF FOUR METHODS OF THE GUTTA-PERCHA ROOT CANAL FILLING INVESTIGATED BY TOOTH-CLEARING TECHNIQUE



SCARLATESCU SINZIANA ADINA[1], ILIESCU ANDREI[2], DIDILESCU ANDREEA CRISTIANA[3], STEFAN DANIELA SIMINA[4], ILIESCU ALEXANDRU [6]

1. **Department of Endodontics, Faculty of Dental Medicine, Carol Davila University of Medicine and Pharmacy, Bucharest**
2. **Department of Endodontics, Faculty of Dental Medicine, Carol Davila University**
3. **Department of Anatomy, Faculty of Dental Medicine, Carol Davila University**
4. **Faculty of Chemistry, Polytechnic University, Bucharest**
5. **Department of Oral Rehabilitation, Faculty of Dental Medicine, Carol Davila University**

ABSTRACT

***Aim**: the assesment of four methods of the gutta-percha root canal fillings performed by warm vertical condensation.*

***Objectives**: comparing the radiographs of the root canal fillings and the real obturated volumes, as visualized by the tooth-clearing technique.*

***Materials and methods**: the root canals of 40 mandibular incisors and premolars, have been rotary shaped with ProTaper files and then divided into 4 groups, each of 10 teeth, which were filled according to 4 distinct vertical warm gutta-percha condensation techniques, as follows: continuous wave, multiple waves, thermal compaction, and hybrid technique. The root canal fillings were assessed both radiographically (2D) and by tooth-clearing method (3D).*

***Results**: the best X-ray pictures were provided by thermal compaction and hybrid techniques. The tooth-clearing method showed the filling of the majority of lateral canals by all 4 techniques of the gutta-percha vertical condensation.*

***Conclusions**: the thermal compaction demonstrated on radiographic and microscopic basis that is the best method of the root canal filling as compared to the other vertical condensation techniques, even if it might have potential risks.*

***Key words**: vertical condensations, lateral canals, tooth-clearing method.*

Correspondence to:

Sinziana Scarlatescu MD
Adress: Department of Endodontics, Faculty of Dental Medicine, Carol Davila University of Medicine and Pharmacy, Bucharest
Phone: 0721-804094
E-mail address: sanzianas@gmail.com

INTRODUCTION

Gutta-percha is to date the most used root canal filling material owing to its plasticity, volume constancy, and biocompatibility [1]. Besides the conventional method of cold lateral condensation of the gutta-percha, since much time in clinical use, the last decade progress of endodontics allowed an increasing use of the vertical condensation techniques of warm gutta-percha.

Because the final objective of an endodontic treatment is to provide an excellent sealing of the root canal, the goal of our study has been to assess this issue comparing 4 usual techniques of warm gutta-percha vertical condensation: multiple waves, continuous wave, thermal compaction, and hybrid technique which consists of the filling of the apical third of the root canal by multiple wave condensation and the more coronal part by thermal compaction.

In that respect we aimed to assess *in vitro* the risc of the apical extrusion, as well as the quality of the root canal filling of the aforementioned techniques comparing the 2D X-rays with 3D stereo-microscopic images provided by tooth-clearing procedure.

MATERIALS AND METHODS

A number of 40 inferior incisors and premolars that preserved their anatomical integrity were randomly shared in 4 groups, each of 10 teeth, having in all 44 root canals.

Shaping and cleaning

The working length was measured for every tooth under direct visual control with a steel K file ISO 08 (FKG Dentaire), watching the moment when its tip appeared at the apical foramen. The first K file that lightly binded at the apical constriction indicated the diameter of the root canal at that level.

We manually peformed the gliding path with steel K files ISO 08-10-15 (FKG Dentaire), while the shaping of the root canal was done with rotary instruments (Ni-Ti files ProTaper Universal, Dentsply-Maillefer). *Canal plus* (Septodont) gel and simultaneous Na OCl 2,5% irrigation of 10 ml/each root canal delivered by Endo-Eze (Ultradent) needles and syringe have also been used. The final apical diameter of the main root canals was between ISO 20-40.

Root canal filling

The root canal has been dried by paper points and lightly coated with sealer, Tubliseal Xpress (Sybron Kerr), using a K file by pumping and counterclockwise movements. The selected gutta-percha point is dimensionally similar to the last ProTaper rotary file that has been used in the apical part of the root canal.

First experimental group: the apical third of the canal has been filled by continuous wave technique using System B Pack (Sybron) and in the more coronal part hot gutta-percha has been injected by System B Fill (Sybron).

Second experimental group: the apical third of the canal has been filled by multiple waves technique and in the middle and coronal parts hot gutta-percha has been injected by System B Fill (Sybron).

Third experimental group: the whole root canal filling has been performed by thermal compaction technique using gutta-condensers (Dentsply-Maillefer).

Fourth experimental group: the apical third of the canal has been filled by multiple waves technique and the

more coronal part by thermal compaction of a gutta-percha point ProTaper F2 Universal (Dentsply-Maillefer).

For every tooth, radiographs have been performed in two incidences, bucco-lingual and mesio-distal, and the access cavities have been sealed by composite resin Filtek Z250 (3M ESPE AG). The tooth-clearing has been performed according to Venturi's protocol [2], the morphology analysis by a stereomicroscope Zeiss Stemi 2000-C (Carl Zeiss Jena) and the photographs by a photo-camera Nikon D60. Our data were statistically analysed by PASW Statistics 18 (SPSS) statistic software.

RESULTS

The extrusions have been evaluated according to 4 categories: A category – no extrusion, B category – sealer extrusion, C category – gutta-percha extrusion, D category – both sealer and gutta-percha extrusion.

The registered values (Table nr.I) are statistically significant concerning the relationship betweeh the filling technique and the category of extrusion (Pearson $\chi^2 = 21.582$, df = 6, $p < 0.005$).

It has to be highlighted that the tooth partition, on morphological basis, has also been statisticaly significant (Pearson $\chi^2 = 8.450$, df=2, $p<0.005$).

Table 1 The extrusion of the root filling materials.

Filling technique	Category				Total number of root canals
	A	B	C	D	
Thermal compaction	0	7	0	4	**11**
Hybrid technique	4	6	0	1	**11**
Multiple waves	7	5	0	0	**12**
Continuous wave	7	3	0	0	**10**
Total	**18**	**21**	**0**	**5**	**44**

Radiographic quality of the root canal fillings has been assessed on a 4 point scale basis [3].

In terms of radiograph density, the thermal compaction prevailed (score 1), followed by the hybrid, continuous wave and mutiple waves techniques, respectively (Table nr. II).

All techniques have shown better results in bucco-lingual than mesio-distal X-ray incidence.

Table 2– X-ray assessment of root canal fillings on 4 points scale basis.

X-rays assessment	Thermal compaction	Hybrid technique	Continuous wave	Multiple waves
1 – well condensed and adapted	95,3%	50%	45%	8,2%
2 – faulty condensed, voids < 1mm	4,6%	45,4%	25%	41,6%
3 – irregularities between 1-2 mm	0	4,54%	25%	32,8%
4 - irregularities > 2 mm	0	0	5%	16,4%

Microscopic assessment of the lateral and accessory canals fillings has been done on a 6 score scale basis, according to a previous study [2], as follows: score 0 - unfilled, score 1 – partially filled with sealer but no gutta-percha, score 2 - partially filled with sealer and gutta-percha, score 3 – fully filled with sealer but no gutta-percha, score 4 - fully filled with sealer and partially with gutta-percha, score 5 - fully filled with both, sealer and gutta-percha.

Table 3 Microscopic assesment of the lateral and accessory canals filling.

Score	Number of canals
0	0
1	14
2	7
3	1
4	4
5	6
Total number of canals	**32**

Only 32 out of 46 in total identified lateral and accessory canals have been partially or completely filled (Table nr.III).

The most effective technique for filling the lateral canals has been the thermal compaction [11], followed by multiple ways vertical condensation [8], continuous wave vertical condensation [7], and hybrid technique [6].

Half of the filled lateral canals have been situated in the middle third of the root [16]. The others have been equally distributed in the coronal [8] and apical third of the tooth root [8].

Fig.1 Lateral canal in the middle third of the root x 0, 8 (score 1)

Fig. 2 Lateral canal in the middle third of the root x 2 (scor 2)

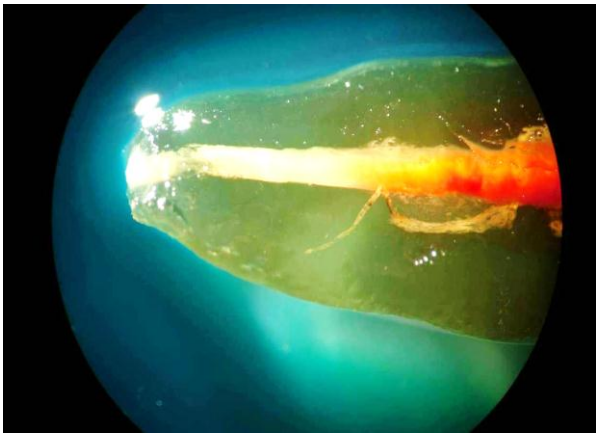


Fig.3 Lateral canals in the apical third of the root x 2 (scor 3)

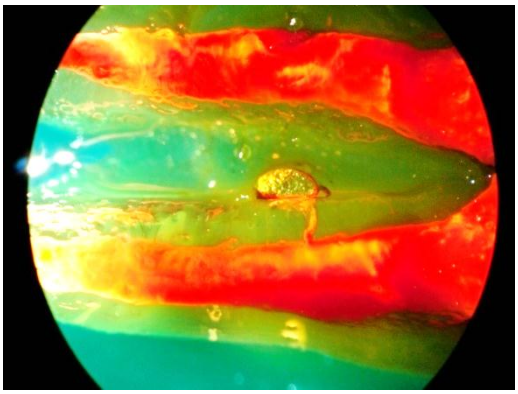


Fig.4 Lateral canal in the apical third of the root x 2 (scor 4)

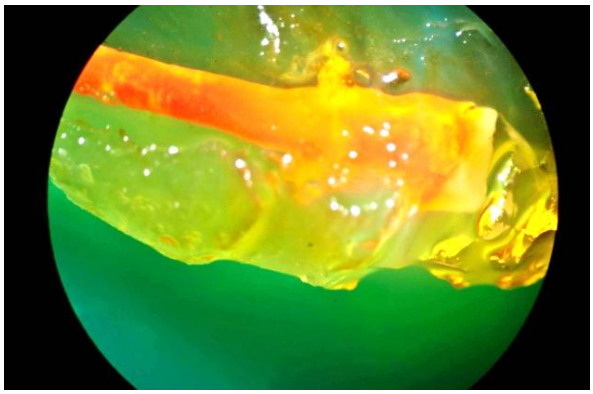


Fig.5 Lateral canal in the coronal third of the root x 1,25 (scor 5)

DISCUSSION

In our study the extrusions were found in over 50% of the root canals (26 out 44). The explanation might be a too far progression of the gutta-condenser to the apex (1,5 mm) as the tehnique of thermal compaction requires. While condensed, the amorphous gutta-percha is replicating the root canal wall, having the tendency to move especially to the area of minimal resistance which is the apical foramen.

The majority of lateral canals, especially the apical ones, seem to be filled mainly by sealer, which is consistent with previous studies showing that gutta-percha may fill large accessory canals, while at the apical level only the sealer is penetrating the lateral canals, consequently to an inefficient warming and codensation of gutta-percha [5,6].

The radiographs exposed in the bucco-lingual incidence pointed out a higher density of the fillings than the mesio-distal one, because more radio-opaque material was encountered by X-rays on their way to the film. This issue is also consistent with other studies [1,2].

Because gutta-condensers plastify the gutta-percha in order to be mixed with sealer, the thermal compaction has shown the best radiographic scores. The homogeneity of the filling material is guiding the vertical forces of compaction inside the main root canal to its walls as it was already depicted in literature [2,4].

Concerning the filling of the lateral canals, as compared to the apical and coronal parts of the root canal, our study has shown high scores mostly in the middle third, regardless the condensation technique, which is not consistent with other previous studies [5,6]. In their turn, the lateral and accessory canals from the middle third of the root were better visualized by thermal compaction, according to higher vertical forces developed through this technique.

CONCLUSIONS

The tooth-clearing procedure gives the opportunity of a 3D study of the gutta-percha adjustment at the root canal walls, obturation homogeneity, and of the filling degree of lateral and accesory canals.

The thermal compaction proved to be an excellent technique in terms of X-

ray control. Together with the hybrid technique, it demonstrated better homogeneity and density, but also developed the most extrusions of the sealer and gutta-percha.

The thermal compaction and multiple waves vertical condensation offer an effective penetration of gutta-percha mostly in the lateral canals of the middle third of the root, as well as Tubliseal Xpress, which secures a good seal of the lateral and accessory canals corresponding to the apical side of the root canal.

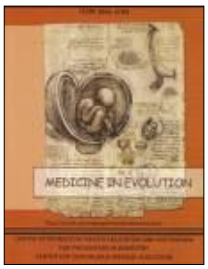
Funding Acknowledgement:

The study was supported by an unrestricted research grant from CNCSIS Romania. Code PD_382, Contract Nr. 36/28.07.2010.

REFERENCES

1. Lea C.S., Apicella M., Mines P., Yancidi P.P., Parker M.H.: Comparison of the obturation density of cold lateral compaction versus warm vertical compaction using the continuous wave of condensation technique, J Endod 2005;31:37-39
2. Venturi M.: An *Ex vivo* evaluation of a gutta-percha filling technique when used with two endodontic sealers: analysis of the filling of main and lateral canals. J Endod 2008;34:1105-1109.
3. Gilhooly R.M.P., Hayes S.J., Bryant S.T., Dummer P.M.T.: Comparison of lateral condensation and thermomechanically compacted warm alfa-phase gutta-percha with a single cone for obturating curved root canals. Oral Surg Oral Med Oral Pathol 2001;91:89-94 .
4. Venturi M., Breschi I.: Evaluation of apical filling after warm vertical gutta-percha compaction using different procedures. J Endod 2004; 30:436-440.
5. Venturi M., Pratti C., Capelli G., Falconi M., Breschi I.: A preliminary analysis of the morphology of lateral canals after root canal fillinf usin a tooth –clearing technique. Int Endod J 2003;36:54-63.
6. Reader C.M., Himel V.T., Germain L.P.,Hoen M.M.: Effect of three obturation techniques on the filling of lateral canals and the main canal. J Endod 1993;19:404-408.

THE INFLUENCE OF ADHESIVE CLINICAL APPLICATION TECHNIQUE ON DENTIN-COMPOSITE INTERFACIAL STRUCTURE



RUXANDRA MATEI[1], ELINA TEODORESCU[2], OLIVIA POPOVICIU[2], VIORICA MILICESCU[2]

1. **Dental Medicine Department, Discipline of Odontoparodontology, Faculty of Medicine and Pharmacy, University of Oradea**
2. **Department of Orthodontics and Dento-facial Orthopedics, Faculty of Dental Medicine, "Carol Davila" University of Medicine and Pharmacy, Bucharest**

ABSTRACT

The adhesion phenomenon has a vital role in dental composite restorations, to obtain a good lifetime resistance. On the other hand, adhesion is dependant on quality of dentin-adhesive zone. The present study wants to investigate the influence of dental adhesive clinical application technique on this interface. The authors made standard cavities on human extracted teeth. The cavities were filled with light curing composite (Charisma®, Heraeus-Kulzer, Germany). Then the teeth were cut and prepared for microscopic investigation (10x, 100x, and 200x magnifications).

Keywords: *dental composites, adhesion, dentin-composite interface, adhesive application technique*

Correspondence to:

Ruxandra Matei MD
Adress: Strada Independenței 24, Oradea
Phone: +40723064949
E-mail address: dr.ruxandramatei@gmail.com

INTRODUCTION

An adhesive bond has two components: an adhesive and a least an adherent on which the adhesive is bonded. These materials, in very close contact, develop intermolecular bonds like van der Waals forces[7]. The micromechanical fixation process between two elements (dentin-adhesive or enamel-adhesive) is realized by substrate etching, adhesive infiltration and polymerization. This phenomenon was first described for dentin by Nakabayashi6 in 1982, and named as hybridization. Therefore a hybrid layer results as resin (adhesive) infiltration into enamel / dentin surface zone. Theoretical conditions imposed to an adhesive stipulate that the resin must realize a good wetting of the acid etched surface (enamel or dentin). The contact angle between enamel/dentin and adhesive has to be as lower as possible, ideally 0^o. But, in the same time, the bonding effectiveness depends on adherence etching model.

The adhesive main function is therefore to fill the remaining spaces between collagen fibers after acid attack on dentin.

Subsequent polymerrization of adhesive monomers will result in the formation of hybrid layer plugs, and achieving the resin micromechanical retention on the substrate[8].

AIM AND OBJECTIVE

Currently, clinical application of adhesive techniques (3 steps systems, 2 steps systems, self-etching systems) focuses on manual "brushing" act, whether the applicator is a brush or an application tip.

The current study hypothesis is that adhesive clinical performance can be influenced by the clinical application modality on dentin substrate: manual brushing or mechanical rotation.

MATERIALS AND METHODS

8 non-carious premolars, extracted for orthodontic purposes, were selected for the study. The teeth were stored in 2% chloramines T solution, until their cleaning with an ultrasonic scaler. Then the teeth were stored in saline solution at room temperature (25^oC).

To expose the dentin, were performed crown cavities with standard dimensions (2 mm depth, 2 mm wide, 2 mm length) [9, 10].

We selected an etch-and-rinse adhesive system in 2 steps, one-bottle type (Gluma®, Heraeus-Kulzer, Germany). Etching was achieved by using ortho-phosphoric acid gel 34% for 20 s, followed by water rinse (20 s) and dentin surface drying. The adhesive was applied according to manufacturer's instructions, in two different manners: manual brushing and mechanical rotation (original method). Then the adhesive was light cured with an LED lamp (Dentmate®, Korea).

The teeth were divided into 4 groups of 2 teeth each, the grouping criteria being: adhesive application method (manual brushing or mechanical rotation) and composite resin application method (in layers or with clear acetate crowns). Finally we obtained:

Group 1: - 2 teeth acid etched; manual brushing adhesive application; light curing composite in layers,

Group 2: - 2 teeth acid etched; mechanical rotation adhesive application; light curing composite in layers,

Group 3: - 2 teeth acid etched; manual brushing adhesive application; light curing composite with clear acetate crown,

Group 4: - 2 teeth acid etched; mechanical rotation adhesive application; light curing composite with clear acetate crown.

Mechanical rotation adhesive application was realized with an "application tip"-like device (originally modified and adapted for the contra-angle hand piece) at a speed of 90-125 rotations/min. (fig.1)

The composite light curing (Charisma®, Heraeus-Kulzer, Germany) was performed 20 s for each layer (increment) with a LED lamp (Dentmate®, Korea). The dental crowns were sectioned in 4 pieces (2 buccal and 2 oral halves). The samples were embedded in cold curing acrylic resin (Duracryl®, Spofa, Czech Republic) (fig.2).

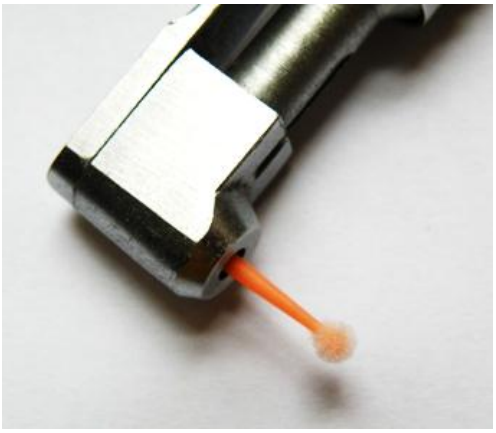


Fig. 1 "Application tip"-like mechanical device

Fig. 2 Dentin sample prepared for microscopic investigation

The samples were stored in a container with saline solution, at 4-5^{o}C.

SiC abrasive papers with P800, P1200, P2200 granulations were used for grinding under running water. To avoid prolonged contact of the sample with the air, the samples were stored in water between grindings. After grinding on SiC abrasive paper P2200, the samples were washed with running water and stored before microscopic examination in a container with distilled water at 25^{o}C, 24 hours.

The surface examination was a qualitative one. We used high power light microscopy (10x, 100x, 200x) through a Neophot type microscope (Microstructural Investigations Lab, Research Centre, INTEC SA, Bucharest, Romania), aiming the hybrid layer homogeneity and continuity, and composite characteristics.

RESULTS

In this situation we noticed remarkable dehiscence between dentin, adhesive, and composite mass (fig.3 a, b, arrows). We assume that these spaces appear due to composite material polymerization shrinkage. Enclosed area (fig.3a) marks a strong adhesion section, attached to the dentin.

***Group 1**: interface dentin-composite*
(manual brushing adhesive application and layered composite)

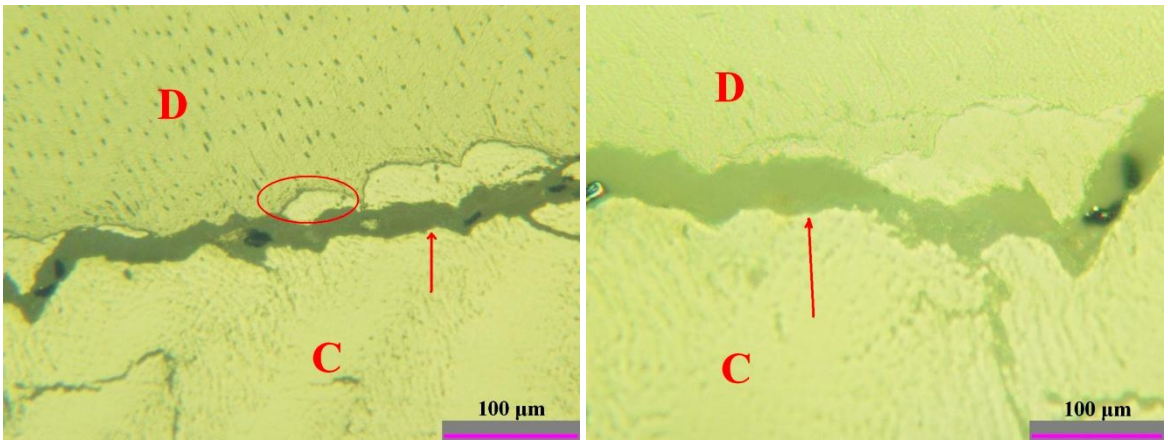


Fig.3 – Dentin (D) – composite (C) interfacial area for two different samples from group 1 (a and b) (100x)

***Group 2**: interface dentin-composite*
(mechanical rotation adhesive application and layered composite)

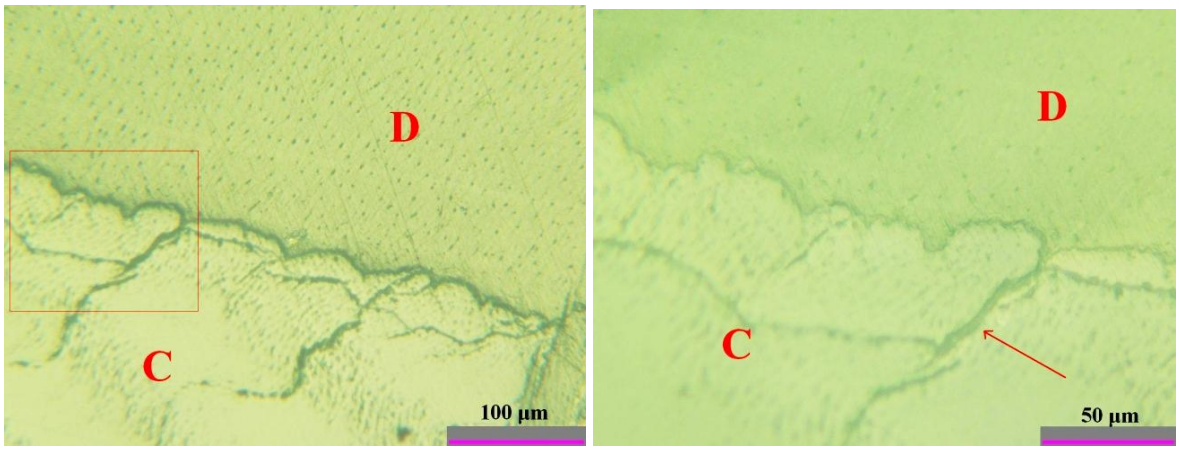


Fig.4 - Dentin (D) – composite (C) interfacial area for the same sample from group 2 (a – 100x; b – 200x)

In comparison with previous images (fig.3) the mechanical rotation adhesive application on dentin surface would result in a more uniform contact between composite and dental tissue. However, and in this case polymerization shrinkage occurs (fig.4a), the composite curing leading to the appearance of cracks into composite resin that extend from the hybrid area to the inner zones, with lengths ranging from 50 to 150 µm (fig.4b, arrow).

Using clear acetate crowns for composite attachment to the dentine led to specific specimens. On the section of the dentin-adhesive interface we noticed a relatively uniform contact between the two structures, both at a 100x magnification (fig.5a) and at 200x (fig.5b). We assume that is the result of exercising a constant and relatively uniform pressure through the acetate crown to the composite mass during light curing.

***Group 3**: interface dentin-composite*
(manual brushing adhesive application and composite with clear acetate crown)

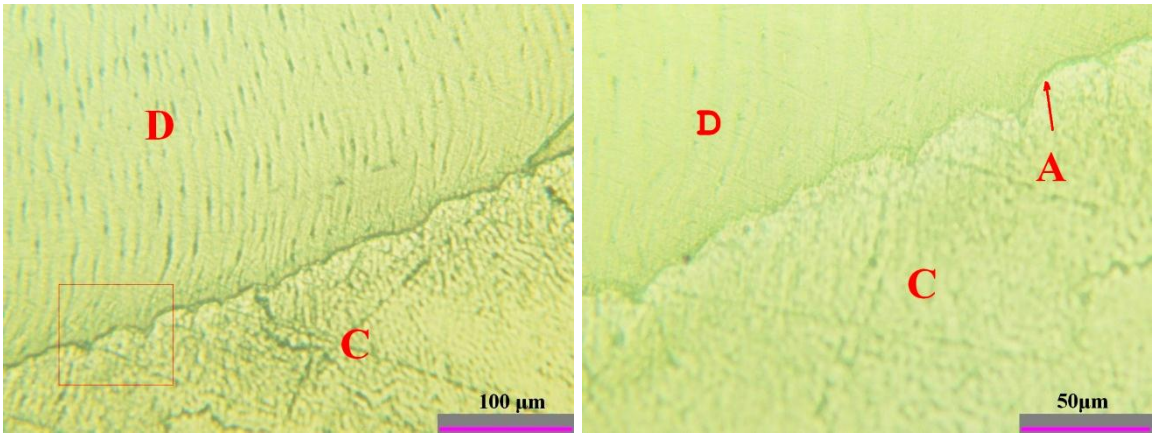


Fig.5 - Dentin (D) – composite (C) interfacial area for the same sample from group 3; adhesive (A) (a – 100x; b – 200x)

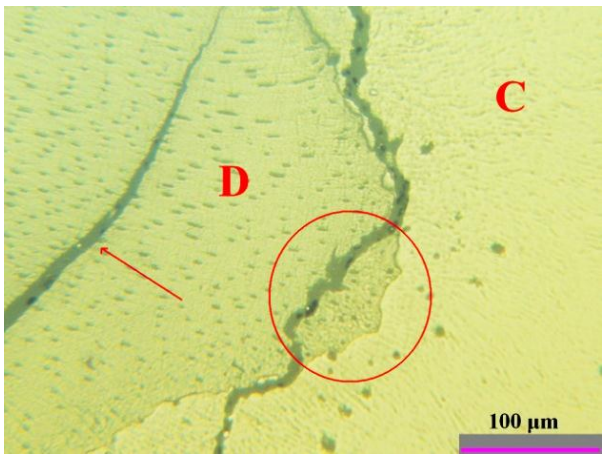


Fig.6 - Dentin (D) – composite (C) interfacial area with dentin fracture (100x)

In fig.6 we present a particular dentin-adhesive interface, also from the group 3.

In this picture there is a win-ding dehiscence due to polymerization contraction even we had used the acetate crown. Into the marked area (fig.6, circle) is visible a dentin detached zone. The arrow shows a dentin fracture.

In this case, microscopic pictures show both at the 100x magnification (fig.7a) and of 200x (fig.7b, arrow) a relative homogeneity of the dentin-adhesive interface, and of the composite mass.

This fact can lead to the assumption that the new technique for adhesive applying (mechanical rotation) could determine a more uniform distribution of the adhesive on the dentine than the classical brushing. From the analyzed optical microscopy ima-ges we can conclude that the combi-nation between rotation technique and acetate crowns may lead to more effi-cient composite systems.

***Group 4**: interface dentin-composite*
(mechanical rotation adhesive application sand composite with clear acetate crown)

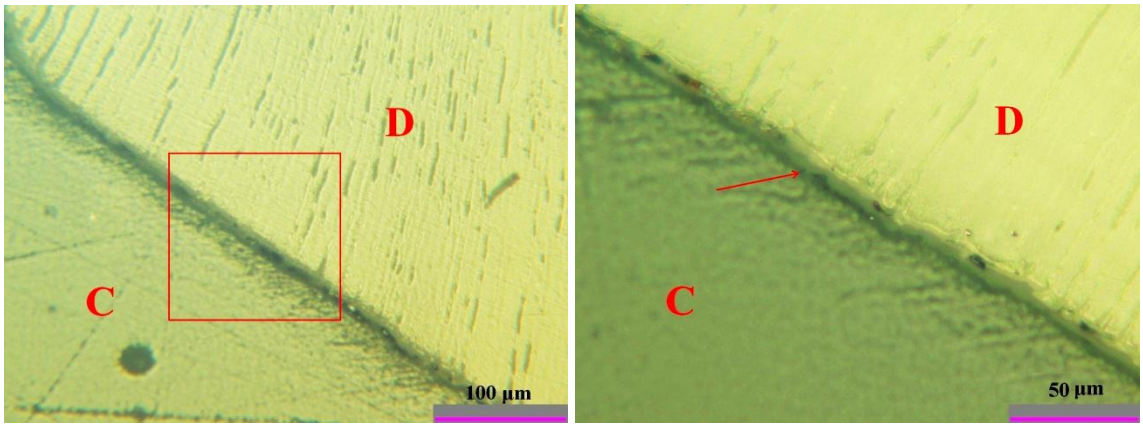


Fig.7 - Dentin (D) – composite (C) interfacial area for the same sample from group 4; (a – 100x; b – 200x)

DISCUSSIONS

Adhesion to dentin involves the formation of a hybrid layer, with quality properties proportionally to the strength of adhesion and sealing capacity[3]. Among the methods mentioned in literature in order to reduce adhesives technical sensitivity are recommended: dry adhesion, the use of acid monomers dissolved in primers containing water-HEMA, etc.[4, 5, 8]. We believe that our results could be included in this category.

CONCLUSIONS

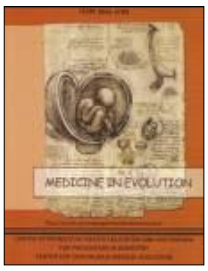
For dentin, the mechanical rotation application had better results than the manual adhesive application. At least, in terms of optical investigation, the hybrid layer has shown a more homogeneous and evenly distributed structure. This fact has led us to the idea that this method can provide some composite systems (composite constructions) with enhanced features, especially when is used with clear acetate crowns for the resin manipulation. On the other hand, we want to emphasize that often composite construction is realized on a very fragile dental substrate, during dental tissue preparation. In our opinion, we consider the optical microscopy images as an argument for using dental composite materials for various purposes, with different techniques, to prevent resistance failures.

REFERENCES

1. Anusavice, KJ, Phillips' science of dental materials, WB Saunders co, Philadelphia, 1995,
2. Bratu, D, Mikulik, L, Munteanu, D, Tehnici adezive în stomatologie, Ed. Facla, Tim., 1982,
3. De Munck, J, Van Landuyt, K, Peumans, M, Poitevin, A, Lambrechts, P, Braem, M, Van Meerbeek, B, A critical review of the durability of adhesion to tooth tissue: methods and results, J Dent Res, 2005, 84(2), 118-132,
4. Gwinnett, AJ, Chemically conditioned dentin: a comparison of conventional environmental scanning electron microscopy findings, Dent Mater, 1994, 10, 150-155,
5. Inoue, S, Vargas, M, Abe, Y, Yoshida, Y, Lambrechts, P, Vanherle, G, et al., Microtensile bond

strength of eleven contemporary adhesives to dentin, J Adhes Dent, 2001, 3, 237-245,
6. Nakabayashi, N, Kojima, K, Masuhara, E, The promotion of adhesion by the infiltration of monomers into tooth substrates, J Biomed Mater Res, 1982, 16, 265-273,
7. Söderholm, K-J, Critical evaluation of adhesive test methods used in dentistry, J Adhes Sc and Tech, 2009, 23, 973-990,
8. Van Meerbeek, B, De Munck, J, Yoshida, Y, Inoue, S, Vargas, M, Vijay, P, Van Landuyt, K, Lambrechts, P, Vanherle, G, Buonocore memorial lecture. Adhesion to enamel and dentin: curreent status and future challenges, Oper Dent, 2003, 28, 215-235,
9. ***, Dental materials – Testing of adhesion to tooth structure, Technical specification, ISO/TS 11405, 2003,
10. ***, ANSI/ADA specification no. 27, 1993/2006.

THE ELECTROMYOGRAPHIC EXAMINATION IN ANGLE CLASS II MALOCCLUSIONS



RALUCA ADRIANA BALAN[1], DANA CRISTINA BRATU[1], MAGDA LUCA[1], ROMINA BITA[2], ELISABETA BRATU[1]

1. **University of Medicine and Pharmacy "Victor Babes" Timisoara, Faculty of Dental Medicine, Department of Pedodontics- Orthodontics;**
2. **University of Medicine and Pharmacy "Victor Babes"Timisoara, Faculty of Dental Medicine, Department of General Medical and Dental Radiology and Imaging.**

ABSTRACT

***Aim**: The study presents an analysis of muscular disorders of the masticators and facial muscles produced by Angle class II malocclusions, using electromyographic methods. We observed the muscular activity by electromyographic means in rest position and maximal contraction. The analyzed muscles are temporalis, masseter, mylohyoid, buccinator, orbicularis oris and mentalis.*

Our study is based on 50 subjects (19 boys and 31 girls), with ages between 6-18 years, who have Angle class II malocclusions.

The subjects in the witness set present the normal values and also variations according to the tested muscles according to age. The values were compared with the ones obtained from the subjects in the active set. The results gathered through the analysis of amplitude, and duration and type of feeder, were calculated by using mathematical and bio statistical assessments.

These data has been supported by the dynamic of growing processes in children, combined with the casual factors that lead to malocclusions.

***Keywords:** electromyography, Angle class II malocclusions, muscular imbalances, maximal contraction*

Correspondence to:

Bălan Raluca Adriana MD
Adress: University of Medicine and Pharmacy "Victor Babes" Timisoara, Faculty of Dental Medicine, Department of Pedodontics and Orthodontics, Timisoara
E-mail address: raluca_balan22@yahoo.com

INTRODUCTION

The studies recording to the etiopathogeny of malocclusions show that most orthodontic syndromes find their origin in muscular imbalances. A disorder levelled at one element of the masticator system may lead to important changes in all the component parts of this particular system. The occlusal stability is directly connected with the neuromuscular closing trajectory of the mandible.

During the treatment, the clinician must solve both dento-skeletal and neuro-muscular system disorders. In order to achieve these desiderates, we must remove the etiologic factors that have led to malocclusions. In most of these cases, the risk of relapse after treatment closing has at its origin the muscular activity[1]. The electromyographic investigation represents the only method that can diagnose disorders at muscular level, and it uses analysing parameters such as: amplitude, time, frequency and types of closing or opening neuromuscular trajectories. The patient should be examined with this method before and after the orthodontic treatment, in order to have the possibility of determining muscular disorders, and their evolution during therapy[2]. The stability of the results depends directly on this evolution after orthodonthic treatment and after ending of the growing period at dento-maxilar level.

MATERIALS AND METHODS

Research has been conducted using a total of 50 children aged between 6 and 18 years, 38% males and 62% females. They were divided into 2 groups, as following: a control group of 24 children with normal occlusal relationships, 10 females and 14 males; and an activ group of 26 children with Angle class II malocclusion, 10 males and 16 females. Prior to orthodontic diagnosis, specific investigations (X-ray, diagnose casts, photographic documentation and electromyographic analysis) were performed.

The orthodontic diagnosis includes skeletal and alveolar discrepancies, dental anomalies, occlusal disturbances [3], muscle pain and dental dystrophies. The active children group presents Angle II/1 malocclusion in a 59, 6% percentage, and 40, 4 % Angle II/2.

The electromyographic examination was realised by detection of maximal contraction of facial and masticatory muscles: the anterior portion of the temporal muscle, the superficial portion of masseter, the mylohyoid, buccinators muscles, orbicularis oris and mentalis. The explored areas of these muscles were chosen and performed in elected points and zones in specific muscular areas, bilateral [4].

To achieve maximum contraction of the examined muscles, several tests were effectuated for obtaining voluntary contraction [5]:

Maximal intercuspidation for testing the temporal muscle and the maseter, everse of upper lip for the mylohioidian muscles, forced smile for the buccinators, whistling for orbicularis oris and lifting of lower lip over the upper lip for the contraction of mentalis muscle. The selection of electric bio potentials from the muscular contractions was realised with the use of flat silver electrodes, and the result is named global electromyography. This study used the device Medicor electromiograph [6].

RESULTS

In resting position of the mandible, the masticator and facial muscles show no electric representation.

In maximal contraction, the electric bio potentials depend on the tested muscle, on the age of the subject and have not been changed by the sex of the subjects. Using these measurements, we were able to establish the normal limits of electric potentials, depending on the type of muscle that is in contraction (Table I).

Table 1 The amplitude values of electric potentials at witness lot

Variables	M_p	*Standard deviation*	*Minimal values*	*Maximal values*
AT	158,33	±42,26	100	200
AM	152,71	±46,51	100	200
AMH	63,33	±18,59	40	90
AB	73,75	±18,01	50	100
AO	114,79	±23,24	90	145
AMS_1	227,50	±21,52	200	260

The amplitude of electric potentials varied according to the growing stage of each subject (6-9 years, 10-13 years, and 14-18 years). This amplitude increases, linked directly to the specific stages of somatic development and dental development (Table II). The clinical symptoms of malocclusion examined electromyograpically led to the founding of other significant results: When the skeletal malocclusion was moderate or minor, the muscular imbalances were found to be severe. When the clinical symptoms show severe gravity, the muscular imbalances were found to be minor [7].

Table 2– Average values of electric potentials at witness lot, age groups

Variables	***6-9 years, n =16***		***10-13years, n=16***		***14-18 years, n=16***	
	M_p	***Standard deviation***	M_p	***Standard deviation***	M_p	***Standard deviation***
AT	135,63	±33,96	168,57	±36,71	174,44	±47,46
AM	115,62	±46,09	163,57	±37,27	173,33	±35,71
AMH	47,50	± 4,63	69,29	±18,13	74,44	±18,78
AB	63,13	±12,23	86,43	±18,42	73,33	±17,14
AO	112,50	±18,90	115,86	±28,26	118,33	±24,87
AMS_1	220,00	±22,04	225,71	±20,70	235,56	±21,28

DISCUSSION

The malocclusions are composed by dental, skeletal, occlusal and functional disorders [8]. These disorders appear in the neuro-muscular growing process of the maxilo-facial complex. First changes are detected by the neuromuscular system that announces the gravity of the future malocclusion [9]. The resting position of the mandible is determined especially by the tonic activity of the temporal muscles. In those cases of Angle class II malocclusions, the electric activity of temporal muscles has electromyographic representation in the mandible resting position [10]. Orbicularis oris and mentalis help an-

terior closing of the oral cavity, when the lips are incompetent because of the skeletal disorders and the position of the frontal teeth [11]. These muscles present an abnormal tonic shape in Angle II malocclusions in rest.

The activity of facial and masticator muscles is influenced by dento-alveolar changes and by the developmental stage of occlusion, affecting the contractibility of the surrounding muscles, as well as the movements and the activity of the mandible [12].

The contraction potential of the raising muscles of mandible is controlled by the feedback of the periodontal receptors.

The activity of facial muscles is being influenced by the incisive occlusion, which determines soft tissue adaptation on the modified dento-alveolar support [13]. The malocclusions determine changes in muscular adaption, inducing new ways of closing of the mandible and also new reflexes. This is the reason that leads to the idea that no orthodontic treatment is 100% successful, until the muscular disorders are analysed and solved 14, because it is very important to create a harmony between the skeletal complex and the surrounding muscular complex.

CONCLUSIONS

In malocclusions, the dental and skeletal modifications determine quick follow-ups. It appears as a vicious circle, in the activity of the compounding elements of the dento-maxilar complex, which sustain each other in a destructive way, having base on the relationship cause and effect. When the adaptation of function to form is made in a short period, the muscular disorders become important. When the adaptation is made over a long period of time, the muscular fibres rearrange, and the activity of the muscles is being found in an unstable balance [15]. The muscular disorders, analysed by electromyography, is becoming a necessity investigation in order to achieve a long-term successful therapy.

Every day new cases add new questions for the clinical approach, because of the compensatory adaptation of the surrounding muscles, considered to be an answer to the multiple modifications that occur in malocclusions.

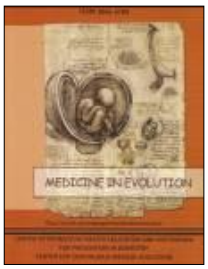
The therapy and correction of malocclusions, with the re-establishment of the muscular balance is a must for the stability of the orthodontic treatment, headed long way.

REFERENCES

1. Gomes AS, Lima EM. Mandibular growth during adolescence. Angle Orthod. 2006; 76(5):786-790
2. Proffit W.R., The facial musculature in its relation to the dental occlusion in muscle adaptation in the cranio-facial region, Edit Carson and McNamara, Michigan, 1978
3. Azarbal M. Comparison of Myo-Monitor centric position to centric relation and centric occlusion. J Prosthet Dent 1977; 38(3):331-77.
4. G. Zegan, S. Hamwi, G Georgescu, Electromiografia in anomaliile dento-maxilare. Semiologie si diagnostic, Chisinau: Edit. Tehnica 1998.
5. G. Zegan, Activitatea muschilor masticatori si oro-faciali la copii cu malocluzii. Studiu electromiografic. 2001
6. Jankelson B. Neural conduction of the myo-monitor stimuls: A quantitative analysis. J Prosthet Dent 1975
7. Dinham G.A. Myocentric a clinical appraisal. Angle Orthod. 1983vol 54, 211-217

8. Bishara SE. Mandibular changes in persons with antreated and treated Class II division1 malocclusion. Am J Orthod. Dentofacial Orthop 1998; 113(6):661-73
9. Monaco A, Cattaneo R, Spadaro A, Marzo G. Neuromuscular diagnosis in orthodontics: effects of TENS on the sagittal maxillo-mandibular relationship. Eur J Paediatr Dent 2008; 9:163-169.
10. Aggarwal P, Kharbanda OP, Mathur R, Duggal R, Parkash H. Muscle response to the twin-block appliance: an electromyographic study of the masseter and anterior temporal muscles. Am J Orthod Dentofacial Orthop 1999;116:405-414
11. Hiyama S, Ono PT, Ishiwata Y, Kuroda T, McNamara JA, Jr. Neuromuscular and skeletal adaptations following mandibular forward positioning induced by the Herbst appliance. The Angle orthodontist 2000; 70:442-453.
12. Cattaneo R, Monaco A, Serafini V, Giannoni M, Birth Delivery Trauma and Malocclusion. J Clin Pediatr Dent 2005;12:123-130
13. Carels C, Van Steenberghe D. Changes in neuromuscular reflexes in the masseter muscles during functional jaw orthopedic treatment in children. Am J Orthod Dentofacial Orthop 1986; 90:410-419.
14. Jankelson B: Functional position of occlusion. J Prosthet Dent. 1973 Oct: 30(4)
15. Jankelson Bernard: Neuromusccular aspects of occlusion Dental Clinics of North America 1979.

A DIGITAL TECHNIQUE FOR SMILE ANALYSIS AND DESIGN-DIGITAL MOCK-UP



ANA PETRE[1], COSMIN CIRCU[2]

1. **Oral Rehabilitation Department, University of Medicine and Pharmacy "Carol Davila", Faculty of Dentistry, Bucharest, Romania**
2. **Oro-dental Prevention Department, University of Medicine and Pharmacy "Carol Davila", Faculty of Dentistry, Bucharest, Romania**

ABSTRACT

Introduction: *Aesthetics has become a common demand in dentistry. As practitioners, we have to understand harmony, beauty, proportion, and symmetry when planning treatment. In Digital Era the analysis of patient's smile and the treatment plan could be done in digital mode.*

Method: *The Aesthetic Smile Criteria and usual software as Microsoft Power Point® were used for developing a method that simplifies the Smile Correction Protocol. As an example, we choose a simple clinical case of a lateral upper incisor situated in wrong position. The result leads us to the correct treatment plan.*

Discussions: *Due to this digital technique we can detect in an accurate way the needful changes of patient's smile and we are able to provide clinical precise information, useful for the dental technician in order to produce the ideal restoration.*

Conclusions: *The use of computer in Smile Design process permits us to achieve better results and to avoid any misunderstanding that can occur during the communication process with the dental laboratory.*

Key words: *smile design, perfect smile, software, aesthetic, digital mock-up*

Correspondence to:

Petre Ana MD
Adress: University of Medicine and Pharmacy "Carol Davila", Faculty of Dentistry, Bucharest, Romania, Oral Rehabilitation Department
Phone: 0722670397
E-mail address: ana.petre@dental-studio.ro

INTRODUCTION

Aesthetics in Dentistry has won its well-deserved place relatively late. Starting with Ronald E. Goldstein in 1976 [2], a number of authors consider that dental aesthetic plays a key role in complete dental treatments and they have developed a set of rules. Using the general criteria of"beauty", experts in Dental Aesthetic have succeeded in standardization of the characteristics of "the perfect smile" with all its components: lips, attached gingiva and teeth [3]. Each of them has a few variables that must be analyzed and corrected in order to achieve the"perfect smile". Usually, we achieve this goal by an analogue analysis of patient clinical features, but nowadays, due to the continuous evolution of Dentistry, dental treatments have to involve some digital features. The availability of digital photography permits digital estimation of existing smile features and an easier way of planning the dental treatment; actually we are able to obtain a"digital mock-up".

AIM AND OBJECTIVE

Our aim is to provide a simple digital method that could ease and specify the Smile Correction Protocol. Due to this technique, the doctor is able to send to the technician directly the "*blue-prints*" of the patient's "*new smile*".

MATERIALS AND METHODS

The Aesthetic Smile Criteria and usual software were used for this process developing. To exemplify the technique, we present a clinical case with one right lateral upper incisor in wrong position due to a small mesio-buccal rotation and a lingual inclination (Fig.1).

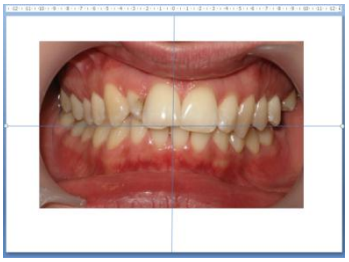


Fig.1 Initial patient's photo

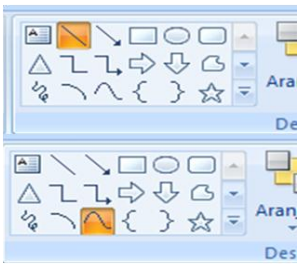


Fig. 2 Line and curvature tools

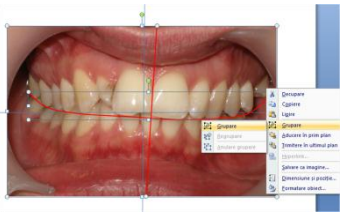


Fig. 3 Midline and incisal curvature

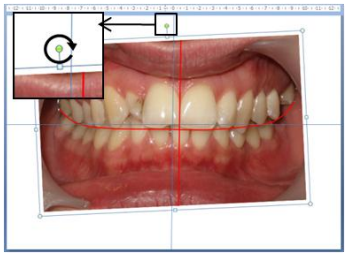


Fig.4 Rotation tool

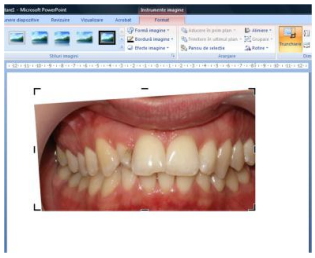


Fig.5 Crop tool

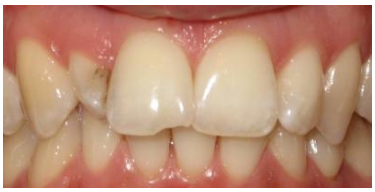


Fig.6 Final pictures

The digital picture of smile area that must be restored is opened with Microsoft Power Point® or similar software. The patient's photo must be checked if is well orientated in both, horizontal and vertical axis-blue lines: facial midline parallel with vertical axis and incisal curvature symmetric to horizontal one. Small rotations can be fixed with draw tools. "Line" and "curvature" tools (Fig.2) are used to mark midline and respectively incisal curvature-red lines (Fig.3). The marked image is all selected and „group". The new image can be rotated using „rotation" tool until the red lines are parallel with blues ones (Fig.4). In the next step we have to „un-group" the ensemble and delete the lines red and blue. The image is saved as pictures and after that the crop tool resizes the picture in correct position (Fig.5). Further, the final picture (Fig.6) is inserted in new slide and with "freeform" tool (Fig.7) we copy the shape of the other lateral incisor, which is in right position (Fig. 8). The new created form is duplicated (Fig.9) and positioned after "horizontal flip"(Fig.10) over the image of right upper lateral incisor (Fig.11).

Filled form shows us the ideal shape of the lateral incisor and the marginal line gives us information about its ideal contour (Fig.12).

Finally, both images are saved as pictures and analyzed.

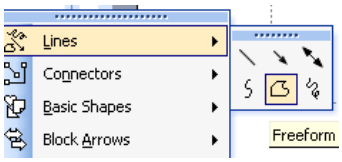


Fig.7 Freeform tool

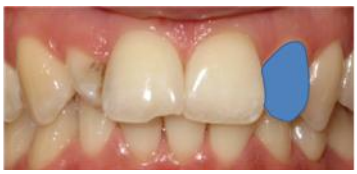


Fig.8 Filled form

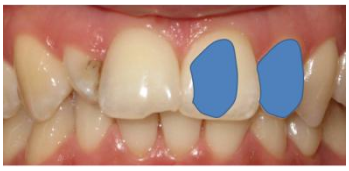


Fig.9 Duplicated form

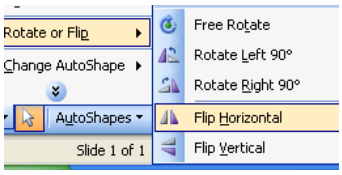


Fig.10 Flip tool

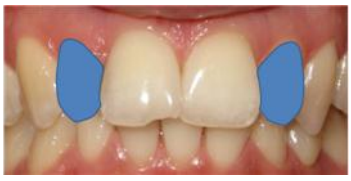


Fig.11 First shape copied end horizontal fliped

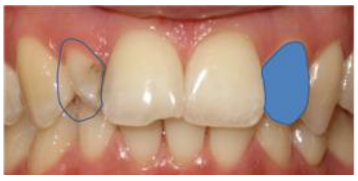


Fig.12 The ideal contour of lateral right incisor

RESULTS

Some of worldwide accepted aesthe-ic criteria [3] are used to analyze the smile: facial midline, gingival embrasures, dental axis, gingival zenith, gingival contour, contact points position, incisal edge line and incisal curvature. All these elements have to be symmetrical to midline and horizontal line (Fig.13). In this particular clinical case, we can observe that, due to the modified position, the appearance of 12 is different from its homologue. The gingival contour is situated in a lower position and the visible contour is significantly smaller affecting the incisal and buccal curvature. It is obvious that the incisal edge of 11 is also affected and a composite direct restoration could easily solve this, but it will be addressed with other occasion.

Regarding to "digital mock-up", in this simple way it can be detected what changes we have to operate in order to obtain an aesthetic smile: small gingival gingival recontouring and a ceramic veneer with shape similar to

22. This technique would improve the communication with technicians who usually build the wax-up on gyps model without any clinical information. By sending these pictures to dental technician we provide very important and exact information about patient's clinical features.

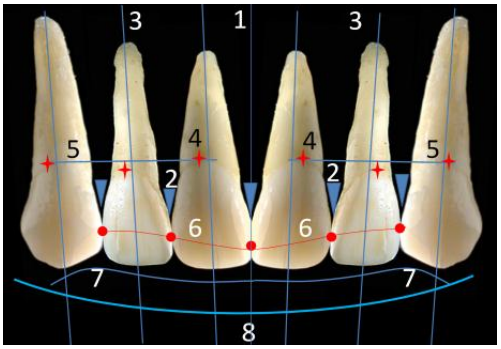


Fig. 13. The facial midline (1), the gingival embrasures (2), the tipping of dental axis (3), the gingival zenith (4), the gingival contour (5), the contact points(6), the incisal edge line (7), the incisal curvature (8)

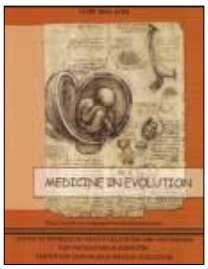
CONCLUSIONS

The utilization of computer in Smile Design process permits us to achieve better results from aesthetic treatments. The Smile Analysis process is less difficult and more accurate. All the information are easily stored, processed and archived. Moreover we could avoid any misunderstanding that can occur during the communication with the dental laboratory.

REFERENCES

1. Geissberger M., Esthetic Dentistry in Clinical Practice,Wiley- Blackwell, Ames 2010, ISBN: 0813828252:9-17
2. Levin El., Dental esthetics and the golden proportion,. J Prosthet Dent. 1978 Sept; 40(3): 244-52
3. Magne P, Belser U, Restauraciones de Porcelana Adherida en los Dientes Anteriores:Método Biomimético, Editorial Quintessence, S.l., Barcelona, 2004:70-2

FRONTAL CEPHALOMETRIC INVESTIGATION IN FACIAL ASYMMETRY RESEARCH



OLIVIA POPOVICIU[1], VIORICA MILICESCU[1], RUXANDRA MATEI[2], ELINA TEODORESCU[1], ECATERINA IONESCU[1]

1. **Department of Orthodontics and Dento-facial Orthopaedics, UMF "Carol Davila" Bucharest**
2. **Department of Dental Medicine II, University of Oradea**

ABSTRACT

Early detection, during growth, of transverse skeletal imbalances of the maxillofacial complex and adequate orthodontic and orthopedic correction contributes to resumption of balanced orthodontic and skeletal development, muscle and functional reequilibration, consecutively reducing the impact of asymmetric facial development on oral health and facial harmony.

Our research objective is to assess the degree and features characterizing skeletal facial asymmetry, potential causality and correlation between asymmetry and malocclusion type or rotational pattern of facial growth, through the examination of the frontal cephalomgrams of a group of growing patients, with moderate and severe malocclusions.

Transverse skeletal asymmetry was examined by evaluation of median landmarks (upper and lower incisale and menton) deviation from the midline and the interpretation of the zygomatic, mastoid, maxillary and antegonion asymmetry indices.

Among numerous clinical and imaging procedures used to assess facial asymmetry, cephalometric measurements allow precise quantification of the involvement of various skeletal areas. The frontal cephalometric investigation contributes to the estimation of the degree of "camouflage" provided by the development the soft tissues, in view of optimizing therapeutic outcomes from the anatomical, functional and aesthetic perspectives.

***Key words**: frontal cephalogram; skeletal facial asymmetry.*

Correspondence to:

Popoviciu Olivia MD
Adress: Street: Eforie nr 4-6, et. 2, cab 3, Bucureşti
Phone: 0723254432
E-mail address: olivia_popoviciu@yahoo.com

INTRODUCTION

Skeletal structures undergo continuous development and changes during growth, principally induced by functional forces, with significant contributions of muscle activity, nutrition, dental and periodontal lesions, clinical signs of general diseases [1-3]; the location, direction and degree of the asymmetrical development constitute valuable indications in understanding the etiology of facial asymmetry, essential for diagnosis and treatment planning of patients with malocclusions [4, 5]. Genetic, environmental or combinations of these factors are incriminated in the occurrence of right / left variations of the growth rate [6-8].

Functional imbalances suffered during growth can lead - in search of new balance - to structural alterations with disruption of harmonious facial development.

Early detection of imbalances in growth and consequent orthodontic re-equilibration contribute to resumption of balanced skeletal development, functional and muscle equilibrium, consecutively reducing the impact of asymmetric development on oral health and facial harmony.

The evaluation of the facial asymmetry includes various clinical and imaging procedures, among which cephalometric measurements allow precise quantification of the involvement of various areas of bone.

Although the value of frontal cephalometry is often asserted, especially in conjunction with facial asymmetry, skeletal inter-maxillary discrepancies and cleft lip and palate, the frequency with which it is recommended - as reflected in published studies - appears to be reduced [9, 10].

AIM

The literature offers no uniform criteria for defining asymmetry. The stage at which asymmetry becomes pathological is not exactly measurable, as clinical parameters should be evaluated in relation to aesthetics and functionality. Our research aims to assess the degree of skeletal asymmetry in a group of growing patients with moderate and severe malocclusions increase of malocclusions and to examine whether the asymmetry depends on certain developmental features as facial growth pattern or type of malocclusion, whether it is anatomical or functional.

MATERIALS AND METHODS

Initial records of 158 patients who started treatment in the Department of Orthodontics and Dentofacial Orthopedics, University of Medicine and Pharmacy "Carol Davila" in Bucharest, between years 2006-2010 were investigated in the preliminary stage our research. Of these patients, the research sample consisted of 26 children aged 8-14 years, 14 female and 12 male, with malocclusions with a dissymmetrical component, whose orthodontic records included frontal cephalomgrams prior to treatment start.

To assess the asymmetric craniofacial characteristics, for the current study we selected and marked on the frontal cephalomgrams a set of landmarks that provide useful and reproducible information [11] - five bilateral

skeletal landmarks, two median skeletal landmarks and two median dental landmarks as follows (Figures 1, 2):

- anterior nasal spine (Nsa) - the center of the intersection between nasal septum and hard palate)
- mentalis / gnathion (Me) - the midpoint of the lower border of the mandibular symphysis
- upper incisale (I) - the midpoint between the upper central incisors at the incisal margin edge
- lower incisale (i) - the midpoint between the lower central incisors to the incisal margin
- lateroorbitale (Lo) - the intersection of the lateral orbital contour with oblique line
- zygion (Zy) - most lateral and superior point of the zygomatic arch
- mastoidale (Ms) - the lowest point on the contour of the mastoid process
- maxilare (Mx) - intersection of the lateral contour of the maxillary alveolar process with the lower contour of the maxillozygomatic process of the maxilla
- antegonion (Ag) - the highest point in the antegonial notch.

Landmarks were recorded manually on the cephalomgrams. The horizontal reference plane was tangent to the upper contours of the orbits, and the vertical reference plane (midline) was perpendicular to the horizontal reference plane through crista galli.

In consideration of evaluating median asymmetry, we measured distances from the points nasospinalis anterior (Nsa), upper incisor (I), lower incisor (i) and menton (Me) to the midline. Records were gathered considering a positive value if landmarks were displaced to the right and a negative value if displaced to the left (figure 2). Deviation of more than 2 mm of any point from the midline was considered a sign of asymmetry, as established by Severt and Proffit (1997 [12]).

We measured the distances from bilateral points zygion, mastoidale, maxilare and antegonion to the midline (figure 2), thus obtaining four pairs of horizontal variables (according to Harvold, Grummons [13, 14]).

Respective asymmetry indices were calculated using the formula:

Asymmetry index (%) = (R-L) / (R + L) x100,

- where R is the distance from the right landmark, and
- L the distance from the left landmark to the midline.

Measured and calculated values of these indices higher than 4% were recorded as a sign of asymmetry, and generally reflect a greater than 3 mm difference between the distances to the midline of the respective pair of bilateral landmarks.

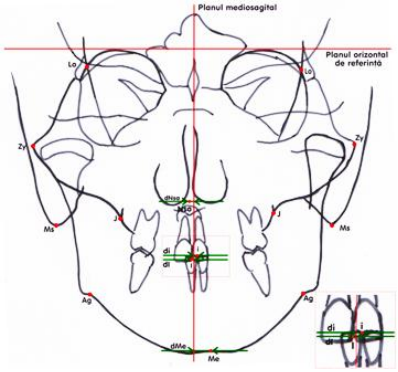


Fig. 1 Median cephalometric landmarks (Nsa, I, i, Me) and reference planes used (horizontal reference plane – tangent to the left and right upper orbital contours, mid-sagittal plane – perpendicular to the horizontal reference plane in crista galli). Horizontal measurements for median facial asymmetry evaluation (dNsa, dI, di, dMe).

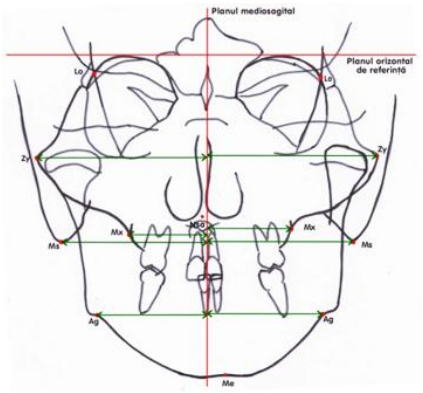


Fig. 2 Lateral cephalometric landmarks (Zy, Ms, Mx, Ag) and reference planes used. Horizontal measurements for lateral skeletal facial asymmetry evaluation, from bilateral cephalometric landmarks to the mid-saggital plane (IZy, IMs, Imx, IAg).

RESULTS

Measured and respectively calculated values of the parameters investigated, describing median and lateral transverse asymmetry for the research subjects are presented in Table I.

Table 1 Measured values for the lateral displacement of median points investigated and lateral transverse asymmetry indices calculated for the research subjects. Median displacements over 2mm and indices over 4% are highlighted.

Case number	Initials	Age	Gender	Malocclusion	Growth pattern	Frontal cephalogram – median transverse symmetry				Frontal cephalogram – lateral transverse symmetry indices				
						Nsa	I	i	Me	Index Lo	Index Zy	Index Ms	Index Mx	Index Ag
1	C.I.	8	M	II/1	h	-1	0	0	1	0,0%	-	-3,4%	-1,5%	0,0%
2	L.A.	8	F	I	N	0,5	-1	-0,5	2	-0,5%	1,8%	2,3%	2,9%	4,0%
3	M.A.	8	M	II/1	H	0	1	1	-3	0,0%	-	-5,0%	-1,6%	-4,9%
4	I.N.	9	F	II/1	H	-0,5	1	-2	-1	0,0%	-2,6%	-4,8%	-2,5%	-2,0%
5	S.A.	9	F	I	H	0	-1	4	5,5	0,0%	1,5%	1,4%	4,3%	1,7%
6	I.I.	10	M	I	N	0	0	-1	-1	0,0%	-2,3%	-3,8%	0,0%	-2,7%
7	L.I.	10	M	II/1	N	0	0	-1	4	0,5%	-0,7%	-0,8%	-2,0%	-3,8%
8	M.A.	10	F	II/1	N	-1	1,5	4	-2	0,0%	-	0,5%	-0,8%	1,7%
9	S.M.	10	M	II/2	h	1	1	3,5	1	0,0%	0,8%	0,0%	2,9%	0,6%
10	M.L.	8	F	III	H	1	0	1	0	1,0%	-2,3%	-4,7%	0,8%	-1,1%
11	B.A.	11	F	II/1	H	0	2	1,5	1	0,0%	0,4%	6,5%	0,0%	1,8%
12	C.A.	11	F	I	H	1,5	1	2	3	0,0%	-0,4%	-1,4%	-1,4%	1,7%
13	D.M.	11	M	III	h	1	1,5	1,5	-3	0,0%	-0,8%	-1,7%	3,9%	-1,1%
14	D.R.	11	F	III	N	-1	-1,5	-0,5	0	-0,5%	-	-4,7%	-1,6%	-3,5%
15	N.A.	11	F	II/1	h	0	1	1	3	0,0%	0,8%	2,8%	5,0%	2,0
16	B.R.	12	M	I	H	0	4	4	5	1,1%	0,5%	-1,6%	5,4%	7,0%
17	C.B.	12	M	I	h	0	0	1	1	0,0%	-	-0,4%	0,7%	-1,1%

18	M.C.	12	M	II/2	H	1,5	1,5	1	0	0,0%	1,5%	1,9%	-1,4%	-1,1%
19	R.A.	12	F	III	h	0	1	0	-2	-1,1%	-3%	2,3%	0,0%	1,8%
20	S.T.	12	F	II/2	N	0	0	1	-1	-0,5%	0,0%	-1,3%	0,7%	-0,5%
21	J.A.	13	F	II/1	H	0	-2	1,5	0	0,0%	0,7%	-2,9%	0,8%	-4,8%
22	M.A.	13	M	II/2	H	1	4	5	-5,5	0,0%	0,4%	-0,9%	-5,2%	-5,6%
23	R.S.	13	M	I	H	0	-2	-2,5	-5,5	-0,6%	-2,0%	-3,5%	-3,1%	-6,8%
24	I.M.	14	F	II/2	N	-2	-6	-3,5	-1	1,1%	-1,6%	-1,9%	-5,6%	-2,2%
25	M.V.	14	M	II/2	h	0	2	-1	-1	0,0%	0,0%	-2,1%	2,4%	-1,7%
26	P.M.	14	F	I	H	0	1,5	-1	3	0,0%	1,6%	4,3%	2,9%	0,0%

DISCUSSION

To assess the median facial asymmetry, we used four anthropometric landmarks established in the literature as having low identification and geometric errors: Nsa, I, i, Me, central landmarks of the maxillary and mandibular bases and of the upper and lower dental arches. Among these, chin deviation from the midline is considered to accurately describe facial asymmetry; Masuoka [15] appreciates chin deviation as the most relevant indicator related to the subjective assessment of facial asymmetry.

We recorded displacements of more than 2 mm from the midline of these points in 69.3% of the cases analyzed. Lateral chin deviation occured in 46.1% of the subjects, with amplitudes up to 5.5 mm. The amplitude at which the chin deviation becomes apparent varies from person to person, but in line with research findings by Edler [16], Haraguchi [17], van Keulen [18] who consider 4 mm as a "threshold" for the perception of asymmetry, we found that 67% of chin deviation cases in our research fall below 4 mm, and 33% between 4 - 5.5 mm.

A correlation between less than 4 mm displacement of the chin and minimal displacement - less than 1.5 mm, considered insignificant - of the the incisive points.

To differentiate between excessive or deficient unilateral development of the mandible and mandibular lateral shift and to evaluate midline symmetry of the dental arches in relation to the facial skeleton we have appreciated the importance of corroborating information obtained by evaluating median asymmetry - displacement from the midline of the central points of the jaws and of the dental arches - with the the skeletal asymmetry in the lateral area.

In our research, measurement of the distances from bilateral landmarks to the mid-sagittal plane and calculation of asymmetry indices revealed the presence of varying degrees of asymmetry between patients. We noted that the distances that characterize the upper facial area are more stable in terms of symmetry, noting symmetry preservation at the level of lateroorbitale and zygion points - 11.5% of cases displayed an above 0 value for the zygomatic index, but less than 4%. Asymmetry of over 4%, occurs at lower levels, concerning the mastoid, maxilla and mandible.

Although most research subjects presented - as measured - a degree of asymmetry greater than zero at the four levels investigated, in assessing lateral transverse asymmetry we considered only index values greater than

4%, as asymmetry of lower magnitude can also occur as a result of potential geometric, patient positioning, recording and measuring errors. Figure 3 presents the values calculated for the zygomatic, mastoid, maxillary and antegonian asymmetry indices for all subjects, patients being ordered according to the malocclusion presented.

Although statistically representative investigation was not feasible, data analysis shows that most cases of asymmetry in our sample belong to the class I and class II division 1 groups:

- 62.5% of class II division 1 subjects and 71.4% of class I presented at least one of the indices evaluated higher than 4%, as compared with 28.6% of the class II division 2 subjects and 50% of class III subjects;
- For 37.5% of class I subjects and 28.5% of class II division 1 subjects the antegonian index was higher than 4%, compared to 14.2% of class II division 2 and none of class III.

Grouping patients by age revealed no significant differences, subject's relatively symmetrical and with varying degrees of mandibular, maxillary or mastoid asymmetry existing in each age group (figure 4).

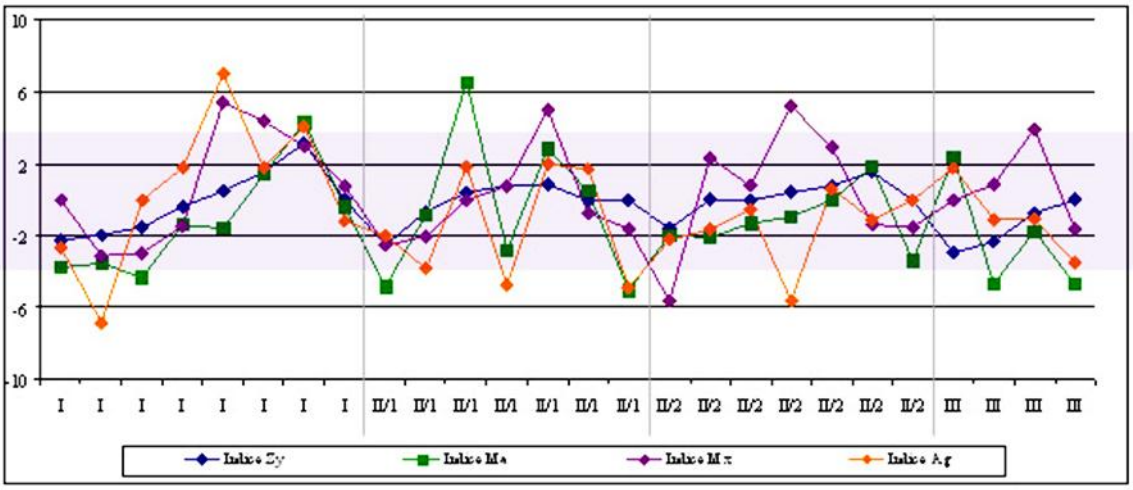


Fig. 3 Asimmetry indices calculated for all subjects, arranged by malocclusion type (n=26).

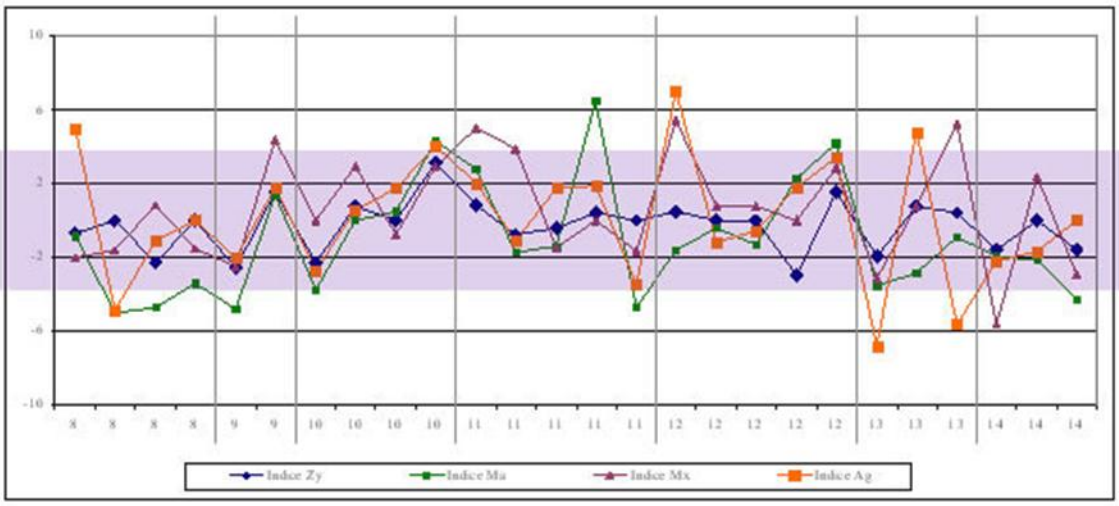


Fig. 4 Asymmetry indices calculated for all research subjects, ordered by age (n = 26).

53.8% of the research subjects had at least one of the indices evaluated over 4% - the mastoid index was over 4% in 26.9% of the cases, the maxillary index in 19.2% and the antegonian index in 23%.

In 38.5% of the sample asymmetry concerned significantly one of the investigated landmarks (19.2% antegonion, 11.5% maxilare, 7.7% antegonion), while for 15.4% of subjects two landmarks were involved in asymmetry (maxilare and antegonion - 7.7%, mastoid and antegonion -7.7%).

Transverse disproportion is discernible, even for people with harmonious or clinically acceptable facial appearance, through the majority of the asymmetry indices [8, 19-21]. Among various indices described for facial symmetry analysis, the ones depicting structures of the lower face present higher degrees of asymmetry compared to those of the upper structures [12, 15, 19], indices concerning mandibular development being particularly disturbed.

Proffit [12, 22] states that facial asymmetry affects 25-34% of the population, 74% of facial asymmetry being detected in the area of the lower jaw. In the group of children we studied the higher incidence of lateral facial asymmetry - 53.8% of subjects, by at least one of the calculated indices greater than 4% - is most likely related to sample composition, as they represented a population with clear need for orthodontic treatment.

Literature reveals the dominance of the right [20, 23] or left [24] side of the face, dominant side variation with growth [16] or lack of dominance. In our research, of the subjects with asymmetry indices over 4%, we recorded larger development of either side of the face in a ratio of right: left = 43: 57, proportion that does not permit assessment of either side dominance.

Assessment of asymmetry indices gives a measure of the degree of asymmetry in the lateral areas of the maxilla and mandible, without differentiating unilateral excessive or deficient growth of the mandible from midline shift or asymmetrical volume changes.

To assess the correlation between lateral and midline mandibular asymmetry we selected, from the research sample, the subjects who fulfilled at least one of the criteria: 3 mm or more chin deviation or 4% or more antegonian index. 46.1% of subjects analyzed were within these criteria.

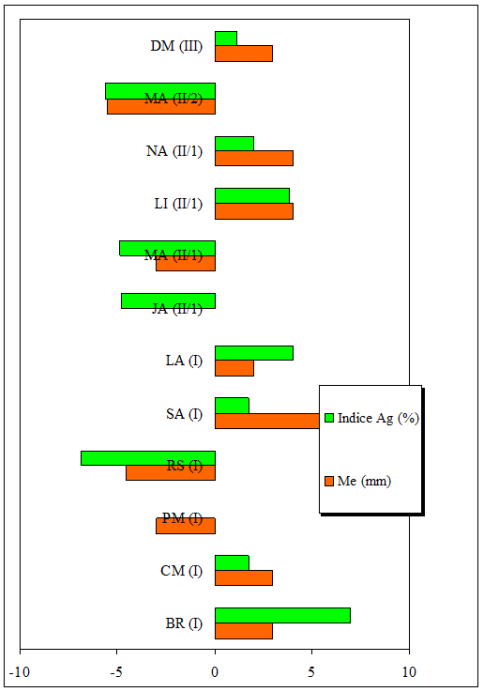


Fig.5 Chin deviation from the midline (in millimeters) and antegonial asymmetry index for subjects with either IAg≥4% or dMe≥3mm, ordered by malocclusion type.

Antegonian index and chin deviation values for these subjects, ordered by malocclusion type, are shown in figure 5. Most of these subjects - with significant changes in perceived mandibular level - are grouped in hiperdivergent class I (50%) and class II division 1 (33%).

Disturbances observed are a consequence of mandibular asymmetric development by unilateral hyperplasia of the mandibular body in 11.5% of the subjects.

In 7.7% of the cases the observed mandibular asymmetry is adaptative, due to transverse asymmetry of the upper jaw and possible asymmetries of the glenoid cavities, as the two halves of the mandibular body are proportionately developed.

For 19.2% of the research subjects the mandibular asymmetry detected is a consequence of the functional shift of the mandible due to crossbites and vicious postural attitudes, although in 40% of these subjects (aged over 12 years) the long-term evolution of the mandibular shift caused anatomical changes - as left-right mandibular dimensional differences were detected and functional examination revealed limitations in centering the jaw.

CONCLUSIONS

As regards facial aesthetics during the skeletal growth period, we found that lateral skeletal asymmetry, although detectable following careful inspection has a lesser impact on the aesthetic perception of facial asymmetry, probably due to the well developed soft tissues characteristic to younger subjects.

Median skeletal asymmetries - mainly chin deviation from the midline - are mostly obvious and immediately detectable, with ongoing visual impact, while dental asymmetries have a reduced effect on facial harmony, upper dental midline shift being inconsistently observed during speech and smile.

A significant aesthetic impact was observed for asymmetries of lesser amplitude than minimum values considered in the study, but of opposite directions on the upper and lower jaws, indicating the need to also assess the amplitude of the asymmetry between the maxilla and mandible.

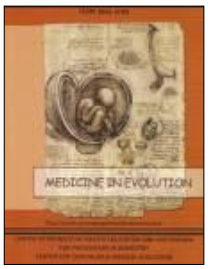
Significant facial asymmetry analysis benefits, in addition to clinical examination and photographs, from frontal cephalometric investigations, that enhance the accuracy of skeletal asymmetries assessment, while allowing estimation of "camouflage" given by the soft tissues' development, with a view to optimize therapeutic outcomes and functional anatomy, and aesthetic. Systematic evaluation of cases with facial asymmetry requires symmetry analysis along lateral contours and at the midline level, at both skeletal and soft tissue levels.

REFERENCES

1. Firu, P. Stomatologie infantila, Bucuresti: Editura Didactică şi pedagogică, 1983.
2. Enlow, D H, Hans, M G. Essentials of facial growth, 2nd. Ann Arbor, Michigan: Needham Press, Inc, 2008.
3. Ionescu, E, Duduca Milicescu, I, Popescu, M, Popoviciu, O, Milicescu, V. Ortodonţie şi ortopedie dento-facială, Bucureşti: Editura Cerma, 2001.
4. Milicescu, V, Milicescu, I D. Creşterea şi dezvoltarea generală şi cranio-facială la copii, Bucureşti: Editura Viaţa Medicală Românească, 2001.
5. Teodorescu, E. Diagrama-reţea în analiza craniofacială tridimensională, Bucureşti: Editura Viaţa Medicală Românească, 2010.
6. Lundström, A - Some asymmetries of the dental arches, jaws, and skull, and their etiological significance. Am J Orthod 1961; 47(2): 81-106.
7. Mulick, J F - An investigation of craniofacial asymmetry using the serial twin study method. Am J Orthod 1965; 51112-29.
8. Melnik, A K - A cephalometric study of mandibular asymmetry in a longitudinally followed

sample of growing children. Am J Orthod Dentofacial Orthop 1992; 101(4): 355-66.
9. Gottlieb, E L, Nelson, A H, Vogels, D S - 1990 JC0 study of orthodontic diagnosis and treatment procedures, part 1: Results and trends. JCO 1991; 25(3): 145-56.
10. Teodorescu, E, Ionescu, E, Milicescu, V, Paun, R - Statistical data regarding the facial asymmetries. Revista Medico-chirurgicală a Societăţii de Medici şi Naturalişti, Iaşi 2008; 112 (1 suppl 1): 443-8.
11. Major, P W, Johnson, D E, Hesse, K L, Glover, K E - Landmark identification error in posterior anterior cephalometrics. Angle Orthod 1994; 64 (6): 447-54.
12. Severt, T R, Proffit, W R - The prevalence of facial asymmetry in the dentofacial deformities population at the University of North Carolina. Int J Adult Orthodon Orthognath Surg 1997; 12(3): 171-6.
13. Harvold, E P, Trugue, M, Viloria, J O. Establishing the median plane in posteroanterior cephalograms. In: Salzmann JA, ed. Roentgenographic cephalometrics 2nd ed. Philadelphia: J. B. Lippincott Co, 1959.
14. Grummons, D C, Kappeyne van de Coppello, M A - A frontal asymmetry analysis. JCO 1987; 21(7): 448-65.
15. Masuoka, N, Momoi, Y, Ariji, Y, etc. - Can cephalometric indices and subjective evaluation is consistent for facial asymmetry? Angle Orthod 2005; 75(4): 651-5.
16. Edler, R, Wertheim, D, Greenhill, D - Clinical and computerized assessment of mandibular asymmetry. Eur J Orthod 2001; 23(5): 485-94.
17. Haraguchi, S, Takada, K, Yasura, Y - Facial asymmetry in subjects with skeletal class III deformity. Angle Orthod 2002; 72(1): 28-35.
18. Van Keulen, C, Martens, G, Dermaut, L - Unilateral posterior crossbite and chin deviation: is there a correlation? Eur J Orthod 2004; 26 (3): 283-8.
19. Hewitt, A B - A radiographic study of facial asymmetry. Br J Orthod 1974; 237-40.
20. Shah, S M, Joshi, M R - An assessment of asymmetry in the normal craniofacial complex. Angle Orthod 1978; 48(2): 141-8.
21. Peck, S, Peck, L, Kataja, M - Skeletal asymmetry in esthetically pleasing faces. Angle Orthod 1991; 61(1): 43-8.
22. Proffit, W R, Phillips, C, Dann, C t - Who seeks surgical-orthodontic treatment? Int J Adult Orthodon Orthognath Surg 1990; 5(3): 153-60.
23. Ferrario, V F, Sforza, C, Miani, A, Tartaglia, G - Craniofacial morphometry by photographic evaluations. Am J Orthod Dentofacial Orthop 1993; 103(4): 327-37.
24. Vig, P S, Hewitt, A B - Asymmetry of the human facial skeleton. Angle Orthod 1975; 45(2): 125-9.

THE IMPORTANCE OF A THOROUGH EXAMINATION IN PLACING AN ORTHODONTIC DIAGNOSIS



ANCA STEFANIA MESAROS[1], DIANA DUDEA[1], MICHAELA MESAROS[1], MINDRA BADEA[1]

1. **University of Medicine and Pharmacy "Iuliu Hatieganu" - Cluj Napoca, Romania**

ABSTRACT

Introduction: *Nowadays, more and more patients are concerned with aesthetic issues.*

Aim: *The present paper wishes to highlight the existence of borderline cases for which the gravity of the orthodontic diagnosis is not always betrayed by the appearance of the patient.*

Material and Methods: *We selected three orthodontic cases, one representative of each class of malocclusion, for which there was considered to be a discrepancy between the facial appearance and the diagnosis set on cephalometric interpretation of the profile teleradiography. For these cases the cephalometric measurements were resumed and were added additional angular measurements to the classical photostatic analysis in order to assess soft tissues.*

Results: *Following our analysis it was observed the presence of a compensation phenomenon of the skeletal discrepancies by the overlaying soft tissues.*

Conclusions: *It must always be considered a series of characteristics given by the configuration of the overlying soft tissue, which sometimes can hide the severity of the malocclusion and may influence the therapeutically approach.*

Key words: *malocclusion, orthodontic treatment, cephalometric.*

Correspondence to:

Anca Stefania Mesaros
E-mail address: mindrabadea@yahoo.com, ancames@yahoo.com

INTRODUCTION

Social integration, psychological well-being and self confidence of every individual are related to physical appearance. The literature contains studies like those of Hershnon L E and Gideon DB's from 198011 or Canut J in 1996 5 which demonstrate a link between an individual's self-esteem and facial a-ttractiveness. Thus, patients' undergoing orthodontic treatment is expecting to improve not only the teeth, but also the facial profile. Therefore, getting a well balanced, harmonious and aesthetic profile is one of the major goals of orthodontic treatment.

However, defining beauty or the aesthetic is not an easy task since it is a subjective issue that involves many factors. Among these factors we include: ethnicity and culture of the individual, age of the patient and of the examiner, global trends and local context. Also today, the media tends to impose certain rules and last, but not least when it comes to treatment planning we must not forget the patient's wishes, which are based on how they perceive themselves. In order to achieve a complex and proper treatment plan in accordance with the aesthetic wishes of the patients, we must carefully analyze both bone structures and soft overlying tissues. After the standardization of the cephalometric technique by Broadbent in 1931 [8], the analysis of soft tissue profile is less used than the analysis of dento-skeletal relationships which has since become the primary factor in orthodontic therapeutic decision. Subsequently, some authors have incorporated in their cephalometric analysis some measurements of soft tissues, by introducing filters to the lateral cephalometric technique to allow visualization of soft tissue. Among these authors we mention Burston CJ in 1958 and 1967, Downs W in 1965, Merrifield L in 1966, and Holdaway RA 1983 [1].

In everyday practice most orthodontists are doing an analysis of facial soft tissues in an unstructured way and often subconsciously.

AIM

An important part of the stage of diagnosis setting and treatment planning is the analysis of the soft tissues, and authors such as JD Subtelny (1958) [15], Burston CJ (1967) [4], Bowker WD and Meredith HV (1959) recommend taking into consideration the soft tissue analysis to achieve a proper assessment of the case because of the existence of individual differences in the thickness of soft tissues. For these reasons, in the present study we wish to emphasize the importance of a thorough examination both in terms of hard tissues and in terms of soft ones whereas the borderline cases exist, for which the gravity of the orthodontic diagnosis is not always betrayed by the appearance of the patient.

MATERIALS AND METHODS

We studied records of orthodontic patients from the Pediatric Dentistry Clinic of the University of Medicine and Pharmacy ¨ Iuliu Hatieganu ¨ of Cluj-Napoca who were seeking orthodontic treatment in 2009-2010. Three cases were selected, one representative of each class of dental

malocclusion, and it was found that there is a discrepancy between facial appearance and diagnosis based on interpretation of the profile teleradiography. Criteria for selection of subjects was the presence of a relatively harmonious and balanced facial appearance, labial slot closed at rest, no accidents, injuries and surgery in maxillo-facial area in the patients' medical history and the existence of complete documentation at the beginning of treatment. For these cases cephalometric measurements were repeated using the cephalometric analysis of Tweed Merrifield and the one developed by Sassouni , to which we added additional angular measurements to the classical photostatic analysis in assessing soft tissues.

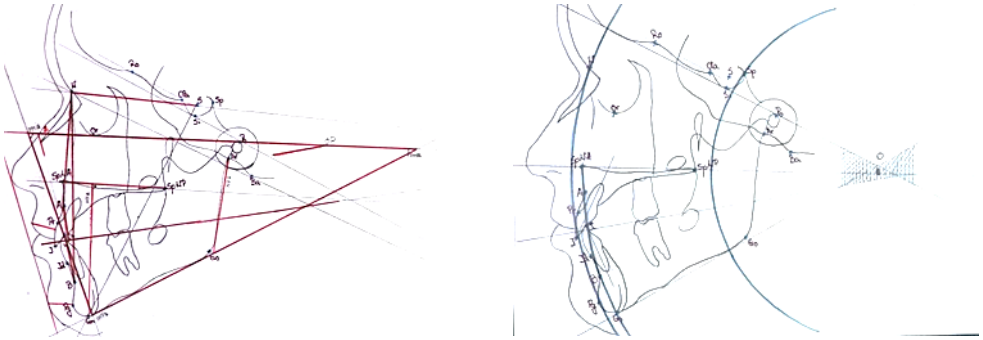


Fig.1 Cephalometric tracings using the Tweed and the Sassouni techniques

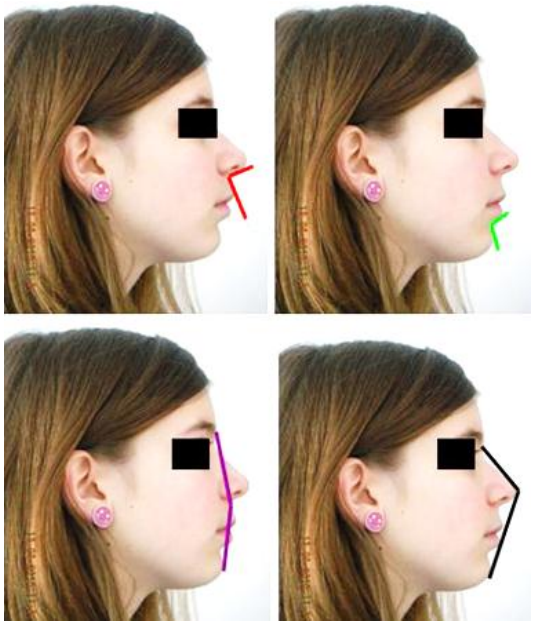


Fig.2 Photostatic analysis-angular mesurements
For the analysis of soft tissue profile the following angles were taken into account:

- Nasolabial angle-Prn-Sn-Ls (Fig. 2 top left)
- Labio-mentonier angle Li-Sm-Pg (Fig. 2 top right)
- Facial contour angle G-Sn-Pg (Fig. 2 bottom left)
- Angle of facial convexity N-Prn-Pg (Fig. 2 lower right)

RESULTS

CASE 1. Patient CA, 14 years of age: A 14-year-old patient presented herself seeking orthodontic solving of a minor crowding the upper frontal arch, and has class I occlusal relationship in the canines and molars.

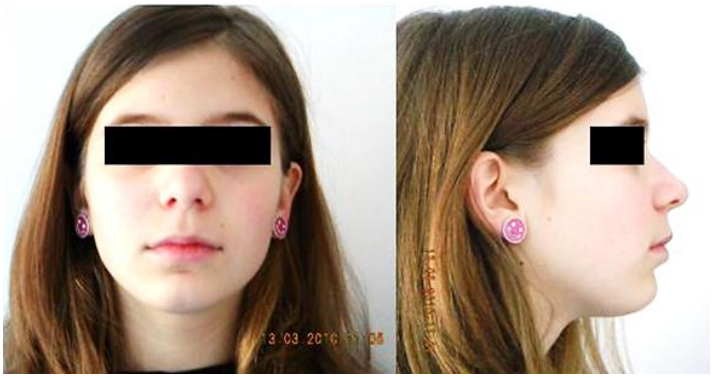


Fig.3 Case 1- Face and profile

The Tweed cephalometric analysis (Table I) shows a normodivergent profile, with a class II skeletal discrepancy compensated by the alveolar bone. Total space analysis (Tables II and III) indicate a moderate degree of severity in solving the case, 116.5, close to the upper limit, due to significant skeletal changes.

The Sassouni cephalometric analysis also shows a proarhial profile with an intermaxillary sagittal discrepancy of 5 mm, a class I dental relationship with vertical normal bite.

Table 1 Cephalometric values - case 1

Measurement	*Normal*	*Observed Values*
FMIA	67	45
FMA	25	27
IMPA	88	108
SNA	82	85
SNB	80	75
ANB	2	10
AO-BO	0	5
OCC PLANE	10	14
Z ANGLE	75	62
UPPER LIP		14
TOTAL CHIN		13
POST FACIAL HEIGHT	45	44
ANT FACIAL HEIGHT	65	68
FAC HEIGHT INDEX	0.70	0.64

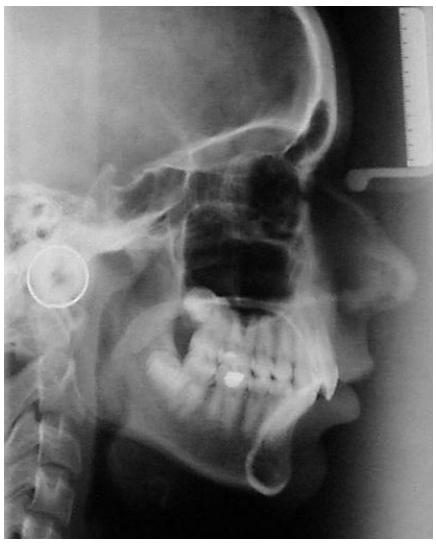


Fig. 4 Teleradiography - case 1

Table 2 Cranial facial analysis-case 1

	Normal Values	**Observed Values**	**Coefficient of Difficulty**	**Difficulty**
FMA	22-28	27	5	0
ANB	1-5	10	15	75
Z	70-80	62	2	16
OCC PLANE	8-12	14	3	6
SNB	78-82	75	5	0
FAC HEIGHT INDEX	0.65-0.75	0.64	3	0
TOTAL DIFFICULTY				91

Table 3 Total Space Analysis-Case 1

ANTERIOR AREA		CEPHALOMETRIC VALUE	DIFFICULTY FACTOR	DIFFICULTY
	DENTAL DISCREPANCY	1	1.5	1.5
	CEPHALOMETRIC DISCREPANCY	16	1	16
	TOTAL	17		17.5
MIDD-ARCH AREA	DENTAL DISCREPANCY	0	1	0
	SPEE CURVATURE	2	1	2
	TOTAL	2		2
	HORIZONTAL OCCLUSAL DISCREPANCY	0	2	0
POSTERIOR AREA	DENTAL DISCREPANCY	-12		
	¨-¨ GROWTH EXPETANCY			
	TOTAL	12	0.5	6
TOTAL SPACE ANALYSIS	31	DIFFICULTY		25.5
TOTAL DIFFICULTY	0-60 LOW 60-120 MODERATE 120-…SEVERE			116.5

On the other hand, the angular phototostatic analysis reveals a harmonious and balanced profile with the nasolabial angle (72 (74 8)), labio-mental angle (116 (122 11.7)) and the facial contour angle (165 (170+5)) with values within the ranges cited in the literature, only the angle of facial conve-xity is slightly diminished 122 (130+ 3.5)).

CASE 2. Patient SS, 16 years old: The second case is a 16 year-old patient presenting a class II malocclusion.

The Tweed Analysis reveals an important hypodivergency, without the presence of a sagittal discrepancy between the two maxillaries but with an evidently increased facial aesthetic angle (Z).

Table 4 Cephalometric values - case 2

Measurment	*Normal*	*Observed Values*
FMIA	67	78
FMA	25	14
IMPA	88	89
SNA	82	85
SNB	80	84
ANB	2	1
AO-BO	0	-1
OCC PLANE	10	5
Z ANGLE	75	82
UPPER LIP		20
TOTAL CHIN		17
POST FACIAL HEIGHT	45	47
ANT FACIAL HEIGHT	65	62
FAC HEIGHT INDEX	0.70	0.75

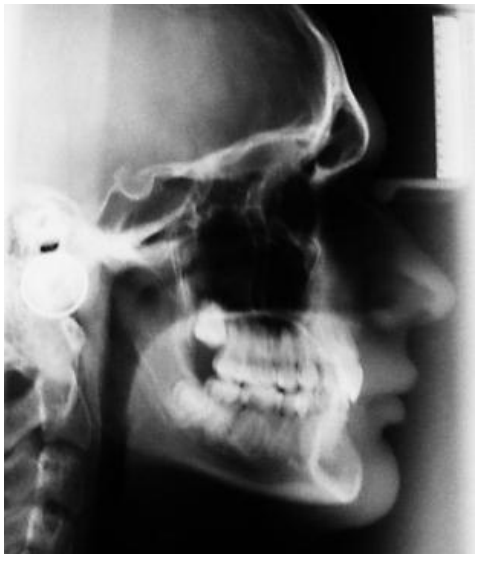


Fig. 5 Teleradiography - case 2

The severity of the malocclusion is moderate 89.5. The Sassouni analysis confirms the lack of sagittal skeletal discrepancy and the presence of a class II alveolar relation of 4 mm with an important deep bite.

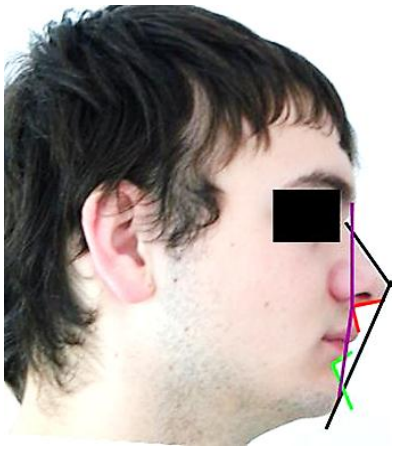


Fig.6 Photostatic analysis - Case 2

The analysis of the nasal complex soft tissues shows normal values for the nasolabial, facial contour and facial convexity angles, but on the other hand we have a labio-mental angle slightly accentuated. 102 (122+11,7).

The Sassouni analysis confirms the lack of sagittal skeletal discrepancy and the presence of a class II alveolar relation of 4 mm with an important deep bite.

The analysis of the nasal complex soft tissues shows normal values for the nasolabial, facial contour and facial convexity angles, but on the other hand we have a labio-mental angle slightly accentuated. 102(122+11,7).

CASE 3 Patient IG, 12 years: The third case refers to a 12 year-old patient with a class III malocclusion.

The Tweed analysis shows a normodivergent profile, with an important sagittal skeletal discrepancy between the maxilla and the mandible (ANB -7).

The total space analysis reveals a severe degree of difficulty 226,5, furthermore since the patient has agenesis of the second premolars (4.5, 3.5) in the lower arch and the first permanent molars are endodonticaly treated and with massive crown destructions.

The Sassouni analysis confirms the impressive sagittal skeletal discrepancy (12 mm) as well as the absence of the alveolar compensation phenomenon.

Table 5 Cephalometric values - case 3

Measurments	Normal	Observed Values
FMIA	67	67
FMA	25	27
IMPA	88	86
SNA	82	83
SNB	80	90
ANB	2	-7
AO-BO	0	13
OCC PLANE	10	22
Z ANGLE	75	67
UPPER LIP		13
TOTAL CHIN		11
POST FACIAL HEIGHT	45	48
ANT FACIAL HEIGHT	65	64
FAC HEIGHT INDEX	0.70	0.75

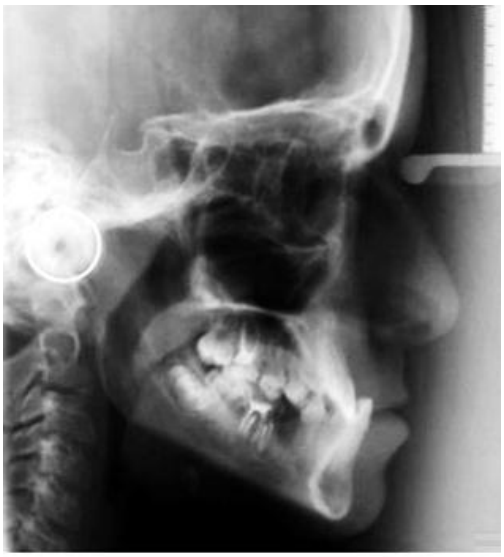


Fig. 7 Teleradiography - case 3

The photostatic angular measurements presents the labio-mental angle (131 (122+11,7)), the angle of facial contour (175(170+5)) and the angle of facial convexity (137(135+ 3.5)) that are between the limits cited in the literature but a nasolabial angle a bit more open (94 (74+8)). For the studied patients it was observed the presence of a compensation phenomenon of the skeletal discrepancies by the overlaying soft tissues as well as the lack of correlation between this phenomenon with the type of malocclusion (dental or skeletal). Furthermore it was observed that the presence of typical cephalometric values for a certain malocclusion is not always implying a less desirable profile.

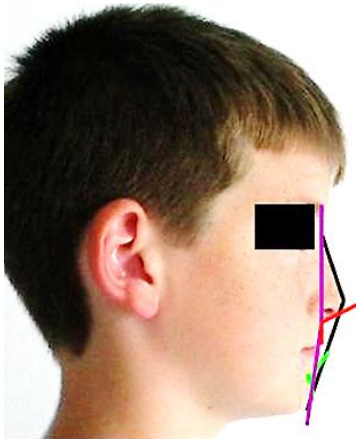


Fig. 8 Photostatic analysis-Case 3

DISCUSSIONS

The advantages of angular readings on the photomontages are many. Thus these measures are not affected by the scale of photography as the cephalometric analysis (Malkoc et al 2005) 13, they can be used both in the pre-treatment phase and during the evaluation of results, they can be achieved through programs and manual tracings but they don't require expensive equipment, nor complex procedures, they provide measurable results and represent a reproducible method. All these advantages are recommending these analyses to be used more frequently in association with other measurements both cephalometric and photostatic in the idea of a thorough examination for each case.

Although the ages of patients in the study take a fairly broad range, we opted for angular measurements of the nasal complex as it is considered that these parameters remain relatively constant between 7 and 18 years of age. (Genecov et al 1989) [10]

CONCLUSIONS

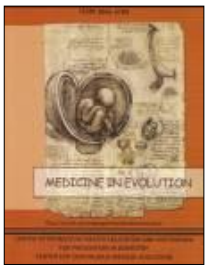
In our survey conducted on three orthodontic cases representative of each class of anomaly, we found that a less desirable skeletal pattern is not always associated with a nonaesthetic facial aspect. This phenomenon is due to the presence of bone defect compensation by superjacent soft tissues. Consideration must be as rigorous and must equally address the hard bony tissues and the soft overlying ones. Knowledge of facial aesthetic standards is important because the treatment established must not harm the aesthetics.

REFERENCES

1. Anić-Milosević S, Lapter-Varga M, Slaj M. Analysis of the soft tissue facial profile by means of angular measurements. Eur J Orthod. 2008 Apr; 30(2):135-40. Epub 2008 Feb 8.

2. Arnett GW, Jelic JS, Kim J, Cummings DR, Beress A, Worley CM Jr, Chung B, Bergman R. Soft tissue cephalometric analysis: diagnosis and treatment planning of dentofacial deformity. Am J Orthod Dentofacial Orthop. 1999 Sep; 116(3): 239-53.
3. Burstone C J 1958 the integumental profile. American Journal of Orthodontics 44 : 1 – 25
4. Burstone C J 1967 Lip posture and its signifycance in treatment planning. American Journal of Orthodontics 53: 262–284
5. Canut J 1996 Un análisis estético dentofacial. Revista Española de Ortodoncia 26: 13–30
6. Clements BS. Nasal imbalance and the orthodontic patient. Am J Orthod. 1969 May; 55(5): 477-98
7. Downs W B 1956 Analysis of the dentofacial profile. Angle Orthodontist 26: 191–212
8. Fernández - Riveiro P, Smyth-Chamosa E, Suárez - Quintanilla D, Suárez-Cunqueiro M. Angular photogrammetric analysis of the soft tissue facial profile. Eur J Orthod. 2003 Aug; 25(4): 393-9.
9. Gavan JA, Washburn SL, Lewis PH. Photography: an anthropometric tool. Am J Phys Anthropol. 1952 Sep; 10(3): 331-53.
10. Genecov JS, Sinclair PM, Dechow PC. Development of the nose and soft tissue profile. Angle Orthod. 1990 Fall; 60(3): 191-8.
11. Hershon L E, Giddon D B 1980 Determinants of facial profile and self perception. American Journal of Orthodontics 78: 279 – 295
12. Holdaway R A 1983 A soft-tissue cephalometric analysis and its use in orthodontic treatment planning. Part I. American Journal of Orthodontics 84 : 1 – 28
13. Malkoç S., Demir A., Uysal T., Canbuldu N. Angular photogrammetric analysis of the soft tissue facial profile of Turkish adults. Eur J Orthod. 2009 Apr; 31(2):174-9. Epub 2008 Dec 8.
14. Merrifield L L 1966 the profi le line as an aid in critically evaluating facial esthetics. American Journal of Orthodontics 52 : 804 – 822
15. 15. Subtenly J D 1959 A longitudinal study of soft tissue facial structures and their profile characteristics, defined in relation to underlying skeletal structures. American Journal of Orthodontics 45 : 481 – 507.

ORTHODONTIC TREATMENT USING FUNCTIONAL DEVICES



ONET MELINDA[1], GRIVU OVIDIU[1], JIANU RODICA[1], LILE IOANA[1], HOSSZU TIBERIU[1], GAMAN SIMINA[2]

1. **University of Medicine Vasile Goldis Arad, Faculty of Dentistry, Department of Orthodontics and Pedodontics**
2. **University of Medicine Craiova, Prosthetic Technology Department**

ABSTRACT

By my contribution I wish to underline the importance of functional orthodontic devices.

Out of 164 patients, 88 aged between 3-45 have been treated with functional devices. With these kind of devices, not only the orthodontist capacity is enough; it also demands an impecable execution of the device as well as the collaboration with the patient who has to exactly follow the doctors instructions. We have selected a few patients, exemplified through extraoral pictures as well as study molds, before and after some treatment steps.

In choosing the treatment a big contribution played the fact that functional therapie has minimal side effects as compared to fixed therapy.

The cost is much lower for functional devices than for fixed appliances. It also has to be underlined that the patients collaboration is very important, without which no result will be obtained.

Key words: *functional devices, interception, utility, effect on the patient.*

Correspondence to:

Onet Melinda MD
Adress: strada Muncii 65, Santana, Arad
Phone: 0744625572
E-mail address: melindaonet@gmail.com

INTRODUCTION

Orthodontics has two important branches that have developed differently in the world. They are fixed techniques and therapy using mobile appliances. Fixed therapy became very important in the USA while in Europe; mobile therapy has been better accepted [4, 8].

Dr Angle was the one who divided anomalies into 3 classes: I, II and III. Schwarz introduced the palatal plate and Andresen-Haupl the activator. Bimler, Stockfisch, Balters, Klammt and Frankel introduced functional devices, very usefull for different types of anomalies [6, 7].

Meanwhile, fixed therapy developped that much that functional devices were left at the side. Dentists have to recognize bad development (excessive or underdeveloped) of the jaws in the three palns, at a young age, because treatment is much easier, faster, without high costs and best results.

CASE REPORT

The moment when orthodontic treatment starts doesn't depend on the cronological age, but on the dental and skeletal age as well as the gravity of the case, given the character of the anomalie.

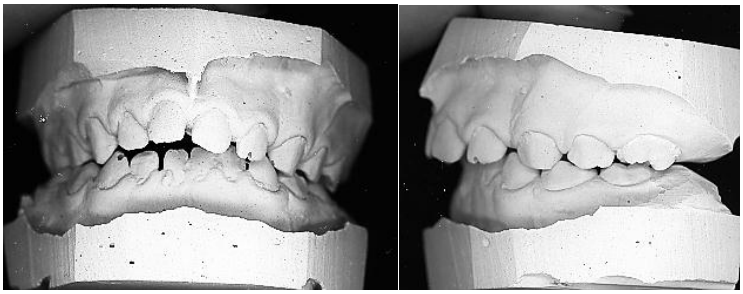
Out of 164 patients, 49 droped out because of different reasons, 88 have been treated with functional devices (exclusive or combined with fixed therapy) and other 27 haven't been well documented.

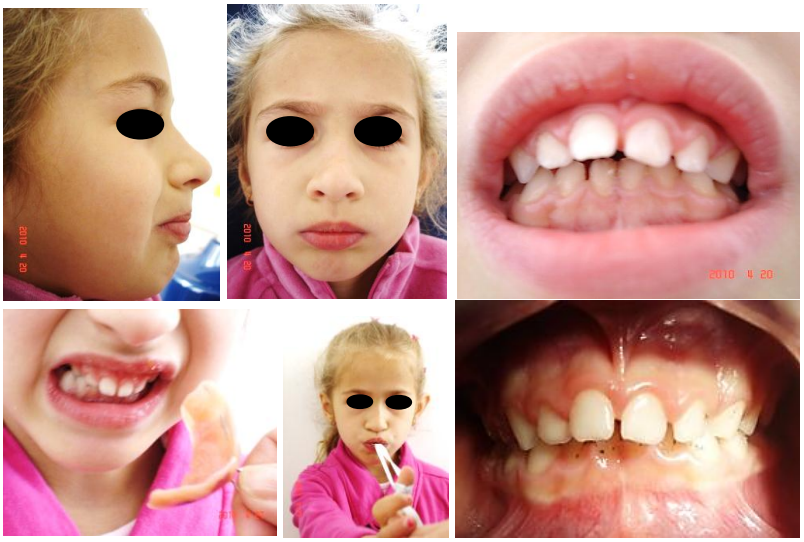
Patients were aged between 3-45. 29% have been treated with fixed appliances, 38% received both techniques and the other 33% have been treated using exclusively functional devices. Check ups have been done every 4 weeks for mono-maxillary devices and every 6 weeks for bi-maxillary devices.

We will describe some of the cases treated exclusively with functional appliances.

INTERCEPTION AND PREVENTION OF DENTO-MAXILLARY ANOMALIES

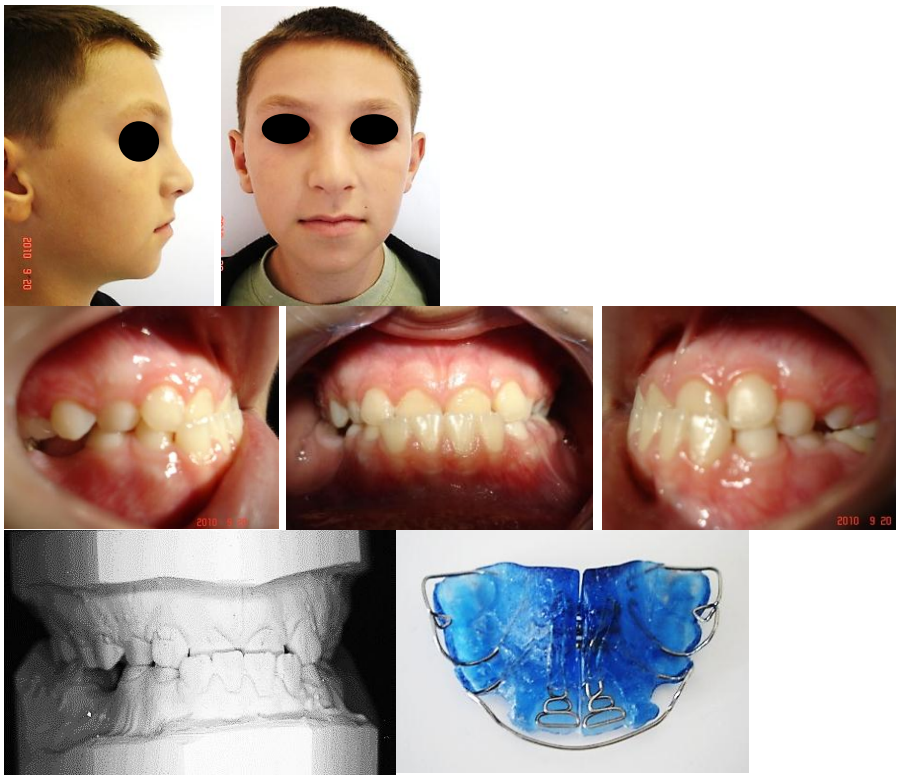
Interception and prevention of dento-maxillary anomalies is realized starting from temporary and mixed dentition because oclusion doesn't develop according to the growing scheme. ***Case 1:*** 3 years old patient with distalized oclusion, 10 mm sagital step, labial incompetence. A vestibular plate which traines the orbicular muscles, as seen in the images. After a few month of treatment the evolution is satisfactory.

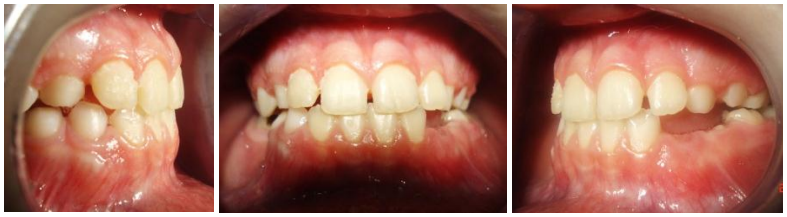




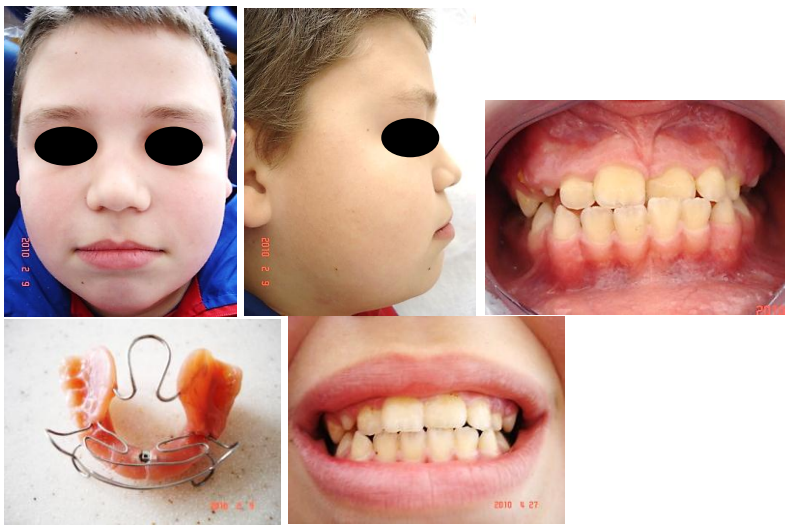
For reversed front occlusion in patients having permanent incisors in reversed relationship, if treatment doesn't star early, the maxillary vestibullum doesn't develop anteriorly, raising a compensation through the palatal leaning of the upper and lower incisors. Once the relationship is removed, the maxillary regaines it's natural development excepting the genetic anomalies.

Case 2: 7 years old patient with reversed frontal occlusion and class II Angle in both molars. We applied a plate with temporary mouth guard in order to achieve normal frontal occlusion, then the mouth guards have been excluded in order to deepen the bite. The evolution is excellent.

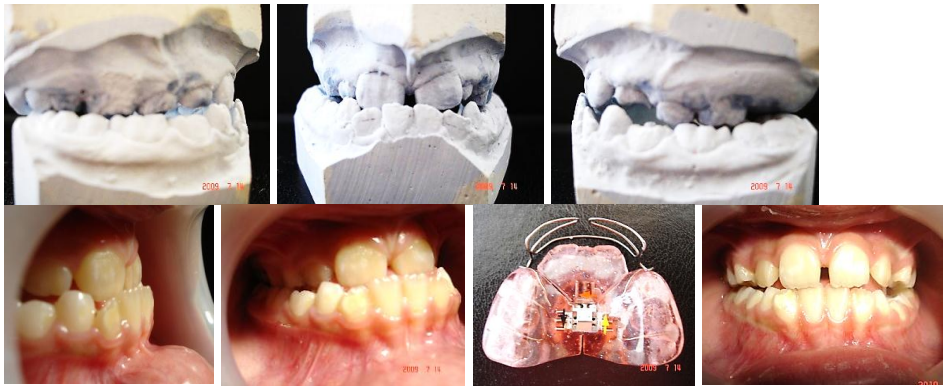




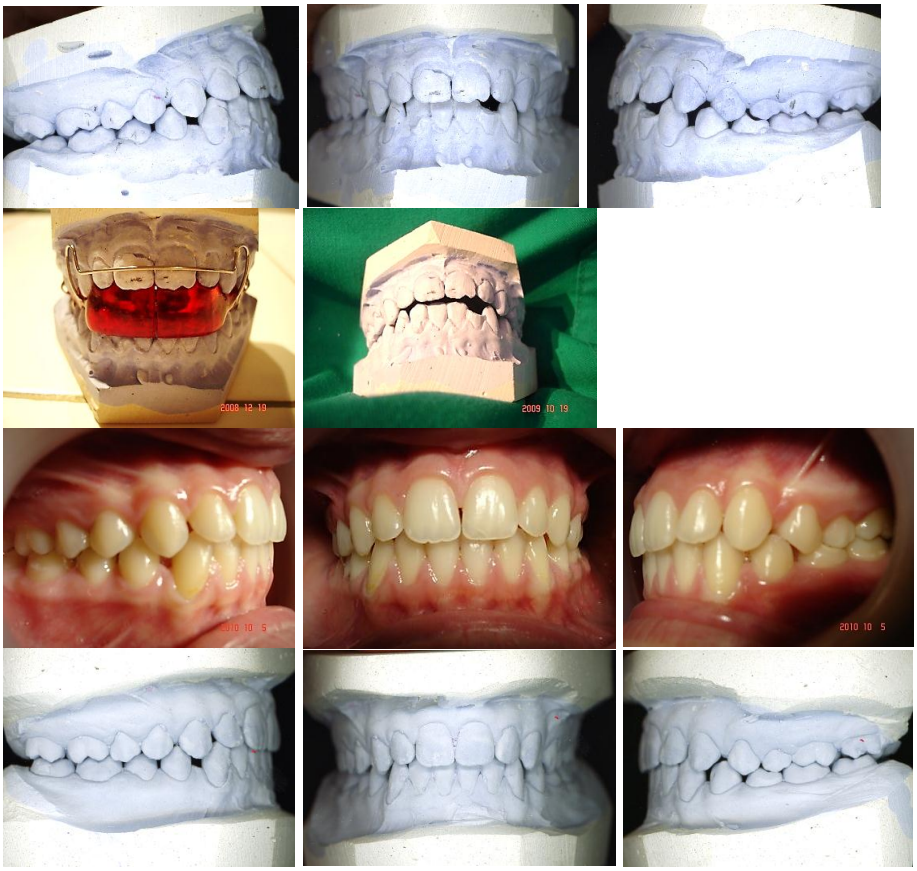
Case 3: 8 years old patient - reversed relationship of 2.1 and a hereditary tendency for class III anomaly. We have applied a plate with a screw and a Coffin wire. The evolution is good. For uni-lateral or bilateral crossbite, delaying the treatment limits the transversal growth of the maxillary and obtaining the circumscription of the inferior teeth.



Case 4: 6 years old patient – having bilateral crossbite with an evolution to genetic class II anomaly. We applied a plate with a 3D screw, vestibular Eschler arch to lingualize inferior incisors. The maxillary disjunction has been obtained in a later stage and now the patient wears a plate with a 3D screw which has predominant action on the upper frontal incisors, in order for them to advance togheter with the alveolar process and obtain enough space for the canine.

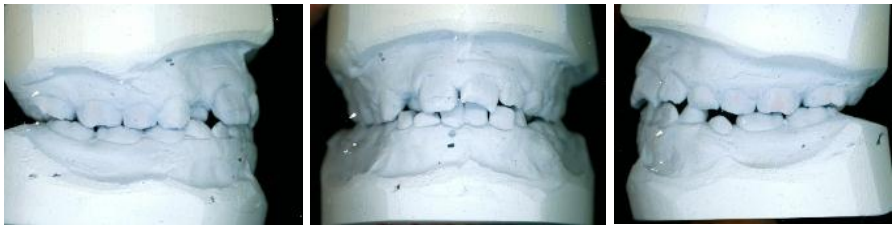


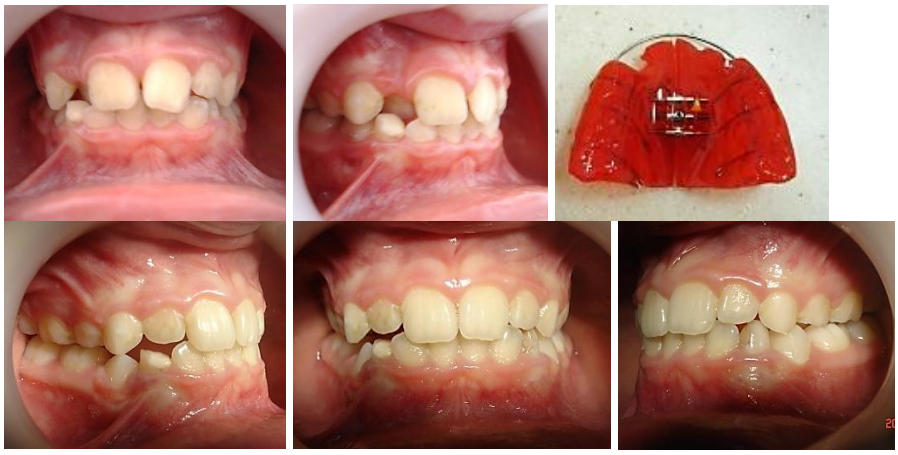
Case 5: 15 years old patient having an open bite. We used an activator to remove the vicious habit and after approximately 1 year the case was finalized. In cases with crowdings, one should intervene as quickly as possible when the sutures are still aproachable. After this time period the enlargement of the arches can only be obtained by axial movements of the teeth or permanent teeth extractions.



Case 6: 8 years old patient crowdings and reversed contact for 1.2 – we used a plate with an auxilliary resort for the vestibularisation of 12. It is an intermediate treatment step. After the oclusal jump the mouth guards have been removed. The evolution is very good.

In cases of class II anomalies with deep bite and positive sagital step, orthodontic treatment must consist of the mesialization of the lower jaw and when this is not possible, extractions in the upper jaw have to be made to cpmpensate the saggital step.





Case 7: 10 years old patient with distalized class II Angle oclusion, deep bite and 5 mm sagital step. We used a twin block with a big median screw, without arch wire to allow the orbicular muscles to tonify. The mandibular plate has no screw. With this appliance we managed to mesialize the lower jaw and open the bite.

We have obtained the mesialization of the lower jaw, modified the upper plate in order to stabilize the oclusion and allow the eruption of the premolars and canines.

RESULTS

The incipient anomaly can be corrected for 35% of the patients using only functional orthodontic appliances.

Approximately 40% of the patients need initially functional orthodontic appliances but the treatment has to continue with fixed techniques in order to achieve the gold standard [1, 2, 3, 5]. The time span of using fixed appliances puts the impression on the stability of the enamel surfaces of the teeth. The shorter the fixed appliances are used, that much less signs of enamel irritation are seen. The gingival retraction and the root resorbtion determine oclusion problems on the long term.

DISCUSSIONS

Functional apliances can be used at any age, starting with the temporary dentition, mixed and permanent. They shoul always be the priority before fixed appliances because they are phisiological appliances and do not have many side effects. One should also take into account that the cooperation with the patien is mandatory, that is why the orthodontist has to explain to the patient the most favorable choice for the given situation. One should also explain that in order to obtain best phisionomic results, treatment with fixed appliances could be also needed. In order to take the right decision the patient has to consider the family antourage, how dental hygiene can be performed and the time needed to wear the appliance.

Our objective is to obtain a functional oclusion that is individualizes for each patient but also a correspunding esthetic aspect. Sometimes an interdisciplinary approach is neede involving a speech therapist or even a psichotherapist. Any unsolved problem could lead to relaps. Dentists send often very complicated cases with deep bites, severe crowdings, cross bites and if we don't get them at the right time, relaps can not be avoided.

CONCLUSIONS

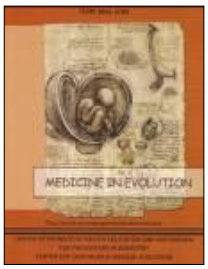
When it comes to orthodontic treatment it is not so important what method we use but how we apply it. We do not only correct the position of teeth and jaws but we also have to keep the soundness of the teeth in mind. The message is addressed to al dentists because they have to send the patient to an orthodontist when they observe some anomalies, but also to the parents who should be informed through add campaignes about the importance of the orthodontic control as soon as possible and a propper interceptive treatment.

REFERENCES

1. Clark J William. Die funktionelle Therapie mit dem Twinblock.1998.
2. Mc Namara JA Jr. Components of class II malocclusion in children 8 – 10 years of age. Angle Orthod.1981; 51:177-202.
3. Korbmacher H, Koch L, Eggers-Stroeder G, Kahl-Nieke B. Associations between orthopaedic disturbances and unilateral crossbite in children with asymmetry of the upper cervical spine. Eur J Orthod. 2007; 29:100-104.

4. Graber T M, Swain B F. Grundlagen und moderne techniken der Kieferorthopadie.1989.
5. O´Brien K, Wright J, and Conboy F, et al. Effectiveness of early orthodontic treatment with Twin-block appliance: a multicenter, randomized, controlled trial. Part 1. Dental and skelettal effects. Am J Orthod Dentofacial Orthop. 2003; 124:234-243.
6. Faltin K Jr, Faltin RM, Baccetti T, Franchi L, Ghiozzi B, Mc Namara JA Jr. Long-term effectiveness and treatment timing for Bionator therapie. Angle Orthod. 2003; 73:221-230.
7. Schulze Chr. Lehrbuch der Kieferorthopadie. Band 2. Berlin; Quintessenz, 1978.
8. Van der Linden F. Orthodontic Concepts and Strategies. 2004.

SEDATION IN UNCOOPERATIVE CHILDREN UNDERGOING DENTAL PROCEDURES, COMPARATIVE EVALUATION OF THREE DIFFERENT METHODS



LAZAR CRISTINA FULGA[1], BABAMETIS ANTONIOS[2], BRATU ELISABETA[1], DEUTSCH PETRU[3], A. DEUTSCH[3], PAPURICA MINODORA[3]

1. **Department of Orthodontics, Faculty of Dentistry University of Pharmacy and Medicine "Victor Babes", Timisoara**
2. **"Sfanta Maria" Dental Clinic, Timisoara**
3. **Faculty of Medicine University of Pharmacy and Medicine "Victor Babes", Timisoara**

ABSTRACT

***Aim**: The aim of the study was to compare the efficiency of three different sedation methods in controlling the behavior of uncooperative children during dental treatment.*

***Materials and methods:** Thirty two children (15 males and 17 females) were selected for this study, with ages between 5 and 10 years. The effectiveness of each sedation method was considered comparing the children's behavior during different stages of the dental treatment. For the assessment of the children's behavior, a scoring system suggested by Houpt et al. (1985) was utilized.*

***Results:** The combination of midazolam and nitrous oxide-oxygen showed superior results in terms of controlling movement and crying during the most stressful stages of the dental treatment. The dental treatment could be successfully carried out with all three different sedation methods.*

***Conclusions:** A combination of oral midazolam and nitrous oxide-oxygen sedation provides more comfort to pediatric dental patients and operators during critical stages of dental treatment.*

***Keywords:** oral midazolam, nitrous oxide-oxygen, mixed sedation.*

Correspondence to:

Lazăr Cristina Fulga MD
Adress: Clinica Stomatologica "Sf. Maria", Str. Romulus Nr.62, Timişoara
Phone: 0744116233
E-mail address: lazartina@yahoo.com

INTRODUCTION

Dental treatment of pediatric patients is difficult, but when the patient also presents behavioral problems, the task becomes extremely challenging.

It has been demonstrated that infants and children experience pain in a similar manner to adults (e. g., Porter, Wolf, Gold, Lotsoff, Miller, 1997). High levels of pain in children may have significant neurophysiological effects (e. g., Ruda, Ling, Hohmann, Peng, Tachibana, 2000) and physiological effects (e. g., Eland, 1993).

In such situations a pharmacological approach in the management of the dental anxiety is needed because inadequately managed pain in children can have psychological consequences, which can turn to lead to higher levels of pain during medical treatments [1]. A variety of oral sedative agents have been used for managing uncooperative children [2]. Midazolams safety margin and its wide toxic/therapeutic ratio, compared to diazepam, do not produce prolonged sedation [3, 4]. It possesses profoundly potent anxiolytic, amnestic, hypnotic, anticonvulsant, skeletal muscle relaxant, and sedative properties [5, 6, 7]. When taken orally, midazolam is absorbed in the gastrointestinal tract and produces its peak effect in about 30 minutes, and has a fast recovery time and is the most commonly used benzodiazepine as a premedication for sedation [3]. It has been demonstrated that, administrated in doses between 0.5 to 0.75mg/kg of body weight it is a useful agent for sedation in paediatric dentistry [8, 9]. The anterograde amnesia property of midazolam is useful for premedication before surgery to inhibit unpleasant memories [10, 11].

Nitrous oxide inhalation produces anxiolytic and mild analgesic effects [12]. Sedation by inhalation of nitrous oxide with oxygen is appropriate for children with mild to moderate anxiety and it enables them to accept dental treatment better and it facilitates co-ping across sequential visits [13]. This method is not adequate in more fearful children, because of their refusal to accept the nasal mask [12, 14].

In the literature there are several studies presenting the efficacy and safety of using inhalation sedation in combination with other sedative drugs, in dental treatment in uncooperative children [15, 16, 17, 18, 19, 20, 21]. But there are little studies comparing the efficacy and safety of a mixed sedation (midazolam and nitrous oxide-oxygen inhalation), comparing to sedation with midazolam alone, and nitrous oxide inhalation sedation alone.

MATERIALS AND METHODS

The study has been realized over a period of 1 year, 02.2009 – 10.02.2010 and has a crossover design, where the same patient received, during three different dental appointments, three different sedation regimens, that is:

- 0,6 mg/kg of oral midazolm
- Nitrous oxide/oxygen inhalation sedation
- 0,6 mg/kg of oral midazolm with nitrous oxide/oxygen inhalation sedation.

The dental treatment was provided by the same doctor, in the same room, and was the same in all the patients and in all the dental treatmens, and it consisted in:

1. Local aneasthesia for temporary molar
2. Preparation of the cavity with rotative instruments
3. Fizionomic obturation of the cavity
4. No pulp therapy needed.

SAMPLE SELECTION

Thirty two children (15 males and 17 females) were selected for this study, and the inclusion criteria was the following:

1. Age between 5 and 10
2. ASA – I Category [22]
3. Child's weight within normal range
4. The behavior category: Frankl [23] Scale #2 (negative: reluctant to accept treatment and some evidence of Negative attitude, not very profound),
5. Needing of restaurative obturation at the tempoarary molars, but with no pulp therapy/enlarged sealing of the temporary molars
6. No cognitive impairment
7. The children had to be healthy, and didn't had recently used medication such as erythromycin or anticonvulsants, or any othe medications that may interfere with the pharmacokinetics of midazolam [3]
8. The respiratory function of the children had to be unobstructed, meaning there had to be no breathing difficulties caused by e.g. adenoid hyperplasia, nasal septum problems, nasal polyps, enlarged turbinates.

Before the treatment was started each child had a general medical examination, clinical and radiological examination and the behavior category was confirmed by a child psychologist.

The parents consented for their child to participate to the study. They received verbal and written explanations of the procedures, and also pre- and postoperative written instructions.

SEDATION PROTOCOL

All the children were examined before each sedation by the anesthetist. The dental treatment had to be postponed if the child was feeling sick and had a cold (28 meetings were postponed). The children's body weight was registered with an electronic weighing scale, and also the blood pressure, the heart rate and the oxygen saturation were recorded before each appointment.

The dose of oral midazolam (Dormicum-La Roche Ltd, Basel, Switzerland) was calculated for each child. The medication was administrated to the children in the recovery room, by their parents with a large amount of water. The treatment was postponed if the child refused the medication (15 meetings were postponed) or if the child expectorated a part, or the whole amount of the medication (no such case occurred). The child waited, after the administration of midazolam, in the quiet recovery room, for 40 minutes with his / her parents and the signs of sedation were observed and recorded by the anesthetist every 10 minutes. After 40 minutes, the child was moved to the dental office, in accompany of his / her parents and the anesthetist.

During the nitrous oxide/oxygen sedation, usig Masterflux automatic AS3000 Tehnogaz, a nasal mask was

used, which delivered first, 100% oxygen, and then gradually nitrous oxide, up to 30-50%, titrated to the patient's need. At the end of the procedure, 100% oxygen was given for 3 minutes before removal of the nasal mask.

The child was monitored continuously during the course of the treatment. The hemodynamic parameters were recorded before any sedation procedure has been made, during the course of the treatment the child was continuously monitored using Siemens sc 7000. At the end of the dental procedure, the child was transferred to the recovery room. The patient was monitored, and was discharged when he / she fulfilled the discharged criteria (ability to maintain a standing posture, absence of dizziness or disorientation, vital signs in parameters).

The time interval between the appointments was one week.

First Appointment: In the first appointment the sedation was carried out only with midazolam. After the child was broughed in the dental office, the following procedures had been carried out:

1. Topical anaesthesia was applied for 1 minute
2. Local anaesthesia (Ubistesin forte 3M ESPE)
3. Preparation of the cavity with rotative instruments
4. Restauration of the cavity using glass ionomers (3M ESPE ketac cem radiopaque)

Second Appointment: In the second appointment the children had sedation with nitrous oxide / oxygen. The dental procedures were the same.

Third Appointment: In the third appointment the sedation was mixed, oral midazolam and inhalation sedation. The dental procedures were the same.

Measurements: A scoring system suggested by Houpt et. al. [24] was utilized for this purpose. The system is consisted of the following scales:

1. Sleep Scale
2. Crying Scale
3. Movement Scale
4. Overall Scale

The evaluations were carried out at the following times:

1. During administration of the local anesthesia
2. During the preparation of the cavity.

RESULTS

Working time: The working time was considered from the moment the child was on the dentalunit chair, until it was brought in the recovery room. The overall working time in the 1st visit was 29,1 minutes, 28,7 minutes in the 2nd visit and 26,4 in the 3rd visit. The difference is not significant, but it showed the time - efficacy of each sedation regime.

Sleep Scale: Only with the mixed sedation regimen deep sleep was reached, and only at the beginning of the treatment, and at the end. There was a significant difference during the most painful procedure, local anesthesia, between the three different sedation regimens. The children were the most comfortable with the dental procedures during the third appointment.

Movement Scale: No patient movement interrupted the dental treatment, only one patient, during the 1st visit was violent during the local anesthesia. A significant difference was observed between the three visits. The children

sedated with midazolam alone exhibited significantly more movement than those sedated with nitrous oxide. During the mixed sedation the children didn't present any movement.

Crying Scale: The scores of crying during all three visits were intermittent crying or no crying. Only in the 1st visit, during local anesthesia 6,25% cried continuously, and 3,1 % in the 2nd visit. The percentage of children not crying was the biggest during the mixed sedation regimen.

Evaluation of Overall Behavior: The percentaje of children with "very good" behavior was the highest during the 3rd visit. Most of the patients showed good behavior. There was no aggressive behavior during any of the visits, and no treatment was aborted. The overall behavior of the children was divided into two categories: acceptable or unacceptable behavior.

Table 1 Sleep Scale

Sleep	**rating**	**scale**	
Dental procedure	Awake	Asleep	Deep sleep
Start of treatment			
1st visit	29	1	0
2nd visit	27	2	1
3rd visit	0	25	5
Local anesthesia			
1st visit	30	0	0
2nd visit	28	2	0
3rd visit	22	8	0
Restoration			
1st visit	29	1	0
2nd visit	25	5	0
3rd visit	0	25	5

Table 2 Movement Scale

	Rating	**Scale**	
Dental procedure	Violent	Continuous	No movement
Start of treatment			
1st visit	0	16	14
2nd visit	0	19	21
3rd visit	0	0	30
Local anesthesia			
1st visit	1	9	21
2nd visit	0	5	25
3rd visit	0	2	28
Restoration			
1st visit	1	8	22
2nd visit	0	3	27
3rd visit	0	0	30

Table 3 Crying Scale

Crying	**Rating**	**Scale**	
Dental procedure	Continuous	Intermittent	No crying
Start of treatment			
1st visit	0	5	25
2nd visit	0	3	27
3rd visit	0	0	30
Local anesthesia			
1st visit	2	16	13
2nd visit	1	13	16
3rd visit	0	4	26
Restoration			
1st visit	0	14	16
2nd visit	0	7	23
3rd visit	0	0	30

Table 4 Evaluation of Overall Behavior

Behavior category	**Behavior ratings**	**1st visit**	**2nd visit**	**3rd visit**
Acceptable	Very good	6	12	28
behavior	Good	19	15	2
	Total	25	27	30
Unacceptable	Fair	5	3	-
behavior	Aborted	-	-	-
	Total	5	3	-

DISCUSSIONS

The present study has attempted to test the efficacy of three different sedation regimens in controlling the behavior of uncooperative children during dental treatment. All three different sedation regimens proved to be effective, all dental treatments could be carried out, and suitable for young dental patients who need minimal restorative treatment. The Houpt [24] Sedation Rating Scale was used to assess the efficacy of sedation because of its demonstrated reliability, simplicity in data interpretation, and frequent successful use by various studies [24, 25].

The mixed sedation proved to be the best during the most painful procedure, local anesthesia, but it could be carried out also with the other sedation methods.

CONCLUSIONS

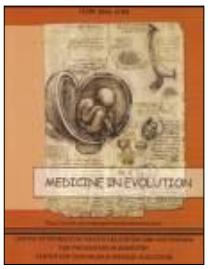
All three sedation methods proved their efficiency in controlling the behavior of uncooperative children during dental treatment. The dental treatment could be successfully carried with all three sedation methods.

The difference could be seen in the comfort of the child, in the recording of the crying and the refusing-movement during the dental treatment.

REFERENCES

1. Roland L. B., Piira T., Cohen L., Cheng P., Behavior Modification, Vol. 30 No.1, January 2006, 24-46.
2. Torres-Perez J, Tapia-Garcia I, Rosales-Berber MA, Hernandez-Sierra JF, Pozos-Guillen Ade J. Comparison of three regimens for pediatric dental patients. J Clin Pediatr Dent 2007; 31:183-6.
3. Kanto JH. Midazolam: The first water-soluble benzodiazepine. Pharmacology, pharmacokinetics and efficacy in insomnia and anesthesia. Pharmacotherapy 1985; 5:138-55.
4. Brandt SK, Bugg JL Jr. Problems of medication with the pediatric patient. Dent Clin North Am 1984; 28:563-79.
5. Mandrioli R, Mercolini L, Raggi MA (October 2008). "Benzodiazepine metabolism: an analytical perspective". Curr. Drug Metab. 9 (8): 827–44
6. Olkkola, KT.; Ahonen, J. (2008). "Midazolam and other benzodiazepines." Handb Exp Pharmacol 182 (182): 335–60.
7. Barash, Paul G.; Cullen, Bruce F.; Stoelting, Robert K.; Cahalan, Michael D. (1 April 2009). Clinical Anesthesia (6 ed.). Lippincott Williams Wilkins. p. 588
8. Silver T, Wilson C, Webb M. Evaluation of two dosages of oral midazolam as a conscious sedation for physically and neurologically compromised pediatric dental patients. Pediatr Dent 1994;16:350-9
9. Smith BM, Cutilli BJ, Saunders W. Oral midazolam: Pediatric conscious sedation. Compend Contin Educ Dent 1998;19 :586-88, 590, 592
10. Riss, J.; Cloyd, J.; Gates, J.; Collins, S. (Aug 2008). "Benzodiazepines in epilepsy: pharmacology and pharmacokinetics." Acta Neurol Scand 118 (2): 69–86
11. Kain ZN, Hofstadter MB, Mayes LC, Krivutza DM, Alexander G, Wang SM. Midazolam: Effects on amnesia and anxiety in children. Anesthesiology 2000;93:676-84
12. Berge TI. Acceptance and side effects of nitrous oxide oxygen sedation for oral surgical procedures. Acta Odontol Scand 1999;57:201-6
13. Hosey, M. T., Managing anxious children: the use of conscious sedation in paediatric dentistry, UK National Clinical Guidelines in Paediatric Dentistry, International Journal of Paediatric Dentistry; 12(5); 359-372.).
14. Lanza V, Mercadante S, Pignataro A. Effects of halothane, enflurane, and nitrous oxide on oxyhemoglobin affinity. Anesthesiology 1988; 86: 591-4
15. Houpt M, Manetas C, Joshi A, Desjardins P. Effects of chloral hydrate on nitrous oxide sedation of children. Pediatr Dent 1989; 11:26-9.
16. Shapira J, Holan G, Guelmann M, Cahan S. Evaluation of the effect of nitrous oxide and hydroxyzine in controlling the behavior of the pediatric dental patient. Pediatr Dent 1992; 14: 167-70.
17. Hartgraves PM, Primosch RE. An evaluation of oral and nasal midazolam for pediatric dental sedation. ASDC J Dent Child 1994; 61:175-81.
18. Houpt MI, Kupietzky A, Tofsky NS, Koenigsberg SR. Effects of nitrous oxide on diazepam sedation of young children. Pediatr Dent 1996; 18:236-41.
19. Litman RS, Berkowitz RJ, Ward DS. Levels of consciousness and ventilatory parameters in young children during sedation with oral midazolam and nitrous oxide. Arch Pediatr Adolesc Med 1996; 150:671-5.
20. Litman RS, Kottra JA, Berkowitz RJ, Ward DS. Breathing patterns and levels of consciousness in children during administration of nitrous oxide after oral midazolam premedication. J Oral Maxillofac Surg 1997; 55:1372-7; Discussion 1378-9.
21. Wilson S, Matusak A, Casamassimo P, Larsen P. The effect of nitrous oxide on pediatric dental patients sedated with chloral hydrate and hydroxayzine. Pediatr Dent 1998; 20:253-8.
22. "ASA Physical Status Classification System". American Society of Anesthesiologists. http://www.asahq.org/For-Members/Clinical-Information/ASA-Physical-Status-Classification-System.aspx. Retrieved 2007-07-09.
23. Frankl SN, Shiere FR, and Fogels HR. Should the parents remain with the child in the dental operatory? J Dent Child 1962;29:22-32
24. Houpt MI, Weiss NJ, Koenigsberg SR, Desjardins PJ. Comparison of chloral hydrate with and without promethazine in the sedation of young children. Pediatr Dent 1985; 7:41-6.
25. Sams DR, Cook EW, Jackson JO, Roebuck BL. Behavioral assessments of two drug combinations for oral sedation. Pediatr Dent 1993; 15:-186-90.

TECHNOLOGICAL ASPECTS OF ZIRCONIA CAD/CAM ALLCERAMIC RESTORATIONS



LAVINIA ARDELEAN[1], LAURA-CRISTINA RUSU[1]

1. **"Victor Babes" University of Medicine and Pharmacy Timisoara**

ABSTRACT

***Aim**: Replacing alloys with alternative materials is a continuous challenge in dental medicine. The study aims to present technological aspects concerning zirconia CAD/CAM all-ceramic restorations, pointing their advantages versus classical metalo-ceramic restorations.*

The authors completed several zirconium oxide-based ceramic restorations using the CAD/CAM method, using Zeno Tec system (Wieland Dental).

Zirconium oxide-based ceramic restorations are definitely superior to metal-based ones, on all counts: esthetic, resistance, mechanical properties, biocompatibility, resistance to corrosion.

Zirconium oxide-based ceramics is a viable alternative as it represents the hi-tech approach that contributes to a superior physiognomic restoration.

***Key words**: zirconium-oxide, CAD/CAM*

Correspondence to:

Ardelean Lavinia MD
Adress: Victor Babes" University of Medicine and Pharmacy Timisoara:
E-mail address: lavinia_ardelean@umft.ro

INTRODUCTION

CAD/CAM systems are computeraided design (CAD) and computer-aided manufacturing (CAM) systems which have been used in dental medicine since 1980. They represent a hi-tech approach used in long-term physiognomic restorations. The dentures produced by using this method represent a high-class alternative to hand made classical metalloceramic or fully ceramic crowns [1, 2].

MATERIALS AND METHODS

Zirconium oxide-based ceramic blocks, the so-called "ceramic steel", are characterised by their high mechanical resistance, therefore being suited for extensive physiognomic restorations, even in the molar area. Zirconium (zirconium silicate), one of the oldest solid terrestrial minerals, was discovered in Australia, and is a natural gem (precious stone in a pure crystal state). Dental medicine uses zirconium oxide (ZrO_2), which is a white biocompatible material, resistant to corrosion, chemically inert, non-toxic, with a high tissue-tolerance, esthetic, wear-resistant, and with very good mechanical characteristics. Most of the times, zirconium oxide is combined with ytrium oxide (Y_2O_2), for a better resistance[7]. The drilling of pre-syntherised is conducted in the incomplete syntherised stage, followed by the stages of heading-contraction-syntherisation, in special ovens, until the final shape and toughness are obtained.

Eventually, the prosthetic piece is plated with a special ceramic in order to cover finer porosities, and after that it is heated at low temperatures for a higher esthetic aspect and to prevent antagonist wear [6]. Plating ceramics are less tough than the ceramic used in manufacturing infrastructure blocks.

The ZenoTec (Wieland Dental) system, used by the authors, is a laboratory system, the model being manufactured based on a conventional print.

The model is scanned with a 3 Shape D tri-dimensional laser system, a fully automated process that lasts for 1-2 minutes.

After scanning, the working model and the antagonists can be visualised on the computer monitor.

An individual scanning has been performed for each abutment, in order to get a high-quality virtual model.

The ends were shaped on the virtual model using the specific Zenotec Manager software. The boundaries of the model are automatically detected by the software, still certain areas will need further adjusting. The areas that after adjusting have become too thin will be marked in red. The insertion axis and the space necessary for the cement are then established.

The next stage is making the ceramic infrastructure of Zeno Zr disks. The restoration piece is placed (virtually) in an optimal position on the zirconium disk and the supporting rods are attached. The presyntherised disk is placed in the drilling unit and the computer-guided drilling starts. The drilling process is fully automated and it lasts for a few minutes. Afterwards, the supporting rods are severed and the frame are submerged in a special ZirColor solution.

Approximately 10 minutes later, the frame have achieved in depth pigmentation and are placed in the syntherisation oven. After selecting the

appropriate zirconium frame syntherisation programme, the process starts and lasts for 11 hours.

Following syntherisation, the frames are processed, removing existing irregularities or material surplus left over from computer-guided drilling. After fitting the denture in the patient's mouth, the frames are prepared for ceramic plating by smoothing the surface that is to be plated.

Plating is done with a special ceramic material, Zirox New generation Ceramics. Syntherisation is carried out in a Vita Vacumat 40T oven, produced by the Vita Company. After syntherisation, the restoration pieces are refined.

The last stage is that of glazing.

In order to obtain special colour effects, certain pigments in the ceramic kit are used, painting the occlusal surface, the grooves and the cervical third of the tooth, thus giving a more natural aspect to the pieces.

The final syntherisation programme eliminates vacuum heating in order to prevent the surface migration of small air bubbles, which would confer a rough aspect.

Fig. 1.a. Scanning of the model

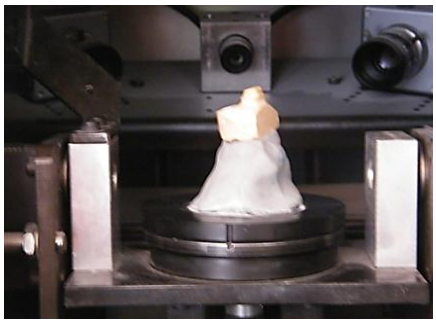


Fig. 1.b. Individual scanning of the abutments

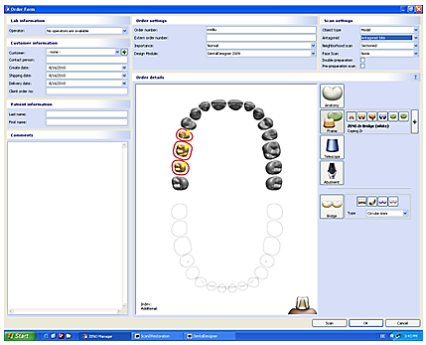


Fig. 2.a. Selecting the restorations

Fig. 2.b. The working model and the antagonists

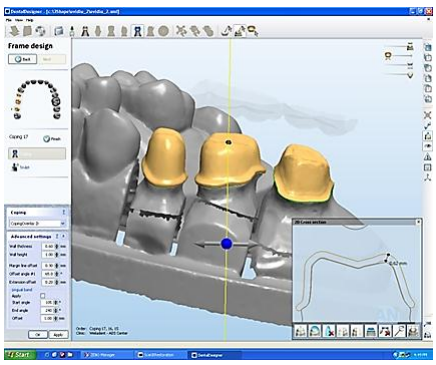


Fig. 3.a. Shaping the ends

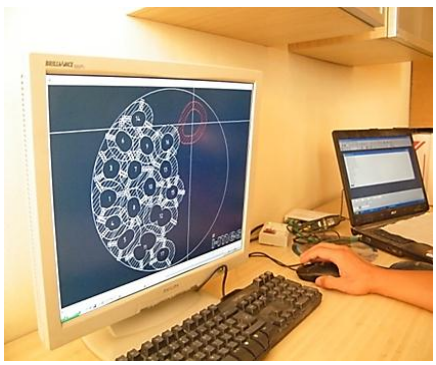


Fig. 3.b. Positioning on the zirconium disks

Fig. 4.a. The drilling unit

Fig. 4.b. The frame in the pigment solution

Fig. 5.a. Syntherisation of zirconium frame

Fig. 5.b. The processed frames placed on the model

Fig. 6.a. Depositing the incisal ceramics

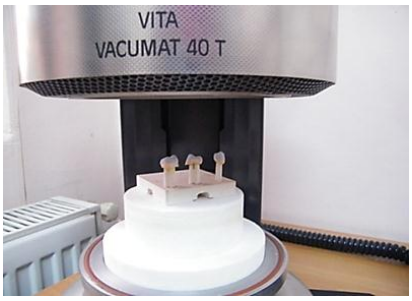


Fig. 6.b. Dentin and incisal syntherisation

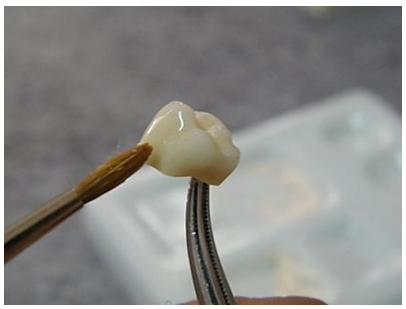


Fig. 7.a. Glazing

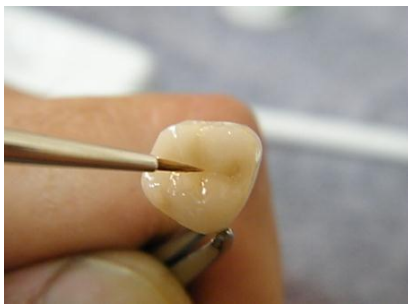


Fig. 7.b. Colour correction

RESULTS

Zirconium processing technique represents a state-of-the-art development in dental restoration as it gives absolute quality, durability and total compatibility[7]. Unlike metal-mounted porcelain, zirconium allows light to penetrate it from all directions, the esthetics of the restoration being exceptional, with a natural and perfect aspect[4]. Zirconium-based crowns are

much lighter than metallic-based ones, consequently the patients' adaptation to the new prosthetic pieces is much faster [1, 3]. This is an extremely important aspect, mainly in multi-element fixed restorations. The precision of zirconium oxide-mounted restorations is granted by the computer-guided process of cutting from a solid zirconium oxide block, which results in an extremely precise adjustment [9].

The use of CAD/CAM systems contributes not only to reducing working stages and, implicitly, the working time, but it also represents an advantage over standard ceramic, being an obvious benefit when one chooses zirconium-based ceramics [7, 8].

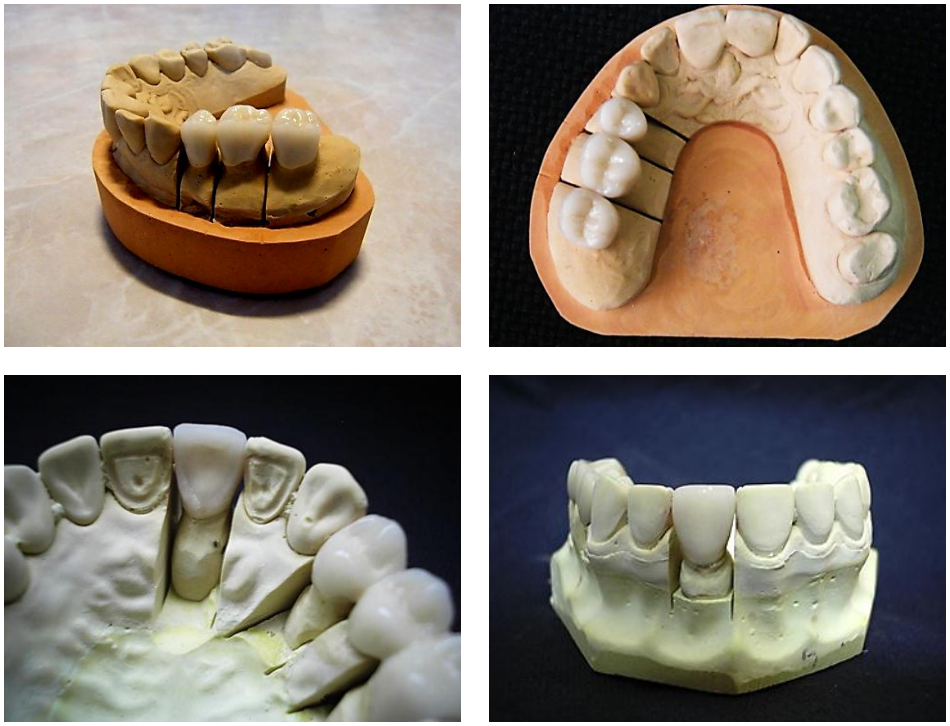


Fig. 8. a, b, c, d. Final restorations

CONCLUSIONS

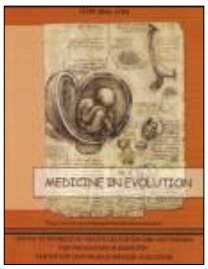
Zirconium oxide is a biocompatible material, very resistant to corrosion, chemically inert, non-toxic, with a very high tissue tolerance, esthetic, wear-resistant, and with excellent mechanical characteristics [2]. Besides being biocompatible, zirconium oxide also has the advantage that, unlike metallic structures, it does not corrode, it does not conduct electricity and temperature, it is white and can be coloured with a special solution to a shade similar to that of dentin, with exceptional esthetic results.

REFERENCES

1. Busson B.: Zirconiul- Ceramica biocompatibilă a viitorului, Tehnica Dentară, 6, 1, 18-20, 2007
2. Chen H.Y., Hickel R., Setcos J.C., Kunzelmann K.H.: Effects of surface finish and fatigue testing on the fracture strength of CAD/CAM and pressed-ceramic crowns, J.Prosthet.Dent., 82, 4, 468-475, 1999

3. Conrad R.S.: CAD/CAM: Changing the Face of All-Ceramic Restorations, Cont. Esth. Rest. Prod., 7,11, 76-78, 2003
4. Drăguş A.: Oxidul de zirconiu în zona frontală superioară, Tehnica dentară, 8, 29, 5-7, 2008
5. Fasbinder D.: Utilising lab-based CAD/CAM technology for metal-free ceramic restorations, Dent. Today, 22, 3, 100-105, 2003
6. Freitag J.R.: Construcția şi placarea unei punți din dioxid de zirconiu, Dentis, 6, 3/4, 11-16, 2007
7. Giordano R.: CAD/CAM: An OverWiew of Machines and Materials. Journal of Dental Technology, 9, 20-30, 2003
8. Kern M.: CAD/CAM a fost doar începutul, Cosmetic Dentistry, 2, 2, 27-31, 2009
9. Moldovan O., Rudolph H., Quass S., Bornemann G., Luthardt R.G.: Precizia internă şi externă pentru punțile din dioxid de zirconiu realizate prin procedeul CAD-CAM, Tehnica Dentară, 25, 2, 2-4, 2007.

EPIDEMIOLOGICAL STUDY ON MOLAR-INCISOR HYPOMINERALISATIONS IN SCHOOLCHILDREN FROM GENERAL POPULATION



IOANA-ANDREEA STANCIU[1], ANETA MUNTEANU[1], RODICA LUCA[1], ECATERINA IONESCU[2]

1. **"Carol Davila" University of Medicine and Pharmacy Bucharest, Faculty of Dental Medicine, Pedodontics Department**
2. **"Carol Davila" University of Medicine and Pharmacy Bucharest, Faculty of Dental Medicine, Orthodontics and Dento-Facial Orthopaedics Department**

ABSTRACT

Aim and objectives: *evaluation of molar-incisor hypomineralisations (MIH) in schoolchildren from 3 localities with different degree of environmental pollution.*

Material and method: *cross-sectional study on 3 samples of schoolchildren: 421 (208 boys) from Slatina, mean age = 10.02 ± 2.32 years; 389 (197 boys) from Fetesti, mean age = 9.52 ±1.92 years; 401 (195 boys) from Patarlagele, mean age = 11.45± 2.38 years. Prevalence (IpMIH), topography and severity of MIH were evaluated.*

Results: *lpMIH: 5.23% - Patarlagele, 3.08% - Fetesti, 0.71% - Slatina. The mean number of affected first permanent molars (FPM): 2.42±0.78 in Patarlagele, 3.33±1.09 in Fetesti, 2.33±2.04 in Slatina. In all groups, the most numerous lesions were located on the FPM cusps and mild defects are most frequently.*

Conclusions: *1) lpMIH is small 2) The mean number of FPM with MIH was similar in all groups 3) MIH etiology is more related to medical history than to the environment.*

Key words: *molar-incisor hypomineralisation, first permanent molar, schoolchildren*

Correspondence to:

Stanciu Ioana-Andreea MD
Adress: Street: Trandafirilor, nr.7, Chitila, Ilfov
Phone: +40723360556
E-mail address: ioana.stanciu@tranger.com.ro

INTRODUCTION

The term "molar-incisor hypomine-ralisation" (MIH) was introduced in 2001 by Weerheijm et al. and defines hypomineralisation of systemic origin of at least one first permanent molar (FPM) frequently associated with the similarly affected permanent incisors [1]. The prevalence of MIH seems to vary between 3.6 and 25% in European countries [2]. For Romania only a few studies have been published on MIH [3, 4].

The etiology of MIH, still unclear, was attributed to the child's medical history, to the variations in the environ-mental conditions or to the atmosphere pollution (the dioxine).

AIM AND OBJECTIVES

This study aims to evaluate MIH in children and adolescents from 3 localities with different degree of environmental pollution: Slatina, Fetesti and Patarlagele. Slatina is a town recognized for the industrial pollution as a result of processing aluminum (fluoride, dioxins etc.). Fetesti is an important crossroad city and a well developed agriculture center. Considerable amounts of dioxins accumulate following the intensive use of pesticides and the removing of the exhaust gas. Patarlagele is a small locality with low industrial activity (textile industry), accordingly with less pollution.

MATERIALS AND METHODS

Study samples consisted in children aged between 6 and 14 years old from schools and high-schools from Slatina, Fetesti and Patarlagele. School's direction and students' parents consent was required. Selection criteria were age 6 at the time of examination and at least one FPM partially erupted in the mouth (at least half of the crown).

Slatina sample consisted in 421 children (208 boys, 213 girls) with mean age = 10.02 ± 2.32 years. Fetesti sample consisted in 389 children (197 boys, 192 girls) with mean age = 9.52 ±1.92 years. Patarlagele sample consisted in 401 children (195 boys, 206 girls) with mean age = 11.45± 2.38 years.

A cross-sectional study was performed. Students were examined in the classroom, in daylight, using dental mirror and probe according to WHO criteria (1997) [5]. The presence, distribution and severity of MIH were recorded. The criteria used for the diagnosis of MIH were those described by Weerheijm et al. (2003) [6]: 1. demarcated opacity; 2. post-eruptive enamel breakdown (PEB); 3. atypical restoration (AR); 4. extracted molar due to MIH. In the case of demarcated opacities, only those exceeding a diameter ≥1 mm were recorded. Diffuse opacities (such as dental fluorosis or amelogenesis imperfecta), incipient caries or dental hypoplasia were not considered to be MIH lesions. Concerning severity, MIH lesions were divided into two categories: mild defects (white-cream opacities, with normal enamel thickness) and moderate/severe defect (yellow-brown opacities, PEB or AR) [7].

The recorded data were electronically filled and processed with the SPSS programme (version 16.0 for Windows). The prevalence index for MIH (IpMIH) was calculated and, for children with MIH, mean number of

affected FPM and incisors and percentage of affected teeth from total number of erupted FPM/incisors were evaluated.

It has been appreciated, as well, the topography and severity of MIH lesions. Independent variables used in the analysis were age and sex of the child. Statistical analysis was performed using Pearson Chi-square test. Statistical significance was set up at $p<0.05$.

RESULTS

Mih Prevalence – MIH prevalence index (IpMIH) was 5.23% in Patarlagele sample, 3.08% in Fetesti one and 0.71% in Slatina one. The most numerous children with MIH are 6 to 8 years old. In each sample, Ip MIH values were similar for boys and girls, differences being not statistically significant ($p>0.05$) (tab I). The percentage of the total FPM affected was 62.19% in Patarlagele sample, 83.33% in Fetesti one and 58.33% in Slatina one. The percentage of the affected incisors was 14.59% in Patarlagele sample, 25% in Fetesti one and 16.66% in Slatina one. The mean number of affected FPM per child was 2.42±0.78 in Patarlagele sample (min 1-max 4), 33.33±1.09 in Fetesti one (min 1-max 4) and 2.33±2.04 (min 2-max 3) in Slatina one.

Mih Lesions Distribution – Exclusive lesions on FPM had 57.14% of the children with MIH in Patarlagele, 41. 66% in Fetesti and 33.33% in Slatina (table II).

42.85% from the children with MIH in Patarlagele, 58.34% from Fetesti and 66.66% from Slatina had both FPM and incisors affected.

The distribution according to the jaws showed some differences. In Fetesti and Slatina samples, the upper molars are more affected (52.5%, respectively 85.71%) than the lower ones (47.5%, respectively 14.29%), while in Patarlagele sample the lower FPM (60. 79%) were more affected than the upper ones (39.2%).

The most affected teeth were 16 in Fetesti (91.66%), 16 and 26 (42.85%) in Slatina and 36 in Patarlagele sample (80.95%). The most affected incisors were 31 in Fetesti (33.33%), 11 and 21 in Slatina (50/50%) and 11 in Patarlagele sample (25%).

Mih Lesions Topography – In all the study samples, the most numerous lesions were located on the FPM cusps, in different levels. In Patarlagele and Fetesti samples, lesions were also found on the occlusal third, with or without the top of the cups' involvement. In addition to those localizations, extended coronal destructions were observed, being difficult to appreciate the initial topography of the lesion (table III).

The Severity Of Mih Lesions – In Slatina sample, only mild defects were found (white or white-yellow demarcated opacity). In Patarlagele sample, 84.32% of affected FPM presented mild defects and 15.68% presented moderate / severe defects, and in Fetesti sample, 70%, respectively 30%. From FPM with moderate / severe defects, PEB percentage was 66.66% in Patarlagele sample and 37.5% in Fetesti sample, yellow-brown demarcated opacity percentage was 25% in Patarlagele sample and 33.33% in Fetesti sample.

Atypical restorations were recorded only in Fetesti sample: 37.5% from FPM with moderate / severe defects.

Table 1 MIH prevalence index according to gender

	Patarlagele	Fetesti	Slatina
Boys	5.12	2.53	-
Girls	5.33	3.64	0.71

Table 2 The affected FPM percentage at children with lesions only on FPM

Sample	*Children with hypomineralisations only on FPM*			
	1FPM	*2FPM*	*3FPM*	*4FPM*
Patarlagele	28.57%	14.28%	-	14.28%
Feteşti	-	16.66%	-	25%
Slatina	-	66.66%	33.33%	-

Table 3 The MIH topography on FPM

The topography of hypomineralisation	*FPM (%)*		
	Patarlagele	*Fetesti*	*Slatina*
Top of the cusp	50.98	77.32	100
Bottom of the cusp	5.88	1.95	-
Entire cusp	23.52	-	-
Occlusal third	13.81	18.2	-
Extended coronal destructions	5.79	2.53	-

DISCUSSIONS

Many authors have attempted to determine the etiology of the development anomalies. Genetic, systemic and environmental factors have been incriminated. In the last 30 years, the environmental pollution (especially by dioxin) has been increasingly discussed as a possible etiologic factor for dental developmental defects. Studies conducted in high polluted areas have shown that the prevalence of MIH is higher than in unexposed regions [8, 9]. In that context, the present study investigates the prevalence, topography, severity and etiology of MIH lesions at children and adolescent from 3 areas with different environmental conditions: Slatina, Fetesti and Patarlagele.

The MIH prevalence was low. The results were among the lowest values reported in other studies made in similar conditions (table IV).

Table 4 MIH prevalence in general population – comparative values

Authors/Country	**Sample**	**Age (years)**	**IpMIH (%)**
Kukleva *et al*. (2008), Bulgaria [10]	2.970	7-14	3.6
Aploz *et al.* (1999), Turkey [11]	250	7-12	14.8
Costa-Silva *et al.* (2010), Brasil [12]	918	6-12	17.6 (UA)/24.3 (RA)
Luca *et al.* (2010), Romania [13]	277	6-12	9.4
Present study – Slatina (2008)	421	6-14	0.71
Present study – Fetesti (2008)	389	6-14	3.08
Present study – Patarlagele (2008)	401	6-14	5.23

It is noted that although the prevalence is close to the lowest values, the mean number of affected FPM is similar to the values reported in other studies. The present study shows that IpMIH values were higher in rural areas than in urban ones. Muratbegovici et al. (2008), in the study in several areas of Bosnia Herzegovina, have also observed variations in MIH prevalence according to geographical location [14] and Costa-Silva et al. (2008) found there is a difference between rural and urban areas [12], an idea strengthened by Rugg-Gunn et al., quoted by [12], who found a higher prevalence of enamel defects in rural areas with low socio-economical level and malnourished population. Instead, Preusser et al. (2007) found no statistically significant differences between urban and rural areas [15]. In the present study, in both Patarlagele and Fetesti samples, the MIH prevalence had quite similar values at boys and girls, the differences being not statistically significant. Similar results were reported by other authors [3, 15-17]. Only one study showed statistically significant differences [18].

Concerning the affected teeth, in the present study, in Fetesti and Slatina samples the most frequent lesions were located both on FPM and incisors (MIH). This result is consistent with those obtained in similar studies conducted in urban areas (UA). At Patarlagele, a rural region, most of the children with MIH had lesions only on FPM (MH), result similar to that found by Costa-Silva et al. (2008) for rural areas (RA) (table V).

In all groups, molars were more affected than the incisors. The more interested teeth were 16 in Fetesti and 36 in Patarlagele. In a study conducted in Iasi, Pasareanu et al. (2006) also found that FPM were 4 times more affected than incisors. The FPM involvement risk was greater as the incisors were most affected [4]. Also, Petrova et al. (2009), in the study on 1320 Bulgarian children aged 7-14 years, found that FPM were more affected than incisors [19]. In addition, Zambrano et al. (2011) observed that the most common affected tooth were 16, 26 and 36 [17]. Calderara et al. (2005) found that the lower molars were the most affected teeth [16].

Regarding the severity, in the present research, in all groups, the mild defects were predominant. The results are consistent with those reported by Preusser et al. (2007) (67.2% mild defects, 32.8%-moderate/severe defects) and Luca et al. (2010) (85%-mild defects, 15%-moderate/severe) [13, 15].

Although it was expected that in Slatina, where the environment is quite polluted MIH prevalence should be highest, the results showed that there was obtained the less value. Balmer et al. (2005) notes that it is possible that fluoride ions in the atmosphere increase the enamel resistance to MIH engraftment [20]. The highest prevalence was recorded in Patarlagele, the region with the smallest pollution. These findings indicate that MIH lesions development was determined by general disturbances occurring during tooth formation and not by environmental pollution. Data from medical records of children with MIH showed that the children' pathological history (hypoxia at birth, low birth weight, premature birth, respiratory/digestive diseases, eruptive fevers, treatment with antibiotics) could be correlated with MIH. Similar results were obtained by other authors [21, 22].

Although the MIH prevalence in the studied groups is small and most lesions are mild defects, it is important to carefully monitor children with MIH to prevent any complications at this level and/or to intervene as soon as possible with prophylactic methods

(sealing, fluoride) or with conservative therapeutic methods.

Table 5 FPM with MIH – the severity defects - comparative values

Authors / year	*Children with MH (%)*	*Children with MIH (%)*
Muratbegovici *et al.*(2008) - UA [14]	17,58	82,42
Zambrano *et al.*(2011) – UA [17]	10,7	89,3
Preusser *et al.*(2007) – UA [15]	42,1	57,9
Costa-Silva *et al.*(2008) - UA [12]	49,07	50,95
Luca *et al.*(2010) – UA [13]	27	73
Costa-Silva *et al.*(2008) - RA [12]	52,36	47,43
Present study (2008) – Fetesti (UA)	41,66	58,34
Present study (2008) – Slatina (UA)	33,33	66,66
Present study (2008) – Patarlagele (RA)	57,14	42,85

CONCLUSIONS

1) The MIH prevalence in the studied groups is quite small, approaching the minimum value reported in the literature for Europe (3.6 to 25%); 2) The mean number of FPM with MIH was about the same in all groups, although the prevalence was different; 3) Etiology of MIH lesions appears to be related to medical history than the action of environmental pollutants.

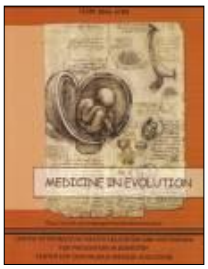
REFERENCES

1. Weerheijm KL, Jalevik B, Alaluusua S. Molar-incisor hypomineralization. Caries Res 2001, 35(5): 390-391.
2. Lygidakis NA, Dimou G, Briseniou E. Molar-incisor hypomineralization (MIH). Retrospective clinical study în Greek children. Part.1 Prevalence and defect characteristics. Eur Arch Paediatr Dent 2008, 9(4): 200-206.
3. Luca R, Prelipcean DDD, Chiș AC, Farcașiu C, Stanciu IA. Anomalii de dezvoltare ale smalțului dinților permanenți în două colectivități școlare urbane. Revista Română de Medicină Dentară 2009, XII (4): 281-290.
4. Păsăreanu M, Bălan A, Maxim A. Systematic impact of MIH syndrom on the child and adolescent. Journal of Preventive Medicine 2006, 14(1-2): 96-102.
5. World Health Organization. Oral health surveys: basic methods. 4th ed. Geneva, Switzerland 1997: 21-46.
6. Weerheijm KL, Duggal M, Mejare I et al. Judgement criteria for Molar-Incisor Hypomineralisation (MIH) in epidemiologic studies: a summary of the European meeting on MIH held in Athens, 2003. Eur Archs Paediatric Dent 2003b, 3:110-113.
7. Chawla N, Messer LB, Silva M. Clinical studies on molar-incisor hypomineralization. Part 1: Distribution and putative association. Eur Arch Paediatr Dent 2008, 9(4):180-190.
8. Alaluusua S, Calderara P, Gerthoux PM, Lukinmaa PL, Kovero O, Needham L, Patterson DG, Tuomisto J, Mocarelli P. Developmental dental aberrations after the dioxin accident în Seveso. Environ Health Perspect 2004; 112(13): 1313-1318.
9. Jan J, Sovcikova E, Ko an, Wsolova L, Trnovec T. Developmental dental defects in children exposed to PCBs in eastern Slovakia. Chemosphere 2007, 67: S350-S354.
10. Kukleva MP, Petrova SG, Kondeva VK, Nihtyanova TI. Molar Incisor Hypomineralisation in 7-to–14-year old children in Plovdiv, Bulgaria – an epidemiologic study. Folia Med (Plovdiv) 2008, 50(3): 71-5.
11. Aploz AR, Ertugrul F. Prevalence of mineralization defects in first permanent molars in a group of 7-12 years old children. Ege Dishekimligi Fakultesi Dergisi 1999, 20:40-44.
12. Costa-Silva CM, Jeremias F, Feltrin de Souza J, Cordeiro RCL, Santos-Pinto L, Zuazon ACC. Molar incisor hypomineralization: prevalence, severity and clinical consequences in Brazilian children. Int. J Paed. Dent. 2010:426-434.
13. Muratbegovic A, Zukanovic A, Markovic N. Molar-incisor hypomineralization impact on

developmental defects of enamel prevalence in a low fluoridated area. Eur Archs Paediatr Dent 2008, 9(4): 228-231.

14. Preusser SE, Ferring V, Wleklinski C, Wetzel WE. Prevalence aand severity of molar-incisor hypomineralization in a region of Germany- a brief communication. J Public Health Dent 2007, 67(3): 148-150.
15. Calderara PC, Gerthoux PM, Mocarelli P, Lukinmaa PL, Tramacere PL, Alaluusua S. The prevalence of Molar Incisor Hypomineralisation (MIH) in a group of Italian school children. Eur J Paediatr Dent 2005, 2: 79-83.
16. Zambrano F, Pinto M, Tapia R. Prevalence of molar-incisor hypomineralisation in the district of San Bernando, Santiago de Chile. Int J Paediatr Dent 2011, 21 (Suppl 1): 74.
17. Jasulaityte L, Veerkamp JS, Weerheijm KL. Molar-incisor hypomineralization: review and prevalence data from a study of primary schoolchildren in Kaunas (Lithuania). Eur Archs Paediatr Dent 2007, 8(2): 87-94.
18. Petrova S, Dimitrova M. Prevalence of molar-incisor hypomineralization (MIH) in children of age 7 to 14 from the town of Haskovo and the community of Yakoruda, Bulgaria Book of abstracts. 14th Congress of BASS, Varna, 2009:166.
19. Luca R, Farcașiu C, Prelipcean DDD, Stanciu IA. MIH in a group of schoolchildren from Bucharest, Romania. Eur Arch Paediatr Dent 2010: 36.
20. Balmer R, Laskey D, Mahoney E, Toumba KJ. Prevalence of enamel defects and MIH in non-fluoridated and fluoridated communities. Eur J Paediatr Dent 2005, 4: 209-212.
21. Van Amerogen WE, Kreulen CM. Cheese molars - a pilot study of the etiology of hypocalcifications in first permanent molars. ASDC J Dent child 1995, 62(4): 266-269.
22. Weerheijm KL. Molar-incisor Hypomineralisation. J Paediatr Dent 2003, 3:115-120.

TO USE OR NOT USE DOXYCYCLINE IN PERIODONTAL TREATMENT?



IOANA FEIER[1], DAN ONISEI[1], OANA VELEA[1], DOINA ONISEI[1]

1. **„Victor Babes" University of Medicine and Pharmacy Timisoara, Department of Periodontology**

ABSTRACT

***Aim**: The aim of the present study is to evaluate the effect of doxycycline on clinical parameters and gingival crevicular level of C reactive protein in chronic severe periodontitis.*

***Matherial and methods**: For this study were selected a total of 31 adult patients, 17 female and 14 male, with severe periodontitis which were diagnosticated and treated in the Clinic of Periodontology, UMF "Victor Babes" Timisoara. The patients were randomly distributed into 2 different samples of 15 and 16 subjects each. One sample (group control) received full mouth supra- and subgingival desinfection into 24 hours and oral hygiene instructions. The 2nd sample (test group) received the same periodontal treatment but in combination with an adjunctive antimicrobial therapy consisting of systemic administration of doxycycline. Supra and subgingival scaling and rootplaning were performed under local anesthesia, using XO Odontogain device and respectively, Hu-Friedy periodontal Gracey curettes.Clinical measurements and C reactive protein estimation were performed at baseline and 3 months later.*

***Results**: Clinical parameters of both groups significantly improved during the study. At 3 months, test group exhibited significantly higher PD reduction at deep sites (baseline PD > 7 mm) compared with control group: 61.4% versus 39.7%, for PD reduction ≥ 4 mm. C reactive protein levels of test group was significantly lower than baseline ($P < 0.0125$) and control group ($P < 0.016$) at 3 months later.*

***Conclusions**: These results ensure further data for beneficial effects of adjunctive doxycicline therapy in the management of severe chronic periodontitis.*

***Keywords**: doxycycline, periodontitis, protein C reactive*

Correspondence to:

Feier Ioana MD
Adress: „Victor Babes" University of Medicine and Pharmacy Timisoara, Department of Periodontology
E-mail address:

INTRODUCTION

Periodontitis is a destructive inflammatory disease of the supporting tissues of the tooth. This condition is caused by a chronic infection of Gram-negative bacteria, such as Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythensis, Aggregatibacter actinomycetemcomitans, and Gram-positive bacteria, such as Peptostreptococcus micros and Streptococcus intermedius [1, 2]. The host responds to the periodontal infections with an array of events involving both innate and adaptive immunity in which activates a large number of complement factors who neutralize invasive pathogens and stimulate repair and regeneration of a variety of tissues. One of the elements with pro-inflammatory properties who received the most attention is C-reactive protein (CRP). CRP is currently regarded as a biomarker of systemic inflammation.

Initial treatment of periodontitis aims to eliminate supra and subgingival bacterial plaque by the institution of proper daily oral hygiene measures and by practicing a meticulous "one stage full mouth desinfection" [3]. However, despite this correct therapy, some patients continue to lose periodontal attachment.

A number of studies have proved the fact that some of periodontal pathogens such as Aggregatibacter actionmycetemcomitans, Porphyromonas gingivalis, and Tannerella forsythia have the ability to invade and "hide" in the tissues (gingiva, root dentine). It is conceivable; therefore, that mechanical instrumentation becomes insufficient for suppressing these aggressive pathogens.

The role of antibiotics using as adjuncts to periodontal therapy has been widely debated in the literature.

Several important authors [4, 5] have shown that the adjunctive use of systemic antibiotics can improve treatment outcomes.

AIM AND OBJECTIVES

The aim of the present study weas to evaluated the clinical effects of systemic administration of doxycycline as an adjunct to mechanical therapy in patients with untreated severe chronic periodontitis. We also observed if this periodontal treatment can reduce CRP levels in periodontal patients.

MATERIALS AND METHODS

For the present study were selected a total of 31 adult patients, 17 female and 14 male, with severe periodontitis which were treated in the Clinic of Periodontology, UMF "Victor Babes" Timisoara. Inclusion criteria were: ≥10 sites with a clinical attachment loss at least 6mm, with bleeding upon probing.

Exclusion criteria were: use of antibiotic therapy in the previous 6 months, requirement for antibiotic premedication, pregnancy or lactation.

A number of 126 gingival crevicular fluid (GCF) samples were collected from the deepest periodontal pockets in the area from all patients before treatment and 1 month and 3 month after treatment. GCF samples were taken after air drying of the site, using isolation with cotton rolls. One paper strip was used for each collection

site. It was introduced into the gingival crevice 5 mm, with care being taken not to traumatize the tissues. The strip was left in place for 30 seconds. CRP level was determined using a high-sensitivity ELISA assay.

Clinical examination – Periodontal examination consisted of percentage plaque index (PI) and bleeding index (BI) and also, two clinical parameters: probing pocket depth (PPD) and clinical attachment level (CAL). The clinical parameters were assessed on 6 sites per tooth (mesio-, mid-, distobuccal and mesio-, mid-, distolingual) using manual Hu-Friedy® periodontal probe.

Treatment – The 31 patients were randomly distributed into 2 different samples of 15 and 16 subjects each. One sample (group control) received full mouth supra- and subgingival desinfection into 24 hours (3) and oral hygiene instructions. The 2nd sample (test group) received the same periodontal treatment but in combination with an adjunctive antimicrobial therapy consisting of systemic administration of doxycycline (GSK®,200mg / day, 7 days).

Supra and subgingival scaling was performed under local anesthesia, using XO Care Odontogain® device under a permanent irrigation with Listerine®. Root planing was performed using manual periodontal Gracey curettes (Hu-Friedy®).

RESULTS

Clinical results – At baseline, no important differences between groups were found for any of the clinical parameters.

After 1 month from active therapy, the periodontal condition in both groups had improved. At the 3 months examination, except for the PI, there was a significantly improve of all clinical parameters in the test group as compared to the control group ($p<0.05$) (Tabel 1 and Graphics 1, 2, 3).

Table 1 Periodontal condition and CRP amount before and after periodontal therapy

Parameters	Baseline		1month after therapy		3months after therapy	
	CG	TG	CG	TG	CG	TG
PI	69,3%	71,5%	11,2%	9,4%	12,4%	9,8%
BI	65,5%	69,2%	10,1%	7,9%	12,6%	7,8%
PPD	8.0±1.1	7.9±1.5	6.8±0.8	5.6±0.4	7.1±1.1	5.2±0.3
CAL	9.1±0.2	8.3±1.1	7.1±1.0	6.2±1.2	7.5±1.4	6.1±1.3
CRP amount(pg)	0.21±0.02	0.22±0.01	0.13±0.02	0.09	0.10±0.03	0.06±0.01

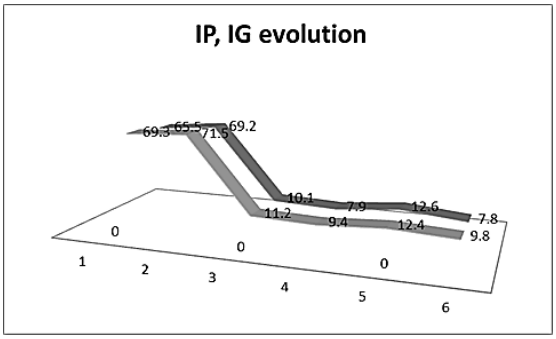


Fig. 1 Graphic 1. IP,IG evolution

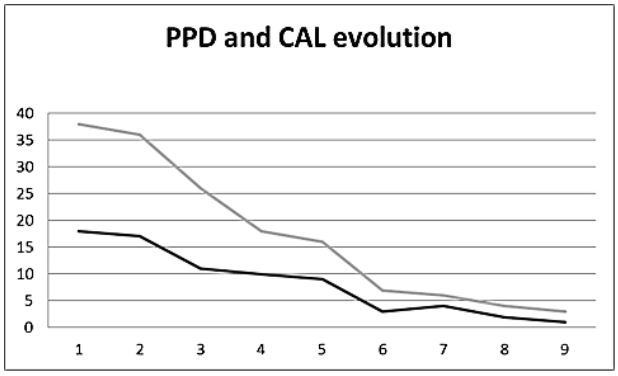


Fig. 2 PPD and CAL evolution

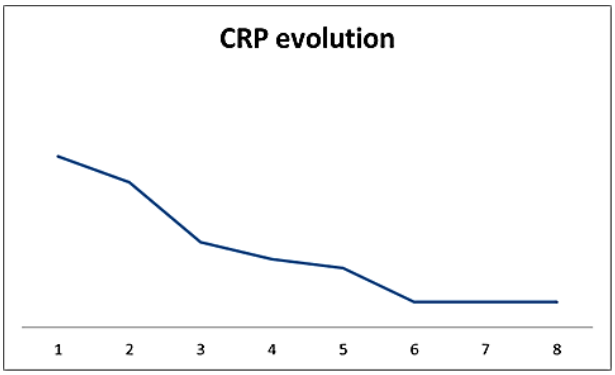


Fig. 3 CRP evolution

DISCUSSIONS

The evaluated studies and the Position Papers by the American Academy of Periodontology clearly suggest that the use of systemic antimicrobials as monotherapy in the treatment of periodontitis is not recommended [6]. All comments stated that the risk of using antimicrobials (systemic side effects, increase in antimicrobial resistance) should lead to restriction in their use in periodontitis in certain patients and certain conditions. Their use should be combined with mechanical debridement.

In the present, the vast majority of periodontitis cases respond well to conventional nonsurgical periodontal therapy: improved oral hygiene, "one stage full mouth desinfection" and supportive periodontal recall. However, certain patients, do not respond favorably to mechanical therapy alone. For these cases, the use of an appropriate adjunctive antimicrobial is often beneficial. In literature, we can find studies who showed very good results, with statistically significant inter-group differences. Systemic antibiotic therapy does not affect supragingival plaque accumulation. Reduction in dental plaque depends mostly on patients' oral hygiene efforts.

In general, the studies using doxycycline in treatment of periodontitis were poor in comparison with those using other antimicrobial agents evaluated.

Current evidence seems to suggest that the use of antibiotics should be limited to specific conditions, such as:

- Aggressive periodontitis;
- Severe chronic periodontitis;
- Refractory periodontitis;
- Necrotizing forms of gingivitis and periodontitis.

In present study, use of doxycycline in conjunction with SRP was shown to significantly reduce PD and improve CAL compared with control group in conjunction with SRP.

The results have indicated important improvements in clinical outcomes and also, in CRP level.

The main source of CRP is acknowledged to be the liver [8] and although several other tissues have recently been shown to produce these are regarded as having minimal contribution to serum levels of CRP.

Systemic CRP detected in GCF and periodontal tissue may be a result of systemic inflammation resulting from disease elsewhere in the body, as well as systemic inflammation induced by periodontitis [9].

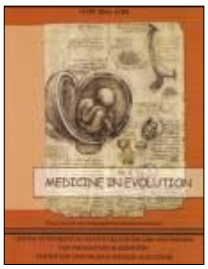
CONCLUSIONS

1. The adjunctive use of doxicycline with FMD is more effective than FMD alone and may represent a new approach in the long-term management of severe periodontitis.
2. Level of CRP in GCP samples is strongly influenced by active periodontal therapy.
3. CRP from GCP may have as source the inflammation from periodontal tissues

REFERENCES

1. Haffajee AD, Socransky SS: Microbial composition of supra and subgingival plaque in subjects with adult periodontitis. J Clin Periodontol 2000; 27: 722–732.
2. Socransky et co. Microbial complexes in subgingival plaque. J Clin Periodontol 1998; 25:134-144.
3. Quirinen m. et co., Benefit of "one-stage full-mouth disinfection" is explained by disinfection and root planing within 24 hours: a randomized controlled trial, J Clin Periodontol, 33, 9:639-647.
4. Kornman et co. The influence of supragingival plaque control on clinical and microbial outcomes following the use of antibiotics for the treatment of periodontitis, J Periodol, 98; 112-117.
5. Sedlacek M. et co. Antibiotic resistance in an in vitro subgingival biofilm model. Oral Microbiol Immunol 2007; 22:333-339.
6. Alex B. Berezow, Microbial shift and periodontitis, Periodontology 2000, 55, 1:36-47.
7. Hurlimann, Formation of C6 by Rabbit Liver Tissue In vitro, Immunology, 1968, 14, 649, 1966.
8. Paraskevas et co., A systematic review and meta-analyses on C-reactive protein in relation to periodontitis, JClin Periodontol, 2008; 35: 277–290.

ALL – CERAMIC INLAY – RETAINED FIXED PARTIAL DENTURES – FROM CONCEPT TO NUMERICAL ASSESSMENT



CANDEA, ADRIAN [1], BRATU DORIN [1], TOPALA FLORIN [1]

1. **Department of Prosthodontics, Faculty of Dentistry, University of Medicine and Pharmacy "Victor Babes", Timisoara, Romania**

ABSTRACT

Aim. *The objective of this study is to investigate different designs of minimally invasive YTZP (yttria stabilized polycrystalline tetragonal zirconia) all-ceramic inlay-retained fixed partial dentures (IRFPDs) using the finite element analysis, in order to evaluate stress distribution and total deformation under occlusal load.*

Materials and methods. *Identical tridimensional maxillary partial models were created, consisting of a first premolar, a missing second premolar, and a first molar, corresponding to four abutment preparation designs: occluso-proximal inlay, proximal box, palatal inlay with wings and palatal inlay. The tridimensional modelling of the abutment teeth, preparations and IRFPDs was achieved using Blender 2.58 and Rhinoceros (McNeel North America) NURBS (Non-uniform Rational B-Splines). Resulting geometry was imported in ANSYS FEA software to be used for structural simulations. Material characteristics (enamel, dentin, zirconia) were entered into the computer program and occlusal loading was performed. FEA was conducted and von Mises and total deformation were calculated.*

Results. *The highest stress concentration was observed on the mucosal aspect of the connector and also in the corresponding cervical shoulder area. The results demonstrate von Mises stresses ranging from 248MPa to 297MPa, with the highest stress for the proximal box preparation. The palatal inlay with wings preparation displayed the lowest total deformation (2.69µm).*

Conclusion: *Considering the occlusal forces in the posterior region of the dental arch it seems clinically possible to replace a posterior missing tooth using zirconia IRFPDs, successfully restoring both aesthetics and function.*

Keywords: *All-ceramic, inlay-retained, minimally invasive, finite element analysis.*

Correspondence to:

Cândea Adrian MD
Adress: Department of Prosthodontics, Faculty of Dentistry, University of Medicine and Pharmacy "Victor Babes", Timisoara, Romania, B-dul Revolutiei din 1989 nr. 9
Phone: +40 723 673790
E-mail address: adrian_candea@yahoo.com, florin.topala@gmail.com

INTRODUCTION

Dentistry nowadays attaches greater importance to preservation aspects rather than to the surgical approach of different clinical situations. Minimally invasive dentistry has brought a change in treatment philosophy and is gaining more and more ground [1].

At the same time, the patients' demand for aesthetic, metal - free, "tooth - like" restorations is growing faster than ever [1]. All-ceramic materials are becoming more and more widespread due to their increasing durability and longevity [2], when compared to fiber-reinforced composites, which present unstable aesthetics and wear of the veneering composite [3]. A great variety of all-ceramic systems is available on the market, with many core materials such as glass-infiltrated alumina (In Ceram), pure alumina (Procera), lithium-disilicate based glass-ceramic (IPS Empress 2, Emax Press) and YTZP yttria stabilized polycrystalline tetragonal zirconia (Lava, ZenoTec), for the CAD/CAM (computer aided design, computer aided machining) technique.

Whenever wanting to replace a missing tooth there are several treatment possibilities, with a varying degree of invasion regarding the adjacent tooth structure. If the patient rejects an implant or the insertion of an implant is not possible, it becomes compulsory to attach to the adjacent teeth. Thus, a certain preparation of the abutment teeth for the retainers becomes necessary. An extensive preparation for an all-ceramic full crown demands 63-73% tooth hard tissue sacrifice [4], and a major risk of pulpal and periodontal reactions [1, 4]. A conservative inlay preparation diminishes the pulpal and periodontal consequences [1]. Therefore, it seems desirable to use more conservative retainers and IRFPDs rather than traditional full-crown retainer FPDs whenever it is possible [4]. Due to its excellent mechanical properties, YTZP seems suitable to be used as a core material for IRFPDs.

Previous studies revealed a relatively high failure rate due to fracture of the connector, delamination or debonding [3-5]. Finding an optimized designs for the fabrication of IRFPDs regarding the abutment preparation for the retainer, the size and shape of the connector and of the gingival embrasure is necessary.

The research conducted looks into various designs of minimally invasive YTZP all-ceramic fixed partial dentures using the finite element analysis, in order to evaluate stress distribution and total deformation under occlusal load. This is achieved by comparing minimally invasive FPDs with occlusal-cervical insertion axis and minimally invasive FPDs with palatal-buccal insertion axis.

MATERIALS AND METHODS

Identical tridimensional maxillary partial models were created, consisting of a first premolar, a missing second premolar, and a first molar. The models included the outer crown surface, and the enamel-dentin interface. Four abutment preparation designs were considered: two with conventional occlusal-cervical insertion axis (1. Occluso-proximal inlay, 2. Proximal box) and two rather innovative ones with a palatal-buccal insertion axis (3. Palatal inlay with wings, 4. Palatal inlay) [6]. The 3D-modelling of the abutment teeth was performed using Blender 2.58. Then, the surfaces were reconstructed and the

pontic and preparations were modelled using Rhinoceros (McNeel North America) NURBS (Non-uniform Rational B-Splines), thus resulting four situations, with five models each.

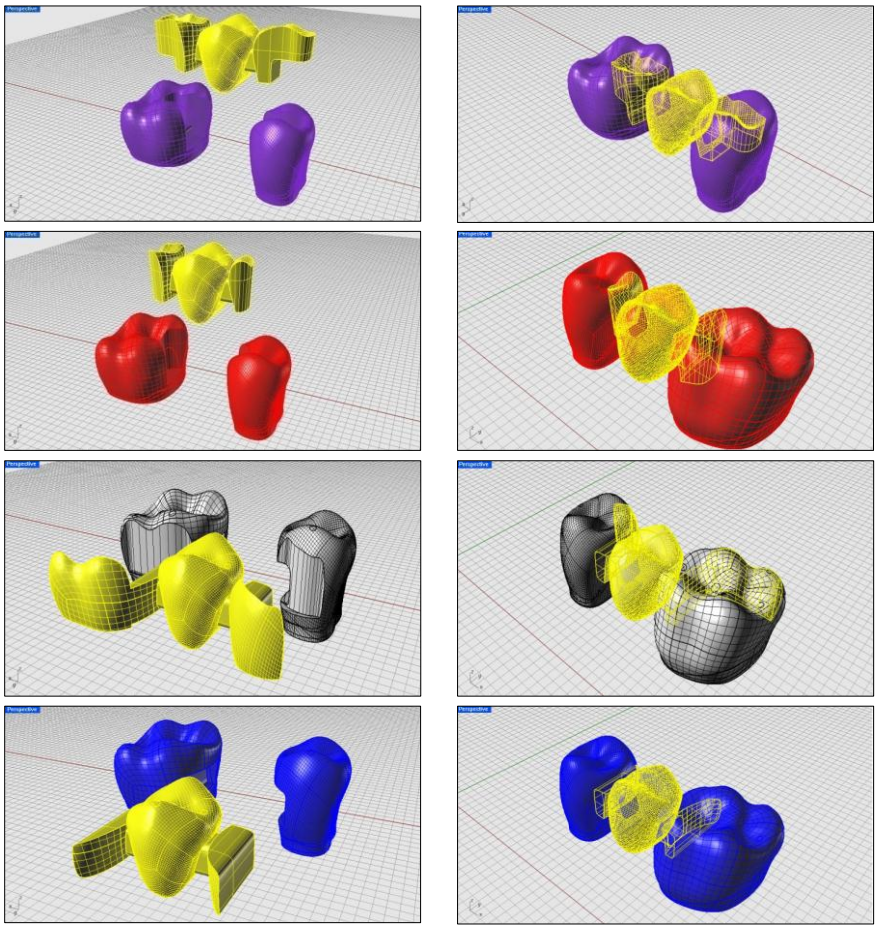


Fig. 1 The tridimensional models of the abutment preparations and the zirconia IRFPDs processed in Rhinoceros 3D v 4.0 (McNeel North America) NURBS (Non-uniform Rational B-Splines) – Occluso-proximal inlay retainers (1), Proximal box retainers (2), Palatal inlay with wings retainers (3), Palatal inlay retainers (4).

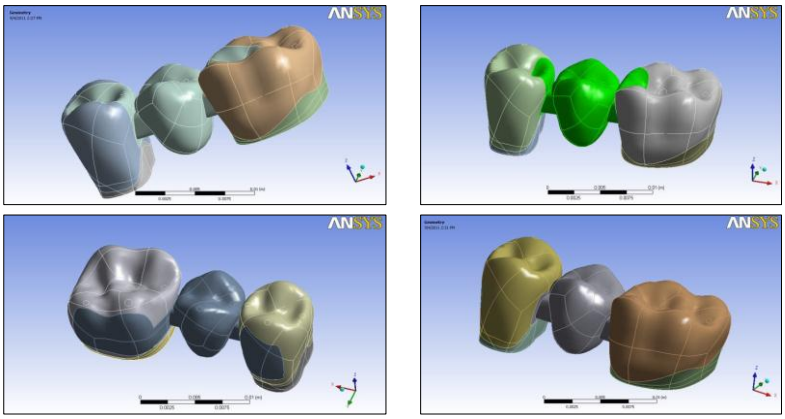


Fig. 2 The tridimensional models of the abutment preparations and the zirconia IRFPDs exported in the ANSYS finite element analysis software to be used for structural simulations

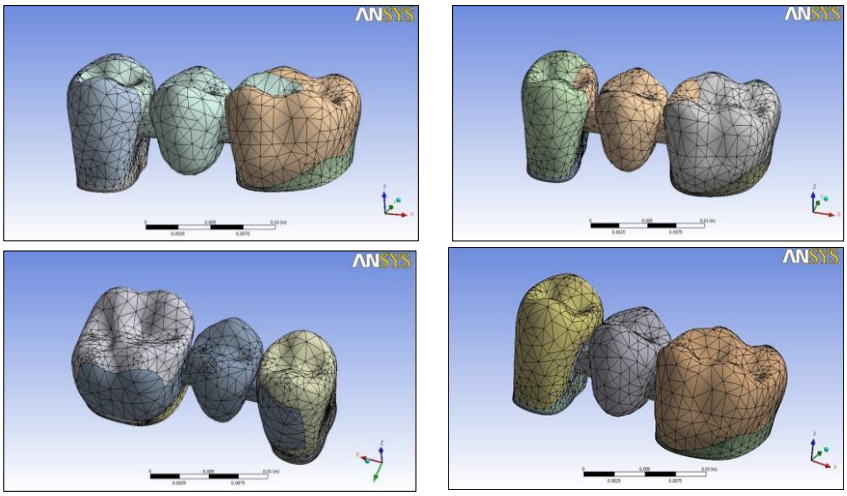


Fig. 3 Tridimensional meshing of the models – Occluso-proximal inlay retainers (1), Proximal box retainers (2), Palatal inlay with wings retainers (3), Palatal inlay retainers (4).

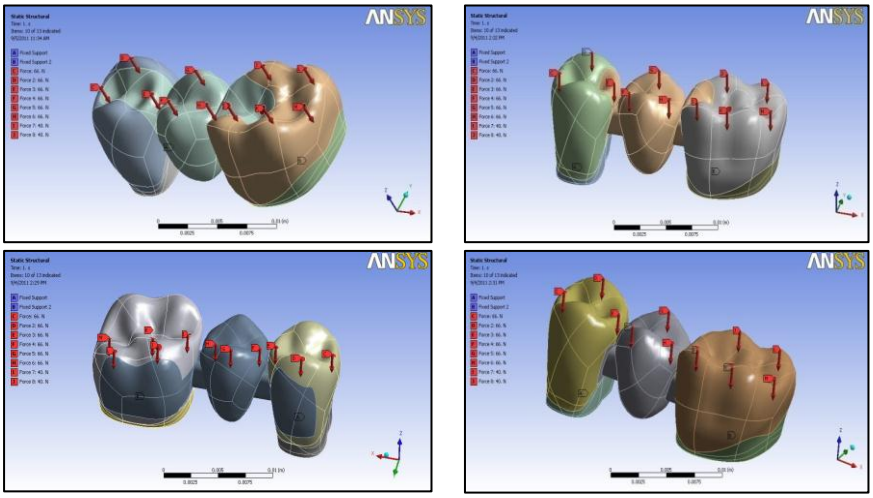


Fig. 4 Application of the occlusal forces – static load force of 200N/tooth

Table 1 Material properties

Material	**Young's modulus of elasticity (MPa)**	**Poisson's ratio**
Enamel	84.1	0.32
Dentine	18.6	0.32
YTZP	204	0.31

RESULTS

The results are illustrated in the figures 5, 6 and 7 and presented in table II and table III. Occlusal load applied on the occlusal surface of the pontic of the zirconia IRFPDs produces a higher stress value in the connector area, in the corresponding cervical floor area and also at the loading point. Von Mi-

ses stress (the equivalent stress) is an indicator that does not define stress as negative (tensile) or positive (compressive) [2] but as an expression of the whole stress field. It indicates the possibility of damage occurrence. The results demonstrate stresses ranging from 248 MPa to 297 MPa, with the highest von Mises stress for the proximal box abutment design.

Total deformation ranged from 2.69μm to 3.02 μm, with the lowest value for the palatal inlay with wings abutment design.

Table 2 The equivalent (von Mises) stress on the gingival aspect of the zirconia IRFPDs.

	First premolar	Connector Pm	Connector M	First molar
1. Class II inlay	35MPa	44MPa	42MPa	33MPa
2. Proximal box	40MPa	40MPa	38MPa	37MPa
3. Palatal inlay with wings	35MPa	46MPa	43MPa	38MPa
4. Palatal inlay	38MPa	43MPa	43MPa	38MPa

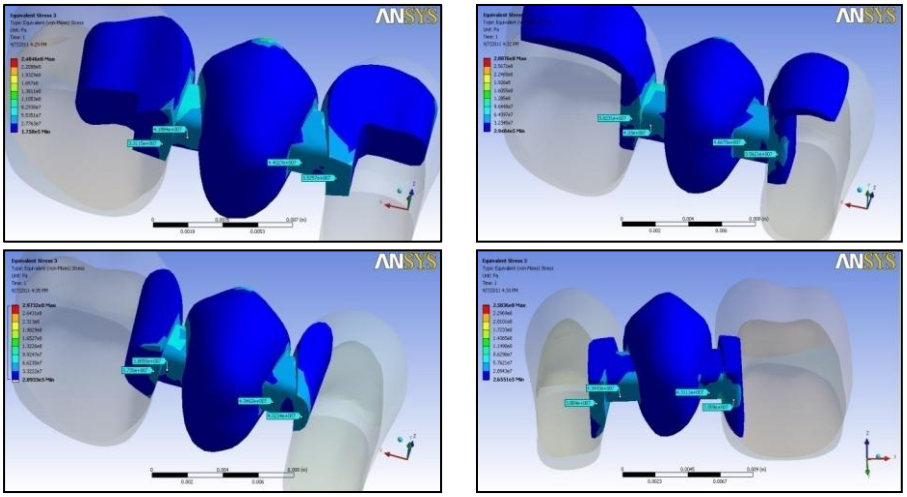


Fig. 5 Distribution of von Mises stress in the zirconia IRFPDs – Occluso-proximal inlay retainers (1), Proximal box retainers (2), Palatal inlay with wings retainers (3), Palatal inlay retainers (4).

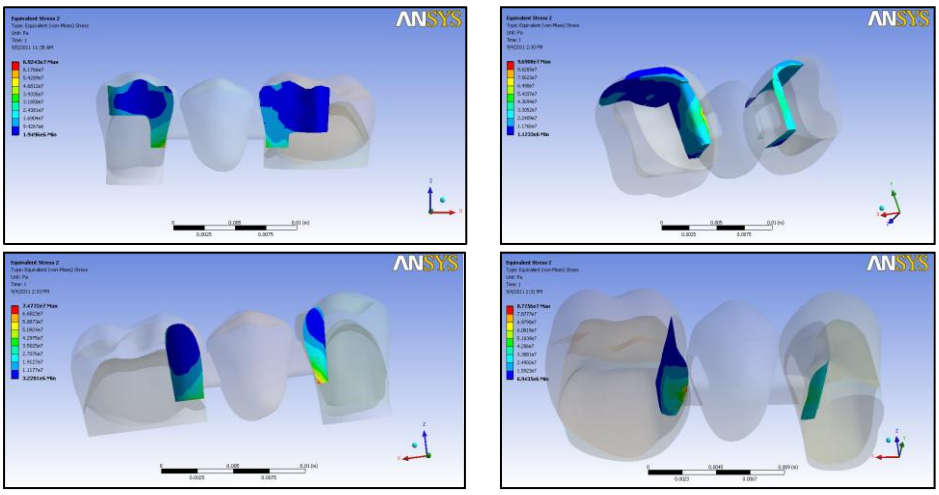


Fig. 6 Distribution of von Mises stress at the interface – Occluso-proximal inlay retainers (1), Proximal box retainers (2), Palatal inlay with wings retainers (3), Palatal inlay retainers (4).

Table 3 The equivalent (von Mises) stress on the gingival aspect of the zirconia IRFPDs.

	First premolar	Connector Pm	Connector M	First molar
1. Class II inlay	35MPa	44MPa	42MPa	33MPa
2. Proximal box	40MPa	40MPa	38MPa	37MPa
3. Palatal inlay with wings	35MPa	46MPa	43MPa	38MPa
4. Palatal inlay	38MPa	43MPa	43MPa	38MPa

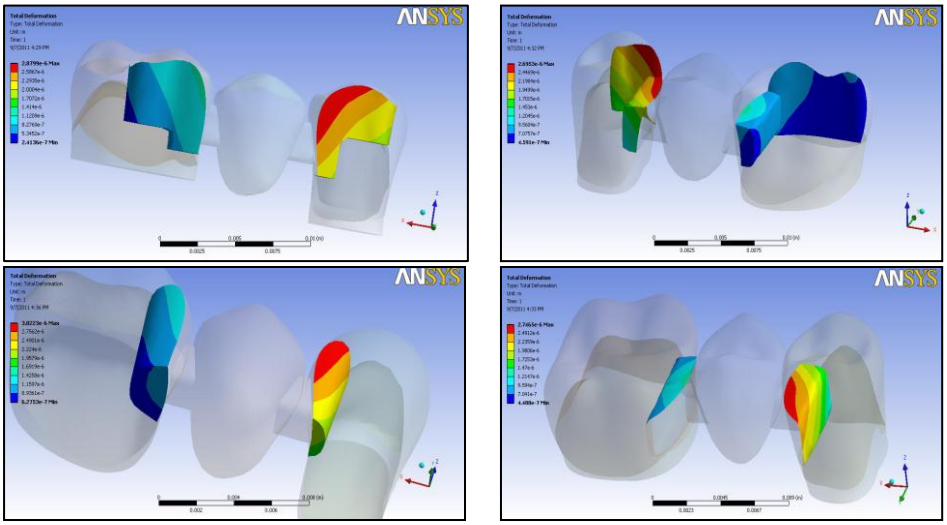


Fig. 7 Total deformation at the interface – Occluso-proximal inlay retainers (1), Proximal box retainers (2), Palatal inlay with wings retainers (3), Palatal inlay retainers (4).

Table 4 The equivalent (von Mises) stress and the total deformation of the zirconia IRFPDs.

	1. Class II inlay	2. Proximal box	3. Palatal inlay with wings	4. Palatal inlay
von Mises stress in the IRFPD	248MPa	297MPa	288MPa	258MPa
von Mises stress at the interface	69MPa	74MPa	96MPa	87MPa
Total deformation	2.87µm	3.02µm	2.69µm	2.74µm

DISCUSSIONS

All four situations display higher stresses in the embrasure between the pontic and the retainer. The stress values were similar for all four types of preparations for the retainers. The von Mises stress values were in accordance with those of Thompson [1, 2]. The registered values of von Mises stress were by a large amount beneath 900-1200 MPa (fracture strength of Y-TZP) [2]. The lower total deformation for the palatal inlay preparations reflects a lower probability of debonding for the latter, when compared to IRFPDs with occlusal-cervical insertion axis.

Despite the limitations of this study (no alveolar support and no periodontal ligament), it seems that YTZP IRFPDs could be a viable treatment option. It is important that various connector shapes and sizes should be investigated.

CONCLUSIONS

Perfecting the connector's shape and adapting its size and also perfecting the preparation and impression techniques for the palatal inlays could render these minimally invasive restorations a routine, for the benefit of the patients. We find our study results rather encouraging, especially regarding the palatal-buccal preparations. We consider it necessary for the spectrum of indications for these fixed restorations to be clarified.

In cases of replacing one posterior missing tooth using zirconia IRFPDs, both aesthetics and function can be successfully restored.

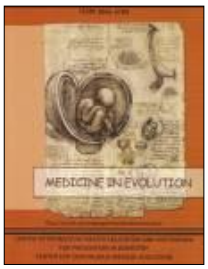
Acknowledgements

This study was supported by the project "BddcAEC" from the POSDRU/88/1.5/S/63117.

REFERENCES

1. Thompson, MC, Thompson, KM, Swain, M – The all-ceramic, inlay supported fixed partial denture. Part 1. Ceramic inlay preparation design: a literature review, Australian Dental Journal 2010; 55: 120-127
2. Thompson, MC, Field, CJ, Swain, MV – The all-ceramic, inlay supported fixed partial denture. Part 2. Fixed partial denture design: a finite element analysis, Australian Dental Journal 2011; 56: 302-311
3. Ohlmann, B., Rammelsberg, P., Schmitter, M., Schwarz, S., Gabbert, O. – All-ceramic inlay-retained fixed partial dentures: Preliminary results from a clinical study, Journal of Dentistry, Volume 36, Issue 9, Pages 692-696
4. Wolfart, S., Ludwig, K., Uphaus, A., Kern, M. – Fracture strength of all-ceramic posterior inlay-retained fixed partial dentures, Dental Materials, Volume 23, Issue 12, Pages 1513-1520
5. Harder, S, Wolfart, S, Eschbach, S, Kern, M – Eight-year outcome of posterior inlay-retained all-ceramic fixed dental prostheses, Journal of Dentistry 2010; 875-881
6. Sampaio-Fernandes, JCA, Silva, CL, Pinho, A, Fernandes, PF – A new system of adhesive fixed partial denture, Revista de odontologia da UNESP 2010; 39(5): 317-322.

CARIOUS EXPERIENCE OF FIRST PERMANENT MOLARS IN CHILDREN WITH PAST SEVERE EARLY CHILDHOOD CARIES



MUNTEANU ANETA[1], STANCIU IOANA-ANDREEA[1], LUCA RODICA[1], CATALINA FARCASIU[1]

1. **"Carol Davila" University of Medicine and Pharmacy Bucharest, Faculty of Dental Medicine, Pedodontics Department**

ABSTRACT

***Aim and objectives**: to analyze frequency and severity of carious pathology of first permanent molars (FPM) in children with past severe early childhood caries (S-ECC).*

***Material and methods**: A case-control study was conducted upon two groups of 76 children 6-12 years old, pair-matched: the study group (SG) – children with S-ECC in primary dentition and the control group (CG) – children with past caries experience only in primary molars.*

***Results**: 64.47% of children from the SG developed caries in FPM versus 38.16% in the CG. Mean DMFT/SFPM indexes were 1.82/2.53 in the SG and 0.93/1.21 in the CG ($p<0.001$).The percentage of the PFM with caries was almost double in the SG (44.74% versus 23.68%).*

***Conclusions**: Caries experience in FPM was significantly higher in the study group. It is important for parents to be aware about the necessity of dental check-ups for earlier application of preventive methods for caries on FPM.*

***Key-words**: carious pathology, first permanent molar, severe early childhood caries.*

Correspondence to:

Munteanu Aneta MD
Adress: Carol Davila University, Pedodontics Department, 12 Ionel Perlea street, district 1, Bucharest
Phone: +40721241181
E-mail address: ivan.aneta@yahoo.com

INTRODUCTION

According to the American Academy of Pediatric Dentistry, severe early childhood caries (S-ECC) is defined as any sign of decay on smooth surfaces in children younger than 3 years of age or, in children aged 3 to 5 years, carious involvement of one or more surfaces of the upper front teeth [1].

In addition to severe damage of primary teeth, this form of decay may have consequences for the child's permanent teeth (carious lesions, eruption disturbances of maxillary incisors, development anomalies of local cause), can be a source of functional disorders (aesthetics, mastication, phonetics etc.) or may influence the child's general development (weight and height lower than the accepted average, iron deficiency anemia etc.) [2 - 6].

Regarding carious involvement of permanent teeth, some studies have shown that children with carious lesions in primary dentition are more likely to develop caries in permanent dentition compared to caries-free children, first permanent molar (FPM) being the most affected tooth (Brook and Winter, 1975, cited by [7, 8, 9]).

AIM AND OBJECTIVES

The aim of this study was to analyze the frequency and severity of first permanent molars' carious involvement in children who had S-ECC compared to children without S-ECC that had caries experience only in primary molars, in order to establish some preventive strategies for FPM involvement in children diagnosed with S-ECC.

MATERIALS AND METHODS

A case-control study upon 2 samples of children examined in the Pedodontics Department - Carol Davila University Bucharest was performed. Each of the 2 samples comprised 76 children, pair-matched in age and gender. The study sample consisted of 76 children (41 boys, 35 girls) aged between 6 and 12 years (mean age = 8.14 ± 1.72 years), who had S-ECC in primary dentition. The control sample consisted of 76 children (41 boys, 35 girls) aged between 6 and 12 years, with a mean age of 8.22 ± 1.63 years, who had a common pattern of caries in primary dentition (caries only in primary molars).

Data regarding dental status was obtained from the dental records of the patients examined and treated by one dentist (M.A.). Collected data was electronically filed using SPSS program - version 10.0 for Windows (SPSS Inc., Chicago, IL, SUA) and processed using PSAW program (formerly SPSS) version 18.0 (2010). Mean dmf-t/s indexes in first dental examination were evaluated and, for the last dental examination, prevalence index of caries in permanent teeth and mean DMF-T/S indexes for all permanent teeth erupted and separately for FPM were calculated.

The results were statistically analyzed using Chi-square, Mann-Whitney and Kruskal-Wallis tests. The limit for statistical significance was set at $p < 0.05$.

RESULTS

I. At the first dental examination - The mean age of the 76 patients from the study sample at the first dental visit was 4.1±1.3 years (ranging from 1 to 5.9 years).

The average follow-up period of these patients was 4.0±1.8 years (range between 1 and 7.6 years). For the control sample, the mean age of the patients was 5.4±0.6 years (ranging between 2.5 and 5.9 years) and the average follow-up period was 2.8±1.7 years, ranging from 1 to 7.5 years.

Mean values of caries experience indexes were significantly higher in the study sample compared to controls ($p<0.001$) (table I).

Table 1 Caries experience indexes at the first dental visit

	dmft		***dmfs***	
	mean	range	mean	range
Study sample	10.41 ± 4.16	2-20	25.55 ± 13.86	2-64
Control sample	2.39 ± 1.32	1-5	3.26 ± 1.40	1-5

II. At the last dental examination

a) Caries prevalence in FPM 64.47% of the children who had S-ECC and 38.16% of those with common pattern of caries in primary dentition developed caries lesions in permanent teeth, differences being statistically significant ($p>0.05$). In both samples, all children with carious experience in permanent teeth had at least one affected FPM. Of the children with carious involvement of permanent teeth, 14.29% of the patients from the study sample and 6.90% of the controls developed caries in other permanent teeth except FPM.

b) Caries experience indexes. In both samples, caries experience indexes for all erupted permanent teeth and separately for FPM were analyzed (table II). Mean DMF-T/SFPM index values in the study sample were almost 2 times higher than values for the control sample, differences being statistically significant ($U= 1967$, $p < 0.001$ and $U= 2004$, $p < 0.001$, respectively).

c) FPM status. In the study sample, 44.73% of the erupted FPM had already treated or untreated caries and only 15.13% were sealed, compared to the control group, where 23.68% of the FPM had caries and 23.02% were sealed (table III). In most of the cases, FPM in children from the study group had caries on one or two dental surfaces, the most frequent caries location being occlusal and in buccal pits of lower molars. In the control group, occlusal caries were the most frequent.

Table 2 Caries experience indexes at the last visit in dental office

	DMF-T		***DMF-S***	
	total	FPM	Total	FPM
Study sample	2.18 ± 2.60	1.82 ± 1.64	2.99 ± 3.70	2.53 ± 2.48
Control sample	0.97 ± 1.45	0.93 ± 1.40	1.25 ± 2.06	1.21 ± 2.00

Table 3 FPM status

	FPM errupted	FPM with treated/untreated caries		Caries-free FPM			
				Total		Sealed	
		n	%	n	%	n	%
Study sample	304	136	44.73	168	55.3	46	15.13
Control sample	304	72	23.68	232	76.3	70	23.02

DISCUSSION

S-ECC is an acute form of decay characterized by multiple carious lesions in primary teeth, including tooth surfaces usually more resistant to decay and by a high concentration of Streptococcus mutans, which creates prerequisites for development of carious lesions in permanent teeth, even shortly after their eruption [10, 11].

In the present study, children with past S-ECC (mean dmf-t=10.41) developed significantly more carious lesions in permanent teeth compared to children who had a common pattern of caries in primary teeth. Moreover, caries prevalence in FPM and caries experience indexes in FPM were significantly higher in the study sample compared to the control sample, children who had S-ECC developing 2.2 times more caries lesions on FPM.

These results are consistent with data reported by other researchers [8, 9, 12-14]. Thus, al-Shalan et al. (1997) found that 40% of S-ECC children aged 4 years old or under had treated or untreated caries on FPM at the last dental examination, when children were around 9 years old [12].

Regarding the predicting value of carious involvement of permanent teeth according to caries experience of primary teeth, it is considered that the identification of children with low risk of developing caries is easy to perform, while accurately determining of high-risk individuals is more difficult (Hausen 2008; Koch et al., 2009 cited by [15]). Regarding the S-ECC children, some studies have shown that in this category of patients a large number of carious lesions in primary dentition has a higher predictive value than the presence of caries only in maxillary front teeth [8, 9, 13]. Thus, in a cohort study over a period of eight years on a sample of 362 Chinese children, Li and Wang (2002) showed that patients having caries in primary teeth were 3 times more likely to develop caries in their permanent teeth (RR=2.6; 95%CI=1.4-4.7; p<0.001). Caries on primary maxillary incisors alone or fewer decayed teeth (dmft<6) in children aged 3-5 years might not necessarily be a sufficient predictive risk indicator for future caries. In contrast, children with S-ECC who had dmft scores equal to or greater than 10 were 3.5 times at greater risk of developing caries in their permanent dentition [8].

Kaste et al. (1992) have demonstrated that a dmf-t≥5 in primary teeth seems to be a risk factor for future caries in permanent teeth 10 years later (DMF-T≥5; RR=2.4; 95% CI=1.4, 4.3). On the contrary, caries on smooth surfaces of upper front teeth are not a representative indicator for subsequent caries in permanent teeth – RR=1.1 (95%CI=1.1, 2.4) [9].

In fact, Heller et al. (2000), using insurance claims data, have found that primary front teeth treatment at ages 0-3 was weakly associated (RR=1.43; 95% CI=1.23, 1.65) with treatment of the FPM at ages 6-8 [13].

On the other hand, Alm et al. (2007) showed in a study on 568 Swedish chil-

dren that children with actual caries at 3 years of age had a higher risk of developing approximal caries in their permanent teeth than caries-free children of the same age (41 vs. 17%) ($p < 0.001$). Additionally, early childhood caries experience (developed before 3 years of age) had a greater predictive value than late childhood caries experience (developed between 3 and 6 years of age) with regard to approximal caries at 15 years of age [14].

All these researches highlight the fact that data regarding the health status of primary teeth in toddlers and preschool children can be very useful in determining caries risk, as in the planning of dental health care methods to be used later [13, 15].

In the present study, the number of FPM with treated or untreated caries was almost 2 times greater in the study sample compared to the corresponding values obtained for the control sample, which demonstrates the increased caries activity of children with S-ECC. Regarding the topography, most of the caries lesions were located on the occlusal surface or in buccal pits of lower molars. These caries location patterns, on those tooth surfaces where decay can be prevented by sealing, point out the need for parents of S-ECC children to be made aware of the increased risk of caries on FPM and of the importance of regular check-ups at short intervals in order to allow early application of local prevention methods. Moreover, in this study, the number of sealed FPM was 30% lower in the study group compared to controls, indicating that further efforts are needed in order to find the best ways to educate parents in general and particularly parents whose children had S-ECC and help them understand the role of periodic preventive dental check-ups.

CONCLUSIONS

1. Caries prevalence index and caries experience indexes on FPM had significantly higher values in children with S-ECC than in children with common pattern of caries in primary teeth;
2. It is necessary for parents to be informed on the importance of early application of caries preventive methods on FPM.

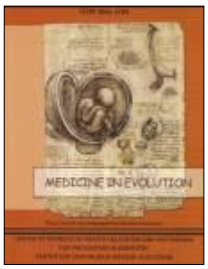
REFERENCES

1. American Academy of Pediatric Dentistry. Policy on Early Childhood Caries (ECC): Unique Challenges and Treatment Options. Pediatr Dent 2009; 31(6) (Reference Manual 2009-2010): 44-46.
2. Luca R. Pedodonție vol. 2. Ed. Cerma, Bucureşti, 2003: 49-69.
3. Ferro R, Besostri A, Meneghetti B, Beghetto M. Comparison of data on Early Childhood Caries (ECC) with previous data for Baby Bottle Tooth Decay (BBTD) in an Italian kindergarten population. Eur J Paediatr Dent 2004; 2: 71 –75.
4. Losso EM, Tavares MCR, da Silva JYB, Urban CA. Severe early childhood caries: an integral approach. Jornal de Pediatria (Rio J) 2009; 85(4): 295-300.
5. Motisuki C, Santos-Pinto L, Giro EMA. Restoration of severely decayed primary incisors using indirect composite resin restoration technique. Int J Paed Dent 2005, 15: 282-286.
6. Tang RC, Huang ST, Huang MC, Chuang FH, Chen HS. The nutritional status of young children with severe early childhood caries in southern Taiwan. Int J Paed Dent 2009; 19 (Suppl. 1): 108.
7. King NM, Anthonappa RP, Ittharagun A. The importance of the primary dentition to children – Part 1: consequences of not treating carious

teeth. The Hong Kong Practitioner 2007; 29: 52-61.

8. Li Y, Wang W. Predicting Caries in Permanent Teeth from Caries in Primary Teeth: An Eight-year Cohort Study. J Dent Res 2002; 81(8): 561-566.
9. Kaste LM, Marianos D, Chang R, Phipps KR. The assessment of nursing caries and its relationship to high caries in the permanent dentition. J Public Health Dent 1992; 52(2): 64-68.
10. Berkowitz RJ. Causes, Treatment and Prevention of Early Childhood Caries: A Microbiologic Perspective. J Can Dent Assoc 2003; 69(5): 304-307.
11. Thibodeau EA, O'Sullivan DM. Salivary mutans streptococci and dental caries patterns in preschool children. Community Dent Oral Epidemiol 1996; 24(3): 164-168.
12. Al-Shalan TA, Erikson PR, Hardie NA. Primary incisor decay before age 4 as a risk factor for future dental caries. Pediatr Dent 1997; 19: 37-41.
13. Heller KE, Eklund SA, Pittman J, Ismail AA. Associations between dental treatment in the primary and permanent dentitions using insurance claims data. Pediatr Dent 2000; 22: 469-474.
14. Alm A, Wendt LK, Koch G, Birkhed D. Prevalence of approximal caries in posterior teeth in 15-year-ol Swedish teenagers in relation to their caries experience at 3 years of age. Caries Res 2007; 41: 392-398.
15. Ollila P. Assessment of caries risk in toddlers. A longitudinal cohort study. Acta Univ Oul., 2010, Oulu: 15-61

EVALUATION OF THE SOFT TISSUE ESTHETIC CHANGES AFTER ORTHODONTIC TREATMENT



ADRIANA MUNTEAN[1], ELISABETA BRATU[2], CORINA VERNIC[3]

1. **University of Medicine and Pharmacy V. Babeş Timişoara, Department of Esthetic Dentistry**
2. **University of Medicine and Pharmacy V. Babeş Timişoara, Department of Pedodontics and Orthodontics**
3. **University of Medicine and Pharmacy V. Babeş Timişoara, Department of Informatics and Biostatistics**

ABSTRACT

***Introduction**: The aim of the study was to evaluate the effects of Angle Class of patients on their change in facial aesthetics following orthodontic treatment. Material and method: 82 patients from the Department of Orthodontics of the University of Medicine and Pharmacy Victor Babes Timisoara were selected. The patients were divided into two groups (41 with ages between 12-16 years old and 41 with ages between 18-33 years old). Cephalometric analysis was used to determine the Angle Class and position of the upper and lower lips to the esthetic line of Ricketts.*

***Results**: Improvement of facial aesthetics by orthodontic treatment was significant for Class II division 1, and Class II division 2 patients, but not for Class I and III patients on both groups.*

***Conclusions**: The relation between the lips and the aesthetic line showed significant changes with increasing age, so this could be an important factor in establishing orthodontic diagnosis and treatment plan.*

***Key words**: Esthetic line, Angle class, orthodontics.*

Correspondence to:

Muntean Adriana MD
Adress: Bd. Revolutiei 1989, Timişoara, Romania
Phone: +40744522118
E-mail address: eamuntean@yahoo.com

INTRODUCTION

The major reason why people seek orthodontic treatment is improvement of facial aesthetics (Birkeland et al., 1999; Kiyak, 2000)[4]. Treatment planning of facial attractiveness is difficult, especially when the two goals of attractiveness and bite correction are combined. Sometimes the orthodontist's will to correct the bite may even result in a decrease of facial attractiveness[1]. All orthodontists nowadays admit that success in orthodontic treatment is closely tied to favourable changes in facial soft tissue[3]. By knowing the soft tissue traits and their normal range, a treatment plan can be designned to normalize the facial traits for a given individual[2].

MATERIALS AND METHODS

The aims of the study were to investigate the esthetic line of the profile before and after orthodontic treatment and to evaluate the possible effects of Angle Class on facial aesthetics following orthodontic treatment.

82 patients divided into two groups (41 with ages between 12-16 years old and 41 with ages between 18-33 years old) from the Department of Orthodontics of the University of Medicine and Pharmacy Victor Babes Timisoara were selected for the study. The inclusion criteria for the patients was treatment time duration up to 3,3 years and no dental or facial trauma.

Cephalometric analysis was used to determine the Angle Class and position of the upper and lower lips to the esthetic line of Ricketts. Neither the severity of the malocclusion nor the chosen treatment modality was important for this study, since the aim was to determine only the change in facial aesthetics after orthodontic treatment. Angle Classifications were defined as follows: Angle Class I: neutro-occlusion and neutro-relationship of the jaws; Class II division 1: disto-occlusion and disto-relationship of the jaws, with proclined upper incisors; Class II division 2: disto-occlusion and disto-relationship of the jaws, with retroclined upper incisors; and Class III: mesio-occlusion and mesio-relationship of the jaws.

The material for the study consisted of lateral cephalometric radiographs that were taken prior to and after the treatment. The patients were in standing position, with teeth in occlusion and relaxed position of lips. All radiographs were traced by the same investigator, using Cephx program of online measurements. Cephalograms were oriented with the facial profile to the right. The parameter evaluated before and after finishing the treatment was the relationship of the upper and lower lips to Rickett's esthetic line. All data obtained was statistically analyzed.

RESULTS and DISCUSSION

The 12-16 age group comprised of 23 women (56.1%) and 18 men (43.9%), with an average age of 13.78 +/-1.458 (12.16). Among these, 51.2% had a malocclusion of Angle class II, 34.1% were in the Angle class I, while 14.6% had a molar relation of Angle class III.

By calculating the distance between the aesthetic line of Ricketts and the upper and lower lips for each pa-

tient, statistically significant differences were observed for Angle class II, as for the distance from the upper lip (p = 0.04825, S). The results are presented in table no. 2.

Table 1 The distribution of the patient lot having ages between 12-16, In Angle classes

	Frequency	Percentage
1	14	34.1
2	21	51.2
3	6	14.6
Total	41	100.0

Table 2 The results of the statistical analysis between the positioning of the lips at the beginning and the end of the treatment for Angle class II, 12-16 age groups

Initial distance aesthetic line – upper lip	Final distance aesthetic line – upper lip	p	Significance
6.89+/ - 3.26	8.49+/ - 1.39	0.04825	S
Initial distance aesthetic line – lower lip	Final distance aesthetic line – lower lip		
6.52 +/ - 2.89	6.22 + / -1.26	0.6663	NS

The 18-33 age group comprised of 23 women (56.1%) and 18 men (43.9%), as well, with an average age of 25.29+/- 4.473(18.33). The distribution in Angle classes is presented in table 3. Table 4 presents the results of the statistical analysis significant for this age group, again at the level of Angle class II and for the position of the upper lip. (p = 0.02704, S).

Table 3 The distribution of the patient lot having ages between 18-33 in Angle classes

	FREQUENCY	*PERCENTAGE*
1	*12*	*29,3*
2	*22*	*53,7*
3	*7*	*17,1*
TOTAL	*41*	*100*

Table 4 The results of the statistical analysis between the positioning of the lips at the beginning and the end of the treatment for Angle class II, 18-33 age groups

Initial distance aesthetic line – upper lip	Final distance aesthetic line – upper lip	p	Significance
5.98+/ - 4.36	8.37+/ - 2.06	0.02704	S
Initial distance aesthetic line – lower lip	Final distance aesthetic line – lower lip		
6.12 +/ - 3.97	5.69 +/ - 1.92	0.6507	NS

CONCLUSIONS

When seeking orthodontic treatment, patients usually have great expectations. The dentists that refer the patients to an orthodontist also expect a big improvement in dental and facial esthetics [4]. This study shows significant improvements only for class II division 1 and class II division 2, but not for class I and III. This means that orthodontists and other clinicians should be cautious with the promises of esthetic improvement that they make to the patients and parents of patients, so that they won't be disappointed that the facial aspect is modified only to a certain degree.

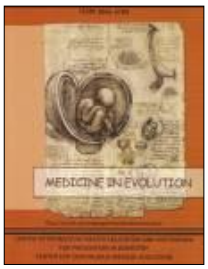
The material for the study consisted of lateral cephalometric radiographs that were taken prior to and after the treatment. The patients were in standing position, with teeth in occlusion and relaxed position of lips. All radiographs were traced by the same investigator, using Cephx program of online measurements. Cephalograms were oriented with the facial profile to the right.

The parameter evaluated before and after finishing the treatment was the relationship of the upper and lower lips to Rickett's esthetic line. All data obtained was statistically analyzed.

REFERENCES

1. Bergman R.T. , Cephalometric soft tissue facial analysis, Am J Orthod Dentofacial Orthop 1999; 116: 373-89
2. Bishara SE, Jakobsen JR, Hession TJ, Treder JE. Soft tissue profile changes from 5 to 45 years of age, Am J Orthod Dentofacial Orthop. 1998 Dec; 114(6): 698-706.
3. Bravo L.A., Soft tissue profile changes after orthodontic treatment with four premolars extracted, The Angle Orthodontist, 1994, 64(1), 31-42
4. Rosemie M. A. Kiekens , Jaap C. Maltha , Martin A. van 't Hof , Huub Straatman and Anne M. Kuijpers-Jagtman, Panel perception of change in facial aesthetics following orthodontic treatment in adolescents, European Journal of Orthodontics, 2008, 30(2): 141-146.

SOLUTIONS FOR EDENTULISM CLASS III KENNEDY WITH MODIFICATION THROUGH MODERN TECHNOLOGIES. REPORTED CASE



PAUL BOLD [1], AUREL BOLD [1]

1. **Dental Medical Faculty . University of Medicine and Pharmacy „Victor Babes" Timisoara**

ABSTRACT

The aesthetic requirements of the last years led to the development of totally ceramic restorations which answer the patient's desires and/or the practitioner's professional satisfaction. Restorations on zirconium support (ZrO2) have gradually won more field in disfavour of restorations on metallic support.

The costs afferent to totally ceramic restorations on zirconium support have made the patients to accept them less willingly, but in the end the physiological and physiognomic benefits have been very convincing.

***Key words**: totally ceramic restorations, zirconium oxide, physiognomy, physiology*

Correspondence to:

Prep. Paul Bold
Adress: Universitatea de Medicina si Farmacie "Victor Babes" din Timisoara, Facultatea de Medina Dentara, departamentul 3 disciplina de protetica dentara
Phone: 0722742112
E-mail address: paul@drbold.ro

CASE REPORT

Patient T.T., 34 years old comes to the clinic for prosthesis for the gaps in his dentition.

During the clinical examination, was confirmed edentulism class III Kennedy with one modification, inappropriate shape of abutments for retention, too conical and presenting a hypertrophy of oral mucosa on the toothless gum, which led to the apparent shortening of abutments.

Because of the apparently too short abutments and implicitly the lack of retention a surgical coronal extension was performed.

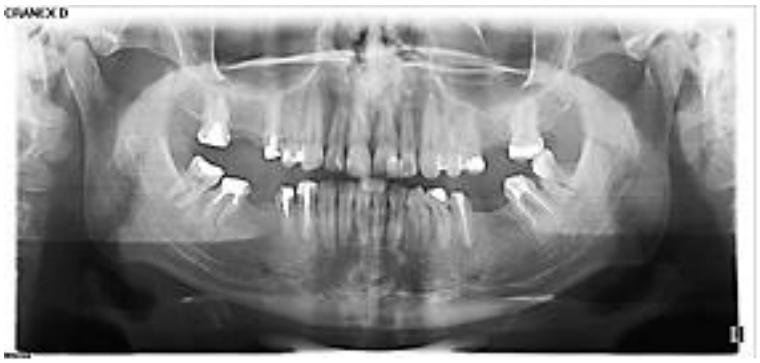


Fig.1

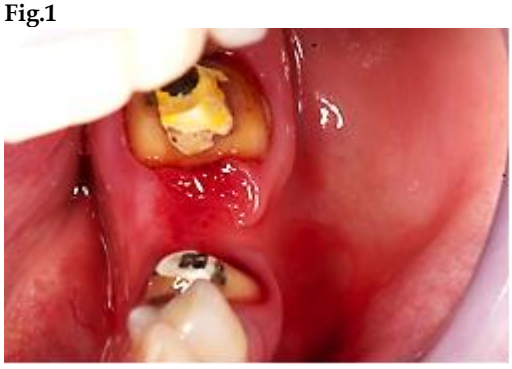


Fig.2

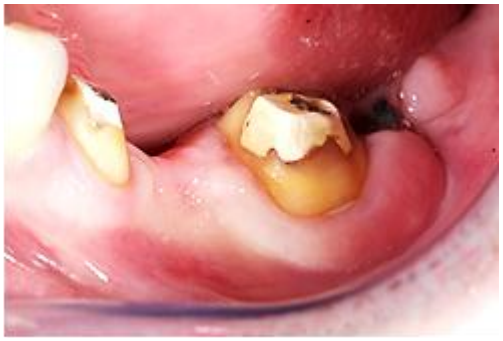


Fig.3

Fig.4

Fig.5

Due to the high aesthetic demands, the choice was made for prosthetic fix restorations on zirconium oxide support.

The abutments were prepared using the kit Modified Chamfer Preparation II, Komet Dr. Domenico Massironi, Italy.

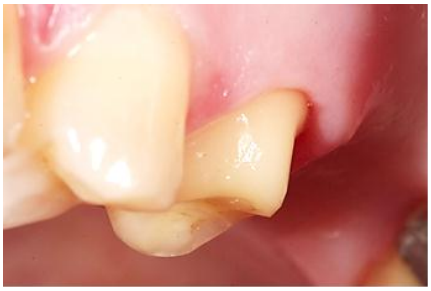


Fig.6

The impression was obtained through the technique of one single retraction courd with addition silicone (Honigum DMG), one step tehnique and the registration of the occlusion was also carried out using addition silicone (Colorbite ZERMACK).

Fig.7

In the lab, the ZrO2 structure was carried out through computer assisted milling, complying with the digital impression. The intra-oral adjustment is checked and afterwards, the ceramic mass is applied to the structure.

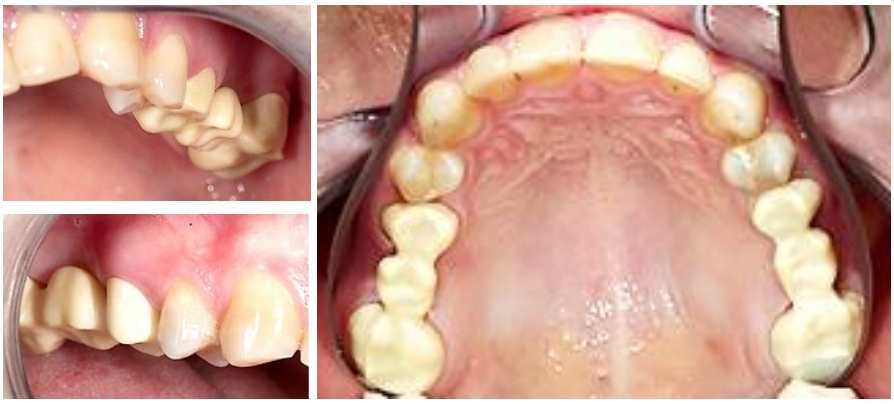


Fig.8

A major step in the technique of fix prosthetic restoration is the occlusal adjustment of the restorations.

Individualization of the restorations was carried out according to the remaining teeth, affected by tetracycline stain.

The fixation was carried out with RelyX Unicem A2 Universal Self-Adhesive Universal Resin Cement (3M).

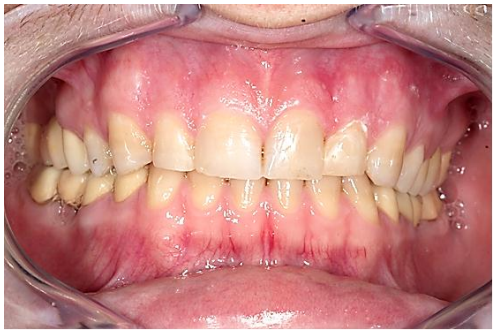


Fig.9

CONCLUSIONS

The aesthetic requirements promoted as an evidence of health contributed to the development of ceramic on zirconium support against ceramic on metallic support. The anatomical impediments can be surgically solved when aesthetic comes first. The presented case is a significant example of how we can combine positive thinking with physiognomic and functional requirements.

REFERENCES

1. M. Fradeani, G. Barducci, Prosthetic Treatment a systematic approach to esthetic, biological and functional integration, 2008, Quintessence Publishing
2. J. Tinschert, G. Natt, Oxidkeramiken und CAD / CAM- Tehnologien, Deutscher Zahnaerzte Verlag Koln.
3. Bratu D., Nussbaum R. - Bazele clinice şi tehince ale potezării fixe, Editura Signata 2001
4. Chu SJ. Precision shade technology: Contemporary strategies in shade selection. Pract Proced Aesthet Dent 2002; 14: 79-83.
5. Dancy WK, Yaman P, Dennison JB, O'Brien WJ, Razzoog ME. Color measurements as quality criteria for clinical shade matching of porcelain crowns. J Esthet Restor Dent 2003; 15: 114-121.
6. Dietschi D, Ardu S, Krejci I. Exploring the layering concepts for anterior teeth. In: Roulet JF, Degrange M (eds). Adhesion: The Silent Revolution in Dentistry. Chicago: Quintessence, 2000: 235-251.
7. Ikeda T, Nakanishi A, Yamamoto T, Sano H-Color differences and color changes in Vita Shade tooth-colored restorative materials. Am J Dent.2003; 16(6): 381-4.
8. Lichter JA, Solomowitz BH, Sher M. Shade selection. Communicating with the laboratory technician. NY State Dent J 2000; 66(5): 42-46.
9. Reich S, Hornberger H. The effect of multicolored machinable ceramics on the esthetics of all-ceramic crowns. J Prosthet Dent 2002; 88:44-49.
10. Rolf Ankil. Zwischen allen Stühlen. Quintessenz Zahntech 2006; 32(3)

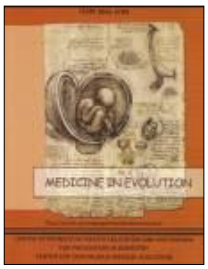
Centrele de Cercetare blend-a-med și Oral-B

Este nevoie de efort - zi de zi - pentru a îmbunătăți cu adevărat sănătatea orală a pacienților. De aceea, P&G Oral Health, cu Centrele sale de Cercetare blend-a-med și Oral-B îi susține pe medicii dentiști atât în cabinet, cât și prin extinderea influenței lor dincolo de unitul dentar. Când pacienții părăsesc cabinetul de medicină dentară, produsele și serviciile noastre sunt un sprijin pentru ei.

blend-a-med RESEARCH CENTERS Oral-B

P&G Oral Health

EFFICACY AND CLINICAL TOLERABILITY OF THE THERAPY WITH FLUVASTATINE RETARD 80 MG IN HYPERTENSION ASSOCIATED WITH CORONARY DISEASE



MARIA SUCIU[1], CARMEN CRISTESCU[1], MIRELA VOICU[1], LIANA SUCIU[1], RALUCA GRADINARU[1], BOGDAN BUMBACILA[1]

1. **University of Medicine and Pharmacy „Victor Babes", Faculty of Pharmacy, Department of Pharmacology – Clinical Pharmacy**

ABSTRACT

Aim: The objective of this study is to monitor the evolution of the efficacy and clinical tolerability of the retard drug, fluvastatin - Lescol 80 mg -, in hypertension and coronary diseases, which are frequently associated in clinical practice.

Material and method: The study has been conducted on 44 patients hospitalized for a period of 6 months. One tablet Lescol 80 mg was administrated to all of the patients, in the evening during the hospitalization and for 1 month after being released.

Results and Conclusions: The benefits of the treatment with HMG-CoA reductase inhibitors, respectively Lescol 80 mg 1 tablet/day used in our study have been observed in 22 patients (59.46%) in a period of approximately 45 days from beginning of the treatment, leaving hope that the results in an interval of 3-6 months are more encouraging.

***Keywords**: efficacy, tolerability, Lescol retard, hypertension, coronary disease*

Correspondence to:

Maria Suciu
Adress: UMF "Victor Babes"Timisoara, Faculty of Pharmacy, Eftimie Murgu Square nr.2 cod 300041
Phone: 004- 0723319050
E-mail address: suciu01maria@yahoo.com

INTRODUCTION

Hypertension is considered a chronic disease that can be present from childhood, but more frequently after the age of 35. It is also called "silent killer" because it often shows no symptoms that can lead to a precise diagnose. When associated to coronary disease, with high cholesterol or diabetes mellitus, the risk of cerebral vascular stroke, congestive cardiac failure or acute myocardial attack is even greater[1].

Low-density lipoprotein cholesterol (LDL-C) is recognized as the primary factor in the development and progression of atherosclerotic disease and elevated LDL-C level remains the primary target of lipid lowering therapy [2, 3]. The two reports, the Third Report of the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III in 2001[2] and the full report in 2002[4], demonstrate the importance of lipid-lowering therapy for both primary prevention of coronary heart disease (CHD) and secondary prevention of cardiovascular events in patients with established CHD. The results of the studies of the next years on the cholesterol lowering medication has led to the appearance of news guidelines, where the statins have an important place, demonstrated by their benefits in slowing progression or even inducing regression of atherosclerosis [3, 5].

Multiple trials demonstrated the efficacy of the statins in lowering the cholesterol, but also the LDL-C6, [7]. Cholesterol lowering medication, represented by the HMG-CoA reductase inhibitors, should be part of the treatment regime of hypertensive patients [8] who also suffer from associated coronary disease according the present clinical guides [9, 10].

It is administered in order to ameliorate the symptoms, to reduce morbidity and cardiovascular mortality, to reduce the number of hospitalizations and even the number of persons retired due to medical causes [7].

Starting from these aspects we have considered necessary monitoring the efficacy of a retard statin, such as fluvastatin, on lowering of the endogen cholesterol synthesis [11, 12].

AIM AND OBJECTIVES

The present study tries to monitoring the efficacy of a retard agent, such as fluvastatin, pharmaceutical name Lescol XL, on lowering of the endogen cholesterol synthesis at patients with primary hypercholesterolemia and mixed hyperlipemia.

MATERIALS AND METHODS

The study has been developed during 6 months: from July 1, 2010 to December 31, 2010, on 44 patients. The assignment on sexes within the study lot has been represented in the following manner: 33 men (75%) and 11 women (25%). The patients within the study lot were between 40 and 80 years old, with an average age of 53 ± 5 – 66 ± 4, assigned as follows: between 40-50 years old: 4 patients (9.1%), between 50-60 years old: 22 patients (50%), between 60-70 years old: 16 patients (36.35%), between 70-80 years old: 2 patients (4.55%). Among the most important symptoms present at the moment

of their hospitalization, we mention: pre-cord pains – 7 patients, retro-stern pains – 4 patients, pain at the level of the post-thorax, with irradiation on the left arm or at the neck basis –7 patients, palpitation –32 patients, sweating –40 patients, dyspnea –12 patients, anxiety –11 patients, bad general health state –7 patients.

Patients admitted for inpatient care were diagnosed with hypertension in different stages and their assignment within the lot has been presented below: HBP I stage – 3 patients (7%), HBP II stage – 33 patients (75%) and HBP III – 8 patients (18%).

All selected patients suffered from associated coronary disease, which has been classified after the electrocardiogram (EKG), echocardiography, Doppler echography in the following stages: monovascular coronary disease: 23 patients (52%), bivascular coronary disease: 17 patients (39%) and trivascular coronary disease: 4 patients (9%).

After performing the standard lab tests, at admission, we've marked the values of lipids, triglycerides, cholesterol and its fractions, which are shown below: cholesterol - 18 patients (41%) normal values; 26 patients (59%) raised values; HDL-cholesterol - 21 patients (48%) normal values, 23 patients (52%) raised values; LDL-cholesterol - 17 patients (39%) normal values, 27 patients (61%) raised values; total lipids - 19 patients (43%) normal values, 25 patients (57%) raised values; triglycerides - 20 patients (45%) normal values, 24 patients (55%) raised values.

After performing the glucose test, the following results were obtained: 23 patients (52%) showed normal blood sugar levels, 18 patients (40.91%) showed values higher than 115mg%, and 3 patients (6.82%) showed levels below 75 mg%. Following the clinical and laboratory investigations during the hospitalization, the following diagnoses have been established within the lot:

Table 1 Distribution of diagnoses in the study lot

Diagnoses	Patients' number	Percentage
Hypercholesterolemia	26	59%
Hypertension	44	100%
Coronary disease	44	100%
Cardiac failure - NYHA I	1	2%
- NYHA II	13	30%
Overweight 1st degree	10	23%
2nd degree	7	16%
3rd degree	1	2%
Diabetes mellitus – type I	0	0
– type II	16	36%

From patients' personal pathological antecedents, we've retained the following: hypertension – 36 patients (81. 82%), ischemic heart disease – 8 patients (18. 18%), angina pectoris 11 patients (25%), myocardial infarction 19 patients (43. 18%), myocardial infarction with coronary stent 10 patients (22. 73%), hypercholesterolemia 16 patients (36.36%), diabetes mellitus type II 18

patients (41%), cerebral vascular stroke 2 patients (4. 54%), cardiac rhythm disorders - atrial flutter type - 1 patient (2. 27%).

Among the significant disease family history we retained the following:

Table 2 Classification of family history within the study lot

Hereditary-collateral antecedents	Number of patients	Percentage
Ischemic heart disease	10	22.73%
Hypertension	37	84.1%
Myocardial infarction	15	34.1%
Hypercholesterolemia	20	45.45%
Diabetes mellitus	5	11.36%
Peripheral artery occlusive disease	8	18.18%
Vascular cerebral stroke	6	13.63%
Angina pectoris	7	15.91%
Cardiac malformation	1	2.27%
No antecedents	7	15.91%

From the patients' history we have observed 3 risk factors, which favor cardiovascular diseases; their appearance within the study lot is presented in the table below:

Table 3 Distribution of three major risk factors in cardiovascular diseases within the study lot

Alcohol	Coffee	Tobacco
16 patients	20 patients	33 patients
Occasionally – 11 patients	Occasionally – 7 patients	Former smokers 22 patients
Frequently – 5 patients	Daily – 13 patients	Smokers – 11 patients

All 44 patients received Lescol cpr. 80 mg 1 tablet/day in the evening as an anti - hypercholesterolemia treatment, regardless of the cholesterol values, either for prevention or for cure, taking into consideration the other associated risk factors. To this, we have associated the corresponding treatment schemes according to diagnoses, which are represented by the medicine classes as follows:

I. Drugs administered for chronic diseases, of the following types: beta-adrenergic blocking agents, calcium channel blocking agents, angiotensin converting enzyme inhibitors, antiarrhythmic agents, antiplatelet agents, vasodilators, diuretics.

II. Drugs administered for different associated diseases, of the following types: gastrointestinal agents, anxiolytic agents, antiasthmatic agents, oral antidiabetic agents, non-steroidal anti-inflammatory agents, vasoprotective drugs. The patients' evaluation has been performed at release and at 30 days

after the release by monitoring cholesterol, triglycerides, total lipids values and of the HDL-cholesterol and LDL-cholesterol fractions, respectively of the cardiac functions. The drug tolerability and compliance has been rated on 4 scales: very good, good, satisfactory, non-satisfactory. We have also carefully followed the possible interactions with other medicines.

RESULTS AND DISCUSSION

The Lescol cpr. 80 mg efficacy, administered 1 tablet/day in the evening has been followed by monitoring the lab tests regarding cholesterol, triglycerides, and total lipids values and of the HDL-cholesterol and LDL-cholesterol fractions at release and at 30 days after release when the patients came for check-up.

The results at release compared to the admission day are shown in the table below:

Table 4 The investigation results during 3 study stages

Paraclinical investigation		**Patients hospitalized**	**Patients released**	**Patients at check-up**
Total cholesterol	normal	18	20	22
	- raised	26	24	15
LDL	normal	17	21	23
	- raised	27	23	14
HDL	normal	21	24	25
	- raised	23	20	12
Total lipids	normal	19	22	24
	- raised	25	22	13
Triglycerides	normal	20	23	24
	- raised	24	21	13

The drug tolerability has been estimated as: very good - 36 patients (81. 82%); good - 6 patients (13. 64%); satisfying - 1 patient (2. 27%) and non- satisfying - 1 patient (2. 27%).

The patients' compliance to the treatment, although sometimes raised due to a poly-pathology, has been estimated as: very good - 29 patients (65.91%); good - 9 patients (20.45%) and moderate - 6 patients (16.34%).

The hospitalization period within the study lot has varied according to the patients' status, with an average of 10 ± 2 days. The following situations have been encountered: 7 days - 5 patients (11. 36%), 8 days - 9 patients (20. 45%), 10 days -18 patients (40. 91%), 12 days - 6 patients (13. 64%), 15 days - 6 patients (13. 64%).

In 30 days from release, only 37 patients (84.1%) from the total 44 initial lot patients came to the check-up, the other 7 were absent. From the lab tests data, 30 days from the release (see table IV) we have established the following development of the cholesterol values and other lipid metabolism investiga-

tions during the treatment with Lescol 80 mg, 1 capsule/day: 3 patients (8. 11%) presented important cholesterol lowering, 19 patients (51. 35%) presented medium cholesterol lowering, 3 patients (8.11%) presented mild cholesterol lowering, 7 patients (18. 92%) presented identical cholesterol values as in hospitalization moment, 5 patients (13. 51%) presented cholesterol increase.

To the treatment regime of the 5 patients who presented cholesterol increase there has been associated also fenofibrate drugs of the type Lipanthyl supra 160 mg (3 patients), and Regadrin-B dragée 200 mg (2 patients).

CONCLUSIONS

1. The Lescol 80 mg efficacy in the study lot at 30 days from release has been very good at 22 patients (59. 46%); good at 3 patients (8. 11%); satisfying at 7 patients (18. 92%) and non- satisfying at 5 patients (13. 51%).

2. The treatment regime in the hospital has significantly improved the hospitalization symptoms at the entire lot of initial patients.

3. The Lescol tolerability during the whole period has been estimated as very good at 36 patients (81. 82%); good at 6 patients (13. 64%); satisfying at 1 patient (2.27%) and non-satisfying at 1 patient (2.27%). No drug interactions of Lescol with other medicines have been observed.

4. Patients' compliance to the treatment schemes during hospitalization has been estimated as very good in 29 patients (65. 91%); good in 9 patients (20. 45%) and moderate in 6 patients (13. 64%).

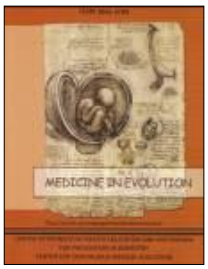
5. The 5 patients (13. 51%) who haven't responded to the treatment with Lescol 80 mg, there have been administered a fenofibrate of the type Lipanthyl Supra 160 mg, respectively Regadrin – B 200 mg, and they should be evaluated in 3 months from treatment.

REFERENCES

1. Ridker P.M., Inflammation, atherosclerosis, and cardiovascular risk: an epidemiologic view, Blood Coagul Fibrinolysis, 1999, 10 (suppl. 1), S9-S12.
2. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III), JAMA, 2001, 285, 2486-2497.
3. Smith S.C., Allen J., Blair S.N., et al., AHA/ACC guidelines for secondary prevention for patients with coronary and other atherosclerotic vascular disease, 2006 update, J Am Coll Cardiol, 2006, 47, 2130-2139.
4. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report, Circulation, 2002, 106, 3143-3421.
5. Grundy S.M., Cleeman J.I., Bairey Merz C.N., et al, Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines, Circulation, 2004, 110, 227-239.
6. Baigent C., Keech A., Kearney P.M., Blackwell L., Buck G., Pollicino C., Kirby A., Sourjina T., Peto R., Collins R., Simes R., Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90056 participants in 14 randomised trials of statins, Lancet, 2005, 366, 1267–1278.
7. Thavendiranathan P., Bagai A., Brookhart M.A., Choudhry N.K., Primary prevention of cardiovascular diseases with statin therapy: a meta-analysis of randomized controlled trials, Arch Intern Med, 2006, 166, 2307–2313.

8. Borghi C., Dormi A., Veronesi M., Immordino V., Ambrosioni E., Use of lipid-lowering drugs and blood pressure control in patients with arterial hypertension, J Clin Hypertens, 2002, 4, 277–285.
9. 2007 Guidelines for the management of arterial hypertension, The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC) European Heart Journal, 2007, 28, 1462–1536.
10. Guidelines on the management of stable angina pectoris: executive summary, The Task Force on the Management of Stable Angina Pectoris of the European Society of Cardiology, European Heart Journal, 2006, 27, 1341–1381.
11. Olsson A.G., Pauciullo P., Soska V., et al, Comparison of the efficacy and tolerability of fluvastatin extendedrelease and immediate-release formulations in the treatment of primary hypercholesterolemia: a randomized trial, Clin Ther, 2001, 23, 45-61.
12. Isaacsohn J.L., LaSalle J., Chao G., et al, Comparison of treatment with fluvastatin extended-release 80-mg tablets and immediate-release 40-mg capsules in patients with primary hypercholesterolemia, Clin Ther, 2003, 25, 904-918.

IN VITRO ACTIVITY OF URSOLIC AND OLEANOLIC ACID ON A2058 (HUMAN MELANOMA) AND A2780 (HEPATIC CARCINOMA)



OFELIA CERGA[1], FLORIN BORCAN[3], CAMELIA SASS[3], ATENA GALUSCAN[3], IULIANA POPOVICI[1]

1. **"Gr.T.Popa" University of Medicine and Pharmacy Iasi, Faculty of Pharmacy**
2. **"V. Babes" University of Medicine and Pharmacy Timisoara, Faculty of Pharmacy**
3. **"V. Babes" University of Medicine and Pharmacy Timisoara, Faculty of Dentistry**

ABSTRACT

Aim and objectives: The main target of this paper was to study the antiproliferative effects of ursolic and oleanolic acids.

Material and methods: Two human cell lines, A2058 (human melanoma) and A2780 (hepatic carcinoma), were used in MTT assay in order to measured the in vitro activity of ursolic and oleanolic acids.

Results: It was found that oleanolic acid present good cytotoxic activities on the two human cell lines, with a significant increase at 96 hours after treatment in the case of A2780 cell line.

Conclusions: Based on the obtained results, it could be assumed that oleanolic acid can be a good treatment on hepatic carcinoma.

***Keywords**: ursolic acid, oleanolic acid, human melanoma, hepatic carcinoma, MTT*

Correspondence to:

Ofelia Cerga
Adress: "Gr.T.Popa" University of Medicine and Pharmacy Iasi, Faculty of Pharmacy, 16th Universitatii Street., Iasi-700115
Phone: 0745625641
E-mail address:

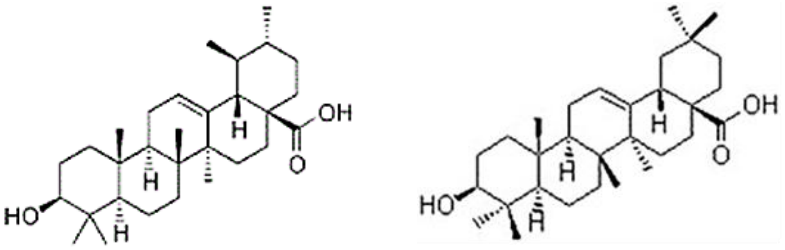
INTRODUCTION

Ursolic and oleanolic acids (UA and OA) are two pentacyclic terpenoids, which were found in many plants, especially in waxy coatings that protect fruits and leaves. Both acids have important phytomedicinal properties and biological activities. It was shown that these acids protect liver [1-4], and have anti-inflammatory effects [5-8] being used in ointments to treat burns.

UA (3-β-hydroxy-urs-12-en-28-oic acid) (Figure 1a), has formula C30H48 O3 and the molecular weight equal with 456.7. It is present in apples, bilberries, prunes, cranberries, peppermint, lavender, thyme, oregano, hawthorn, elder flower. Ursolic acid has topically and internally medicinally action. There are many UA-based cosmetic preparations for its anti-inflammatory, antitumor and antimicrobial properties. Ursolic acid has antibacterial and antifungal activity. Tests have shown that UA inhibits the growth of Candida albicans and Microsporium lenosum. It was demonstrated that topical application of UA inhibits TPA-induced initiation and promotion of tumor growth.

For their effects, medicinal plants containing UA have been used in folk medicine. In the literature, it was revealed a few pharmacological effects of UA, such as, anti-tumor, hepatoprotective, anti-inflammatory (oral and topical), anti-ulcer, antimicrobial, antihyperlipidemic and antiviral [9].

OA (Oleanolic acid; 3beta-Hydroxyolean-12-en-28-oic acid) (Figure 1b), has the same formula and molecular weight with UA. It has antifungal [10, 11], insecticidal [12], anti-HIV [13, 14], diuretic [15], complement inhibitory [16], blood sugar depression [17] and gastrointestinal transit modulating [18] activities.



A B

Fig. 1. Chemical structure of (a) UA and (b) OA

AIM AND OBJECTIVES

The main target of this paper was to study the antiproliferative effects of ursolic and oleanolic acids.

MATERIALS AND METHODS

Ursolic acid (UA) and oleanolic acid (OA) were purchased from Sigma Aldrich (Taufkirchen, Germany). DM SO was used as solvent and it was purchased from Fluka (Buchs, Switzerland). The concentration of stock solution of UA and OA in DMSO for all tests was 10 mM, but it were also prepared diluted solutions of the two active compounds with concentration equal with 5 mM in order to compare the cytotoxic activities.

MTT assay – Antiproliferative effects were measured in vitro on two human cell lines (ECACC; Salisbury, UK): A2058 (human melanoma) and A2780 (hepatic carcinoma) with the MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]) assay. The cells were cultivated in minimal essential medium supplemented with 10% fetal bovine serum, 1% non-essential amino acids and an antibiotic-antimycotic mixture. All media and supplements were obtained from PAA Laboratories (Pasching, Austria). The cells were grown in a humidified atmosphere of 5% CO2 at 37 °C. Cancer cells (5000/well) were seeded onto a 96-well microplate and attached to the bottom of the well overnight. On the second day, 200 µL of new medium containing the test substances was added. After incubation for 72 h, the living cells were assayed by the addition of 20 µL of 5 mg/mL MTT solution. MTT was converted by intact mitochondrial reductase and precipitated as blue crystals during a 4 hours contact period. The medium was then removed, and the precipitated crystals were dissolved in 100 µL of dimethyl sulfoxide (DMSO) during a 60 minutes period of shaking. Finally, the reduced MTT was assayed at 545 nm, using a microplate reader; wells with untreated cells were utilized as controls. All in vitro experiments were carried out on two independent microplates with at least five parallel wells. The MTT evaluation was developed on the same time and the same preparation of cells. Stock solutions of the tested substances (10 mM) were prepared with DMSO and the highest DMSO concentration (0.3%) of the medium did not have any significant effect on the cell proliferation.

RESULTS

It was observed that cells from the cancer cell lines underwent different alterations.

It is well-known that in the case of A2058 cells, after four hours it started the modification process of cytoplasm and another two hours later the level of adhesion is influenced (many cells detached easily from the plastic flasks and moved into medium).

Table 1 Cytotoxic Effect of UA on the studied cell lines

A2058 (human melanoma)								
	24 h		48 h		72 h		96 h	
Conc, mM	10^5 live cells	Survival, %	10^5 live cells	Survival, %	10^5 live cells	Survival, %	10^5 live cells	Survival, %
0	7.1±0.1	100.0	9.8±0.2	100.0	14.8±0.2	100.0	18.4±0.3	100.0
5	3.8±0.1	53.5	4.8±0.1	48.9	5.1±0.3	34.4	3.9±0.1	21.2
10	1.4±0.2	19.9	1.5±0.2	15.8	1.4±0.2	9.7	1.4±0.2	8.1
A2780 (hepatic carcinoma)								
	24 h		48 h		72 h		96 h	
Conc, mM	10^5 live cells	Survival, %	10^5 live cells	Survival, %	10^5 live cells	Survival, %	10^5 live cells	Survival, %
0	9.2±0.2	100.0	7.6±0.1	100.0	11.3±0.2	100.0	14.1±0.2	100.0
5	5.6±0.1	60.8	4.1±0.3	53.9	4.5±0.2	34.4	3.4±0.2	24.1
10	2.3±0.1	25.2	1.6±0.1	21.5	2.2±0.1	19.7	2.3±0.1	16.3

Started density 105 cells/mL. Results are means + S.D.

In other studies 2, it was observed that at the same time disintegration of the nuclei and cell lysis started; these can be observed in the period between

6 and 8 hours after treatment. Dividing cells are completely absent.

All changes and cell damages were irreversible: elimination of the extract 4 hours after treatment, followed by trypsinisation and transfer of the cells in a fresh culture medium not restored their normal status.

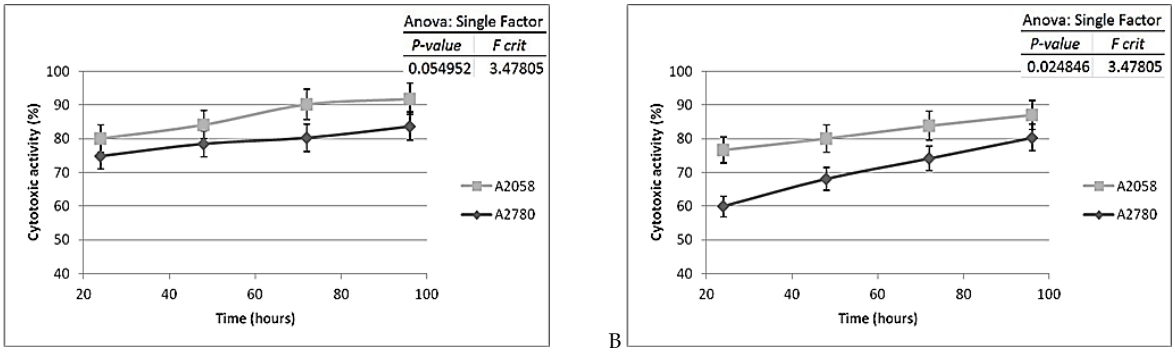
Cells were still unable to attach to the plastic flasks and formed multicellular aggregates, which floated in the medium. Two hours later died. It was observed that cells not attach to the flask and died rapidly when DMSO solutions were applied at the beginning of incubation (alterations were more drastic in this case). The results showed that the viability of cells depends of concentration and time. For the studied cell lines, the dependency was valid for almost all concentrations and the whole period tested (Table I and II), with no exceptions.

The cytotoxic activity for the stock solutions (10 mM active substances in DMSO) is present in Figure 1.

Table 2 Cytotoxic Effect of OA on the studied cell lines

A2058 (human melanoma)								
Conc, mM	24 h		48 h		72 h		96 h	
	10^5 live cells	Survival, %	10^5 live cells	Survival, %	10^5 live cells	Survival, %	10^5 live cells	Survival, %
0	9.9±0.2	100.0	9.7±0.1	100.0	11.4±0.3	100.0	15.7±0.2	100.0
5	4.7±0.2	47.7	3.9±0.1	39.9	2.9±0.2	25.3	2.8±0.1	17.8
10	2.3±0.1	23.3	1.9±0.1	19.9	1.8±0.2	16.1	2.0±0.2	12.9
A2780 (hepatic carcinoma)								
Conc, mM	24 h		48 h		72 h		96 h	
	10^5 live cells	Survival, %	10^5 live cells	Survival, %	10^5 live cells	Survival, %	10^5 live cells	Survival, %
0	9.3±0.1	100.0	9.5±0.2	100.0	12.3±0.1	100.0	16.6±0.3	100.0
5	5.8±0.2	62.2	4.3±0.2	45.1	3.8±0.3	31.2	3.9±0.2	23.6
10	3.7±0.1	40.1	3.0±0.1	31.9	3.2±0.2	25.8	3.2±0.2	19.6

Started density 105 cells/mL. Results are means + S.D.



A B

Fig. 1. Citotoxic activity of (a) UA and (b) OA on A2058 and A2780 cell lines

DISCUSSION

Analyzing the results obtained in this study, it is easy to observe that the best cytotoxic activity was recorded for the oleanolic acid on A2780 cell line. In the Figure 1, there is shown that OA have a better activity than UA; there were recorded for OA a significant increase of 20% in 96 hours on the A2780 cell line and a normal increase of 10% in 96 hours on the A2058 cell line. In the case of treatment with UA, there were obtained low increase of cytotoxic activity in 96 hours: 12% on the A2058 cell line and only 8% on A2780 cell line.

Results from this proliferation test showed unambiguous cytotoxic effects and antitumour activity of UA and OA on treated in vitro cells. The substances caused typical changes in the shape of the examined cancer cells, altered their ability to attach to plastic flasks and to form monolayers. Considerable reduction in the number of the viable cancer cells was observed in other study [9] together with irreversible structural damages–vacuolization of the cytoplasm, disintegration of the nuclei and lysis of the cell. These changes caused cell death, which set in as early as the first hours of treatment.

CONCLUSIONS

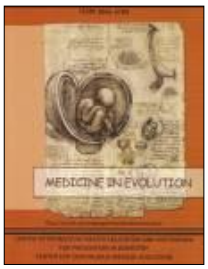
The ursolic and oleanolic acids are well-known pentacyclic terpenoids with important phytomedicinal properties and biological activities. Their antiproliferative effects were measured in this study on two human cell lines: A2058 (human melanoma) and A2780 (hepatic carcinoma) with the MTT assay. Taking into account the correlation between cell survival and concentration of the active substances / time after treatment, it could be assumed that oleanolic acid can be a good treatment on hepatic carcinoma.

REFERENCES

1. Yim TK, Wu WK, Pak WF, Ko KM. Hepatoprotective action of an oleanolic acid-enriched extract of Ligustrum lucidum fruits is mediated through an enhancement on hepatic glutathione regeneration capacity in mice. Phytother. Res. 2001; 15: 589-592.
2. Saraswat B, Visen PK, Agarwal DP. Ursolic acid isolated from Eucalyptus tereticornis protects against ethanol toxicity in isolated rat hepatocytes. Phytother. Res. 2000; 14: 163-166.
3. Latha PG, Panikkar KR. Modulatory effects of ixora coccinea flower on cyclophosphamide-induced toxicity in mice. Phytother. Res. 1999; 13: 517-520.
4. Jeong HG. Inhibition of cytochrome P450 2E1 expression by oleanolic acid: hepatoprotective effects against carbon tetrachloride-induced hepatic injury. Toxicol. Lett. 1999; 105: 215-222
5. Ismaili H, Tortora S, Sosa S, Fkih-Tetouani S, Ilidrissi A, Della Loggia R,Tubaro A, Aquino R. Topical anti-inflammatory activity of Thymus willdenowii. J. Pharm. Pharmacol. 2001; 53: 1645-1652.
6. Giner-Larza EM, Manez S, Recio MC, Giner RM, Prieto JM, Cerda-Nicolas M, Rios JL. Oleanonic acid, a 3-oxotriterpene from Pistacia, inhibits leukotriene synthesis and has anti-inflammatory activity. Eur. J. Pharmacol. 2001; 428: 137-143.
7. Ryu SY, Oak MH, Yoon SK, Cho DI, Yoo GS, Kim TS, Kim KM. Antiallergic and anti-inflammatory triterpenes from the herb of Prunella vulgaris. Planta Med. 2000; 66: 358-360.
8. Baricevic D, Sosa S, Della Loggia R, Tubaro A, Simonovska B, Krasna A, Zupancic Topical anti-inflammatory activity of Salvia officinalis L. leaves: the relevance of ursolic acid. J. Ethnopharmacol. 2001; 75: 125-132.
9. Liu J. Pharmacology of oleanolic and ursolic acid. J. Ethnopharmacology 1995; 49: 57-68.
10. Tang HQ, Hu J, Yang L, Tan RX. Terpenoids and flavonoids from Artemisia species. Planta Med. 2000; 66: 391-393.
11. Jeong TS, Hwang EI, Lee HB, Lee ES, Kim YK, Min BS, Bae KH, Bok SH, Kim SU. Chitin synthase II inhibitory activity of ursolic acid, isolated from Crataegus pinnatifida. Planta Med. 1999; 65: 261-263.
12. Marquina S, Maldonado N, Garduno-Ramirez ML, Aranda E, Villarreal ML, Navarro V,Bye R, Delgado G, Alvarez L. Bioactive oleanolic acid saponins and other constituents from the roots of Viguiera decurrens. Phytochemistry 2001; 56: 93-97.
13. Kashiwada Y, Nagao T, Hashimoto A, Ikeshiro Y, Okabe H, Cosentino LM, Lee KH. Anti-AIDS agents 38. Anti-HIV activity of 3-O-acylursolic acid derivatives. J. Nat. Prod. 2000; 63:1619-1622.
14. Ma C, Nakamura N, Hattori M, Kakuda H, Qiao J, Yu H. Inhibitory effects on HIV-1 protease of constituents from the wood of Xanthoceras sorbifolia. J. Nat. Prod. 2000;63:238-242.
15. Alvarez ME, Maria AO, Saad JR. Diuretic activity of Fabiana patagonica in rats. Phytother. Res. 2002;16:71-73.

16. Assefa H, Nimrod A, Walker L, Sindelar R. Enantioselective synthesis and complement inhibitory assay of A/B-ring partial analogues of oleanolic acid. Bioorg. Med. Chem. Lett. 2001; 11: 1619-1623.
17. Yoshikawa M, Matsuda H. Antidiabetogenic activity of oleanolic acid glycosides from medicinal foodstuffs. Biofactors 2000; 13: 231-237.
18. Li Y, Matsuda H, Yoshikawa M. Effects of oleanolic acid glycosides on gastrointestinal transit and ileus in mice. Bioorg. Med. Chem. 1999; 7: 1201-1205.

CONTRIBUTIONS TO THE CHEMICAL STUDY OF THE ESSENTIAL OIL ISOLATED FROM CARAWAY FRUITS



TRIFAN ADRIANA, APROTOSOAIE ANA CLARA, SPAC AADRIAN, CIOANCA OANA, DRUTU CATALINA, STANESCU URSULA

1. **Department of Pharmacognosy, "Gr. T. Popa"University of Medicine and Pharmacy, Faculty of Pharmacy**
2. **Department of Physical Chemistry, "Gr. T. Popa"University of Medicine and Pharmacy, Faculty of Pharmacy**
3. **Agricultural Research and Development Centre, Secuieni, Neamț, Romania**

ABSTRACT

Introduction: *Caraway fruits (Carum carvi L., Apiaceae) are used in phytomedicine as a digestive, anti-foaming, spasmolytic and galactagogue, due to its essential oil content. Many parameters such as genetic and climatic factors, the maturity stage of the plant or agronomical practices can influence the yield and composition of the essential oil. The plant spacing is an important factor that affects especially the productivity of organic cultures such as the ones included in our study.*

Materials and methods: *We included in our research three samples of caraway fruits harvested in 2008 from plants cultivated in the field of the Agricultural Research and Development Centre, Secuieni (Neamt, Romania). The volatile fraction was isolated by hydrodistillation and characterized using gas cromatography coupled with mass spectroscopy (GC/MS). The samples were obtained from caraway plants with different nutritional spaces. An essential oil isolated from caraway fruits (2008 harvest) commercialized in Czech Republic by an authorized european medicinal plants producer: (Fyto Kralik, Brno), was used as a standard.*

Results and discussion: *The main components of the three Romanian oils were L-carvone (55.26-60.31%) and limonene (29.75-33.88%), meanwhile for the Fyto Kralik standard the content was 64.87% and 24.13%, respectively.*

Conclusions: *The essential oils obtained from the Romanian caraway fruits have a similar chemical composition and they meet the requirements mentioned in the European Pharmacopoeia, 6th ed. Although, the Fyto Kralik standard possesses a more complex composition than the Romanian samples, it does not comply with the pharmacopoeial provisions, due to its low content in limonene.*

Key words: *Carum carvi L., essential oil, chemical composition*

Correspondence to:

Aprotosoaie Ana Clara
Adress: Gr. T.Popa University of Medicine and Pharmacy, Iasi, Universitatii Str., No.16, 700115, Iasi, Romania
Phone: 040232-301600 / 1816
E-mail address: anaclara70@yahoo.com

INTRODUCTION

Caraway (Carum carvi L., Apiaceae) is mainly cultivated for the fruits that have medicinal value and are used also as a spice and a flavoring agent in the food industry. Ripe caraway fruits are harvested and stored for three weeks for postmaturation, afterwards the material is suitable for use or for the essential oil isolation [1].

The biennial caraway is naturally found in pastures around Europe, but also in Siberia, Caucasian Mountains, Himalaya Mountains, Mongolia and even in Morocco; due to its alimentary and therapeutical significance, the plant was acclimatized in North America. Nowadays, the pharmaceutical drug is cultivated in The Netherlands, Poland, Hungary, Turkey, Egypt, Morocco and Czech Republic.

The dried fruits contain essential oil (3-7%) with (+)-carvone (40-65%) and (+)-limonene (30-45%) as main constituents. Myrcene, α-phellandrene, p-cymene, β-caryophyllene, cis/trans-carveol, cis/trans-dihydrocarvone and trans-dihydrocarveol are present as minor components. Environment conditions and soil type influence both the qualitative composition and the yield of the essential oil [2, 3].

The occurence of phenol acids (0.35%) in caraway fruits such as caffeic acid, ferulic acid, p-cumaric acid, syringic acid, sinapic acid, vanilic acid is substantiated by several authors; esters of caffeic and quinic acid (clorogenic acid, 4-caffeoylquinic acid) were identified as well. The flavonoids content in Carvi fructus is extremely low (0.06%); the predominant constituents are quercetine, isorhamnetine, isoquercitrine, astragaline and michelianine. Additionally, the fruits contain fatty oil (around 20%), proteins (up to 25%) and polysaccharides (13%), traces of vitamin C and furanocoumarins (bergapten, xanthotoxin).

Caraway fruits and the extracted essential oil have been used in therapy as spasmolytic, carminative, cholagogue, antimicrobial and antifungal agents. Consequently, the volatile fraction and the various extracts from caraway (infusions, tinctures) are recommended as a drug for the treatment of digestive tract disorders such as cramps, satiety sensation, flatulence, and also as a remedy for the gastrocardiac symptoms (Roemheld sindrome) and infants stomach aches. Caraway tea favours milk secretion, meanwhile Carvi Aetheroleum is a component of mouthwash as a breath-freshener and of hyperemiant liniments for external use.

Carum carvi and its essential oil is well tolerated in medicinal doses and does not have toxic effects towards humans.

However, carvone it is mentioned to have sensitising properties [4], caraway being classified among the plants causing contact dermatitis.

High oral doses of caraway oil can lead to migraine, vertigo, conscious disorders, followed by anxiety and finally by paralysis [5].

MATERIALS AND METHODS

In the present paper, we investigated the essential oil composition of three Romanian caraway variants (V1, V2, V3), harvested in 2008 from plants cultivated in the experimental field of the Agricultural Research and Development Centre, Secuieni (Neamt, Romania). An essential oil isolated from ca-

raway fruits (2008 harvest) commercialized in Czech Republic by an authorized european medicinal plants producer (Fyto Kralik, Brno) was used as a standard (FK). The Romanian variants were grown in the same conditions, but with different nutritional spaces; the cultivation parameters do not represent the focus of the present study.

Essential oil isolation

The volatile fraction was isolated by hydrodistillation of powdered dried fruits in a Clevenger type apparatus (3 h). The essential oil samples were subsequently analyzed.

Analysis of essential oils

The essential oils have been characterized using gas chromatography coupled with mass spectroscopy (GC-MS). GC-MS analyses were carried out on an Agilent type 7890A gas chromatograph, equipped with an Agilent 5975C mass spectrometer and a DB-5MS capillary column (25 m x 0.25 mm, 0.25 µm).

The oven temperature was increased with a rate of 10°C/min from 40°C to 280°C (where kept isothermally for 5,5 min). The other characteristics of the analysis program were: Inlet temperature: 250°C; Split ratio: 100:1; Carrier gas: helium (flow rate: 1 mL/min); Injected volume: 0.2 µL of essential oil.

Volatile compounds identification

The compounds were identified comparing their recorded mass spectra with those stored in the Wiley mass spectral library.

RESULTS

Essential oil yields (mL/kg dried fruits) of the Romanian caraway varied from 32 to 36 mL, meanwhile for the Fyto Kralik fruits we obtained 33 mL (Fig. 1).

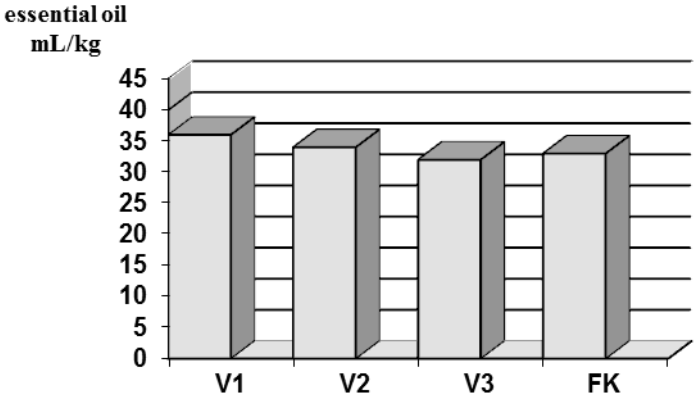


Fig. 1 Caraway essential oil yield/kg dried fruits
Samples (Secuieni): V1= caraway variant no.1; V2= caraway variant no.2; V3= caraway variant no.1; Standard: FK= caraway Fyto Kralik Brno

As shown in figure 1, the essential oil content of the three variants with different nutritional spaces (from wide to narrow) varies a lot especially if we express our results per ha. Still, if we consider also the fruits productivity in correlation with the nutritional space of the caraway plants the differences are significant as the fruits yield is lower for a wider nutritional space.

In order to evaluate the pharmaceutical quality of the volatile fractions isolated from the four samples of caraway (accordingly with the requirements found in *Carvi aetheroleum* monograph from Ph. Eur., 6th ed.), GC-MS analysis was carried out; the results of the identification and semiquantitative determination of the compounds are presented in table I.

Table 1– Chemical composition of the essential oils obtained from caraway fruits (2008)

RT (min.)	Compound	Composition (%)			
		V1	**V2**	**V3**	**FK**
6.320	sabinene	0.04	0.04	0.04	0.04
6.554	**myrcene**	**0.28**	**0.29**	**0.28**	**0.25**
6.813	m-mentha-1,8-diene	-	-	-	0.20
6.978	cis-ocimene	-	-	-	0.13
7.116	cymene	0.04	0.04	0.02	0.04
7.212	**limonene**	**33.88**	**30.23**	**29.75**	**24.13**
7.445	trans-ocimene	0.31	0.22	0.54	-
7.644	γ-terpinene	-	0.06	0.19	0.07
7.774	α-terpinolene	-	-	-	0.16
8.138	α-thujone	0.44	0.08	-	-
8.250	linalool	0.19	0.19	0.13	-
8.822	cis-p-mentha-2,8-dien-1-ol	0.34	0.49	0.40	-
8.337	trans-p-2,8-menthadien-1-ol	-	-	-	0.45
8.873	trans-limonene oxide	0.24	0.34	0.30	0.36
9.635	cis-p-mentha-1(7),8-dien-2-ol	-	0.23	0.14	-
9.679	β-fenchyl alcohol	-	-	0.08	0.12
9.722	neodihydrocarveol	0.28	-	-	-
9.765	**trans-dihydrocarvone**	**1.05**	**1.72**	**1.54**	**0.74**
10.033	izodihydrocarveol	0.51	0.87	0.78	-
10.094	**trans-carveol**	**0.53**	**0.78**	**0.67**	**0.87**
10.241	dihydrocarveol	0.49	0.75	0.74	0.33
10.284	geranial	-	-	-	0.27
10.328	**cis-carveol**	**0.24**	**0.43**	**0.90**	**0.41**
10.518	**L-carvone**	**55.26**	**60.31**	**59.45**	**64.87**
10.682	carvacrol	-	-	-	0.35
10.778	D(+)-carvone	0.25	-	0.46	-
10.899	methyl benzoate	0.67	0.75	0.93	0.73
11.020	trans-anethole	3.42	0.18	0.49	-
11.955	β-bourbonene	-	-	-	0.13
12.414	β-caryophyllene	-	-	-	0.31
12.846	α-humulene	-	-	-	0.09
12.950	aromadendrene	-	-	-	0.22
13.193	germacrene-D	-	-	-	1.29
13.383	bicyclogermacrene	-	-	-	0.72
13.435	β-bisabolene	-	-	-	0.08
13.660	Δ-cadinene	-	-	-	0.08
	Other constituents	1.54	2.00	2.17	2.56

RT= retention time

L-carvone and limonene were the major compounds of these essential oils amongst many other minor constituents. *Carvi aetheroleum monograph* (Ph. Eur., 6th ed.) specifies the limits of five compounds; the correlation of our results with the requirements found in caraway oil monograph is shown in table II.

Table 2 Pharmaceutical quality of caraway fruits essential oil [6]

Compound	Ph. Eur. 6th ed. requirements	Composition (%)			
		V1	V2	V3	FK
myrcene	**0,1-1** %	0.28	0.29	0.28	0.25
limonene	**30-45**%	33.88	3023	29.75	24.13
dihydrocarvone	**max. 2.5** %	1.05	1.72	1.54	0.74
carveol	**max. 2.5** %	0.77	1.21	1.57	1.28
L-carvone	**50-65**%	55.26	60.31	59.45	64.87

The analysis revealed that two of the investigated essential oils (isolated from V3 and FK samples) do not meet the requirements for limonene (less than 30%) mentioned by Ph. Eur., 6th ed.

Nevertheless, the V3 sample presented values close to the pharmacopoeial minimum, meanwhile the FK sample, that is certified by the quality of the producer, lacks of more than 5% limonene.

But, myrcene, dihydrocarve-ol, carveol and L-carvone comply with the ranges specified in the Carvi aetheroleum monograph.

In conclusion, the essential oil isolated from the dried caraway fruits (2008) meets the pharmaceutical qualities imposed by the 6th Ph. Eur., therefore it can be used in phyto-therapy and for medicinal purposes. Although the essential oil extracted from the Fyto Kralik caraway fruits is poor in limonene, but this sample proved to be the richest in L-carvone.

The sum of the constituents according to their classes is presented in table III. Only 32 out of 46 in total identified lateral and accessory canals have been partially or completely filled (Table nr.III).

Table III - Chemical composition of *Carvi aetheroleum* grouped by compound classes

Volatile compounds classes (%)	Sample			
	V1	V2	V3	FK
Monoterpenes	34.51	30.84	30.80	24.98
Monoterpene-ketones	57.00	62.11	61.45	65.61
Monoterpene-alcohols	2.58	3.74	3.84	2.18
Monoterpene-oxides	0.24	0.34	0.30	0.36
Monoterpene-aldehydes	-	-	-	0.27
Sesquiterpenes	-	-	-	2.92
Aromatic compounds	4.13	0.97	1.44	1.12

The caraway fruits essential oil consists mainly of monoterpene-ketones and monoterpenes. Essential oils rich in **monoterpene-ketones** have high afinity for the central nervous system, but also for the epithelial tissue and mucous membranes [7], being used for its healing properties as it favours the development of the granulation tissue. Monoterpene-ketones are efficient mucolytic, digestive, spasmodic and anti-foaming agents, stimulating at the same time the choleresis. Regarding the central nervous systems uses, these compounds are stimulants of the cerebral metabolism, the administration

of low quantities could improve serotonine and acetylcholine levels.

Monoterpenes are tonic, anti-inflammatory, antalgic and immunomodulatory. Acting as local irritant, monoterpenes would stimulate the analgezic and anti-inflamatory mediators release, being recommended for rheumatic pains. Psychologically, are tonic, stimulant of the concentration and memory activities.

Consequently, caraway fruits and volatile oils therapeutic uses are justified and maybe still less exploited.

CONCLUSIONS

The chemical analysis of the three essential oils isolated from Romanian caraway fruits, cultivated with different nutritional spaces, revealed that the samples comply with the pharmacopoeial provisions.

The standard e-ssential oil obtained from Fyto Kralik caraway fruits (Brno, 2008 harvest) also meets the requirements from the Ph. Eur., 6th ed., except the limonene content (24.13% compared to 30% minimum).

Eventhough the Romanian caraway variants meet the pharmaceutical qualities required by Ph. Eur., 6th ed., still the local producers should confirm the pharmaceutical quality by chemical investigation for each batch of the raw material or of the essential oil.

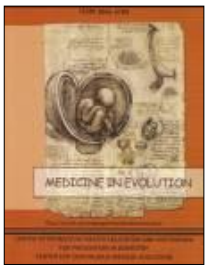
Funding Acknowledgement:

The work was supported by PN- Biodiv 4.01/2009, financed by National Authority for Scientific Research-Romanian Government.

REFERENCES

1. Saller R, Reichling J, Hellenbrecht D. Phytotherapie. Haug, Heidelberg, 1995.
2. *** Hagers Handbuch der Drogen und Arzneistoffe, CD-ROM. Springer Medizin Verlag, Heidelberg, 2006.
3. Czygan FC. Carvi fructus. In: Teedrogen und Phytopharmaka, Wichtl M editor. WVG, Stuttgart, 1997, 134-135.
4. Hausen B. Allergiepflanzen-Pflanzenallergene. ecomed. Landsberg, Munchen, 1988.
5. Teuscher E, Lindequist U. Biogene Gifte, 2nd edition. Gustav Fischer, Stuttgart, 1994.
6. 6. *** European Pharmacopoeia, 6th edition. EDQM, Strasbourg, 2008.
7. 7. Werner M, v. Braunschweig R. Praxis Aromatherapie, Haug, Stuttgart, 2006.

CONTRIBUTIONS TO THE PHARMACOGNOSTICAL STUDIES OF THE SPECIES ALLIUM CEPA L. VAR. CEPA (ALLIACEAE) FOR THERAPEUTIC PURPOSES



MIHAELA - ZENAIDA RISTACHE[1], VIORICA ISTUDOR[1], MARIA – LIDIA POPESCU[1]

1. **University of Medicine and Pharmacy ,,Carol Davila" Bucharest, Faculty of Pharmacy, Pharmacognosy. Phytochemistry. Phytotherapy Department**

ABSTRACT

The Allii cepae L. bulbus var. cepa (Alliaceae) were studied for therapeutic purposes based on its antibacterial, cicatrisant and antimitotic properties in order to obtain a pharmaceutical product for dermatological use. Research aims consist in description, setting of the anatomical characters, identification and dosing of the main classes of active principles.

Were considered as specific the following anatomical elements: liliaceous type epidermis with stomata (with 4 annexes cells, parallels), parenchyma with reserve substances (sinistrin).

The qualitative chemical analysis (specific reactions and TLC) showed the presence of flavanoids (quercetol, rutoside), sterols (β-sitosterol and/or stigmasterol), sterolic saponosides, mono and polysaccharides.

This vegetal product contains: from 0.10 - 0.12 g% flavonoids (expresed rutin), polyholosides 9.80 - 10.04 g%, soluble substances 4.20 - 4.26 g% in water, 7.15 - 7.27 g% in alcohol.

***Keywords**: Allii cepae bulbus, flavonoids, polysaccharides, anatomical characteristics.*

Correspondence to:

Mihaela - Zenaida Ristache
Adress: University of Medicine and Pharmacy ,,Carol Davila" Bucharest, Faculty of Pharmacy, Pharmacognosy. Phytochemistry. Phytotherapy Department
E-mail address: mihaela_zenaida@yahoo.es

INTRODUCTION

Allium cepa L., var. cepa, onion (Alliaceae family), is a biennial herbaceous plant, cultivated on all continents and used in food, but also in the phytotherapy of skin or respiratory diseases, hypertension, diabetes, tumors [1-7], antimitotic effect of the product being reported since 1949 by D'Amato and Avanza [1, 3, 9],.

According to the literature, the fresh bulb contains aliine [sulfoxides of E (+)-S-propene-1-yl-L-cysteine or isoaliin (0.2%) (+)-S-methyl-L-cysteine and (+)-S-propyl-L-cystein], flavonoids (heteroside of quercetol: 3,4 '-diglucoside = 50-1300 mg / kg 4'-β-D-glucoside = 36-394 mg / kg, 7.4 '-diglucoside, rutoside and isoramnetin-4'-glucoside), phenol carboxylic acids (1-2% protocateic acid in the external tunic), steroid saponosides = 0.04% (a-spirosids aliil A and B, ceposids AF) sterols (cholesterol, cicloartenol, lofenol, β-sitosterol), phyitoalexins (cyclopentane derivatives), polysaccharides (10-40% fructosans, respectively sinistrin), 9% other oligoside, 10-15%, simple oses [1].

The sulfur constituents of the onion bulbus inhibits platelet and thrombocyte aggregation, increase fibrinolytic activity reducing the risk of myocardial infarction), reduce hyperglycemia and hyperlipidemia. Thiosulphinates (produced by hydrolysis of the cysteine-sulfoxides) reduce the formation of thromboxane (favoring of platelet aggregation). Cepaenes and thiosulphinates inhibits in vitro 5-lipoxygenase and cyclooxygenase, thereby reducing allergen-induced bronchoconstriction [8].

Sulfoxides (S-methyl-L-cysteine sulfoxide, S-L-cysteine sulfoxide aliil) reduce glycemia for people with diabetes by increasing serum insulin levels [8].

Onion extracts exhibit antagonistic properties towards 4-nitroquinoline-N-oxide and 2-amino-fluorene (carcinogenic nitrosamines) thus suppressing cytotoxic properties [8].

Pressed juice and volatile oil obtained by steam drive has antimicrobial activity. The volatile oil inhibits development of pathogenic bacteria in the oral cavity (Streptococcus mutans and S. sobrinus) [8].

Aqueous extracts of onion have an androgenic effect in male mice, anti-inflammatory (showed in rats paw edema assay) and immunosuppressive (rabbit) [8]. The purpose of the study was to verify the identity, chemical composition and microscopic characters of the Allium cepa bulb grown in Romania, in order to exploit the phytotherapy for psoriasis. Arguments in favor of such information are the antimitotic, antiinflammatory and antimicrobial properties of this vegetal product.

By microscopic analysis were observed: fragments of epidermis with elongated cells and liliaceous type stomata (4 cells annexes, two short and two elongated, arranged in extension of stomata) (Fig. 2a, c), fragments of parenchyma with reserve substances (possibly sinistrin) (Fig. 2b, c, d).

MATERIALS AND METHODS

The raw material consists of Allii cepae bulbus, harvested at their full maturity in august 2010, from Ilfov county (Romania). After harvesting the product was preserved under silage conditions (temperature of 10 - 15 °C). To verify the identity and determine the quality was applied the pharmacognostic analysis. Identity was assessed by macroscopic examination (organo-

leptical and morphological characteristics) and microscopic examination (preparation "concissum" clarified with a solution of sodium hydroxide 50 g / L and examined under a Zeiss Research Microscope Imager D1) microchemical examination (microsublimation), qualitative chemical analisys and thin layer chromatography (TLC) for sterols / triterpenes and flavonoids.

For qualitative analysis were made successive extractions from raw material in different solvents (ethyl ether, methanol, water). Half of alcoholic and aqueous solutions were hydrolysed. Aglycones results were extracted with diethyl ether. On the resulting solutions were made specific reactions to identify their different actives principles [3, 10, 11].

Parameters of TLC analysis

Preparation of test solutions

1) 1g plant product to reflux with 10 mL distilled water for 15 min; aqueous solution was hydrolysed with HCl 100 g / L for 60 min. and extracted with ether; the ethereal solution was concentrated to 1 mL (solution A);

2) 1g fragmented plant product was extracted with 10 mL methanol for 30 min, filtered and concentrated to 1 mL (solution B);

3) 1g fragmented plant product was extracted with 10 mL chloroform for 30 min., Filtered and concentrated to 1 mL (solution C).

stationary phase: ready to use silica gel GF254 Merck plates activated by keeping the oven at 105 ° C for one hour.

mobile phase

- ethyl acetate: formic acid: acetic acid: water/100:11:11:27 (solvent 1);
- ethyl acetate: formic acid: water/80: 8: 12 (solvent 2);
- chloroform: acetone/8:2 (solvent 3);
- formic acid concentrated.: water: methanol: ethyl acetate / 2,5:4:4:40 (solvent 4).

reference substances (0.1% methanolic solution)

- rutoside (Fluka), quercetol (Merck)
- for flavones;
- caffeic acid (Merck), chlorogenic acid (Merck) - for polyphenolcarboxylic acids;
- ursolic acid (Sigma), oleanolic acid (Sigma) - for triterpenes;
- beta-sitosterol (Sigma), stigmasterol (Sigma) - for sterols;
- scopoletol (Merck) - for coumarins.

revelation reagents

- diphenylboryloxyethylamin (1% methanol solution), propylene glycol 400 (5% methanol solution), sprayed in succession (reagent 1);
- mixture of acetic anhydride and sulfuric acid concentrated ethanol concentrated (1:1), heated to 100°C for 10 min. UV λ = 366 nm (reagent 2);
- diphenylboryloxyethylamin (1% methanol solution), heated to 100 °C for 5 min. (reagent 3).
- UV at λ = 366 nm (reagent 4).

The chromatograms was examinated under visible light and under UV light at 366nm (using a Camag UV lamp). Solutions for analysis and reference substances were submitted to the start band, 5 ml each, as follows: 1-solution A, 2-B solution, 3-reference substance. Quality assessment was performed by determining: loss on drying, water soluble substances and alcohol by maceration of 24 hours, the flavanols, spectrophotometric method based on coupling with AlCl3/CH3COONa, λ = 427 nm, using a standard curve rutoside) [8] polysaccharides homogeneous type mucilage (gravimetric method based on precipitation in methanol concentrated) [9-10]. Results were reported on dry weight basis.

RESULTS

The results obtained are shown in Figures 1 - 5 and in Table I. The macroscopic analysis confirmed the identity of raw material (Fig. 1). By microscopic analysis were observed: fragments of epidermis with elongated cells and liliaceous type stomata (4 cells annexes, two short and two elongated, arranged in extension of stomata) (Fig. 2a, c), fragments of parenchyma with reserve substances (possibly sinistrin) (Fig. 2b, c, d). Methanolic solution obtained by leaching of the microsublimate presented blue fluorescence (at λ = 366 nm), which in alkaline solution became yellow - green. It may contain coumarin or derivatives of the o-hydroxycinamic acid.

Through specific chemical reactions were identified: flavones, sterols / triterpenes, saponosides sterol / triterpenoid, reducing compounds, polysaccharides (mucilage) and non-alkaloids nitrogen-containing compounds.

Reactions to polyphenolcarboxylic acids and tannins were negative.

Fig. 1 The bulb of Allium cepa var. cepa

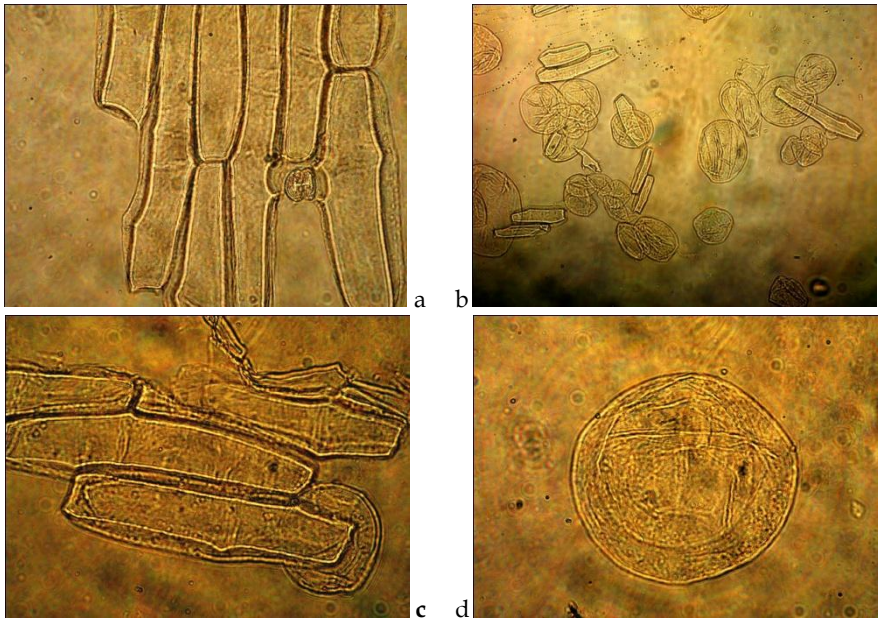


Fig. 2 Anatomical elements of the Alii cepae bulbus a) fragments of epidermis with stomata (Ob 40 x), b) palisade cells and lacunar parenchyma cells (Ob 10 x) c) epidermal cells (Ob 40 x) d) parenchyma with reserve substances (Ob 40 x).

Comments for TLC analysis – Two spots with flavonoid properties (yellow colour and yellow fluorescence; Rf = 0. 78 and Rf = 0. 74) were separated from methanolic solution, in solvent 1 and 2 (fig. 3). Quercetin (Rf = 0. 94; yellow colour and yellow fluorescence) and other two flavonoid aglycones (Rf = 0. 91, Rf = 0. 84) were separated from eteric solution (after hydrolysed aqueous solution) (fig. 3). Caffeic acid (Rf = 0. 82 blue colour and blue fluorescence), chlorogenic acid (Rf = 0. 36 blue colour and blue fluorescence) and rutoside (Rf = 0.23 yellow colour and yellow fluorescence) were not identified in these solutions (fig. 3). Beta-sitosterol / stigmasterol (Rf = 0. 76 in solvent 3 violet colour in visible and yellow fluorescence in UV, after pulveration with acetic anhydride and H2SO4/ethanol) was identified by TLC in all three samples analysed (fig. 4).

Other two composites with comportment of sterol / triterpene (Rf = 0. 78, Rf = 0. 79) were separated from these three solutions. From cloroformic and eteric extracts were separated another composite sterol /triterpen with Rf = 0. 98. The two triterpene aglycones, used as reference substances, oleanolic acid (Rf = 0. 73) and ursolic acid (Rf = 0. 72) weren't identified, in this conditions TLC, in this vegetable product.

Scopoletin (Rf = 0,89 in solvent 4) was identified in methanolic extract.

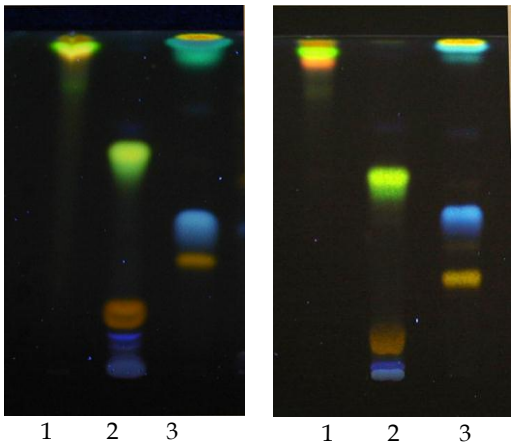


Fig. 3 TLC Separation of polyphenols (a reagent and UV -366 nm): a = solv.1, b = solv.2 1) solution A, 2) solution B, 3) reference materials (from top to bottom: quercetol, caffeic acid, chlorogenic acid and rutoside)

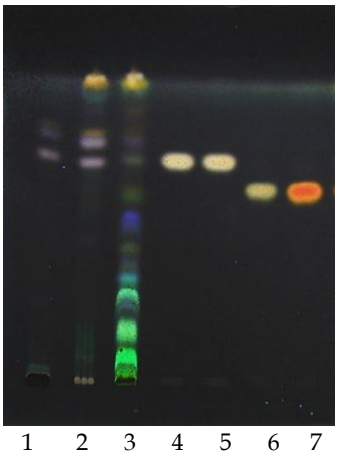


Fig. 4. TLC chromatogram of sterols / triterpenes (reagent 2 solvent 3).

1) solution B,
2) solution C,
3) solution A,
4) beta-sitosterol,
5) stigmasterol,
6) oleanolic acid,
7) ursolic acid.

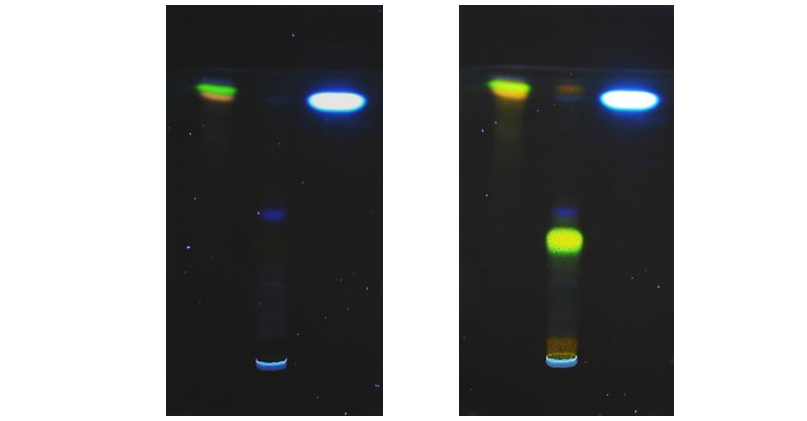


Fig 5. TLC chromatogram of coumarins
1) soluția A; 2) soluția B; 3) scopoletin, solvent 4 reagent 4

Table 1 The results of quantitative chemical analysis

Parameter		Method / quality reference	Results (g %)
loss on drying		European Pharmacopoeia 5th ed.	86.71 – 89.74
matter extractable	by water	Romanian Pharmacopoeia 10th ed.	4.20 – 4.26
	by alcohol		7.19 – 7.27
flavonoids		spectrophotometric method / Romanian Pharmacopoeia 10th ed.	0.10 – 0.12 (expressed in rutin)
polysaccharides (mucilage)		gravimetric method	9.80 – 10.04

Results for quantitative analysis

Loss on drying (88.23 g%) are within the recommended limits for fresh bulbs (not more than 85 - 90 %). Substances soluble in water are approximately 4. 23 g% and soluble in alcohol of about 7. 23 g%. This small content than could be explained by the technique applied (cold maceration for 24 hours). Flavone content is approximately 0.11 g% (expressed in rutoside). Polyholoside content is relatively high (about 9.92 g%).

CONCLUSIONS

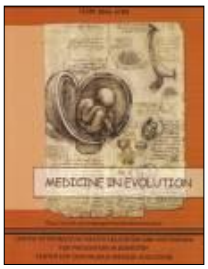
The bulb of the species Alium cepa L. contains flavonoids (quercetol, rutin), sterols (beta-sitosterol /stigmasterol), steroid saponins, reducing-compounds, monosaccharides, polysaccharides (mucilages) and nonalkaloids nitrogen-containing compounds. It was determined 0.11 g% flavonoids (expressed as rutin) and 9.92 g% polysaccharides.

In the future we will establish if phytochemical data support the therapeutic indications in traditional medicine.

REFERENCES

1. Bruneton J., Pharmacognosie, Phytochimie, Plantes medicinales, TEC&DOC, Paris, 2005, 209-211,
2. D'Amato F., Avanzi N.G, O comparison of the cytologic activity of seven essential oils. Caryologia (1949), 1,175-193 recenzat în C.A. (1949), 43, 9170c
3. Duțu L.E., Comparative Study about Herbal Drugs and Their Analysis Methodology in Romanian Pharmacopoeia and European Pharmacopoeia. Note1. Commun Monographs about Underground Organs. Farmacia, 55 (1), 2007, 61-72
4. Galeone C, Pelucchi C, Levi F et al.- Onion and garlic use and human cancer, Am J Clin Nutr. 2006 Nov; 84(5): 1027-32
5. Gîrd C.E., Duțu L.E., Popescu M.L., Pavel M., Iordache A.T.– Experimental researches regarding the active extracts polyphenols-standardized, Note II. Meliloti herba – pharmacognostic analysis of the raw material, Farmacia, 2009, 57(2), 184-191
6. Humadi S., Istudor V. – Lythrum salicaria (purple loosestrife). Medicinal use, extraction and identification of its total phenolic compounds – Farmacia, 2009, 57(2), 192-200;
7. Shams-Ghahfarokhi M, Shokoohamiri MR, Amirrajab N et al. - In vitro antifungal activities of Allium cepa, Allium sativum and ketoconazole against some pathogenic yeasts and dermatophytes, Fitoterapia. 2006 Jun; 77(4): 321-3
8. Teuscher E., Anton R., Lobstein A. - Plantes aromatiques, epices, aromates, condiments et huiles essentialles, Lavoisier, TEC&DOC, Paris, 2005, 348-353
9. Xiao H, Parkin KL. - Isolation and identification of potential cancer chemopreventive agents from methanolic extracts of green onion (Allium cepa), Phytochemistry. 2007 Apr; 68(7): 1059-67
10. xxx - European Pharmacopoeia, 5-th ed., Council of Europe, Strasbourg, 2006, 4929
11. xxx – Farmacopeea Română ediția a X-a. Ed. Medicală, Bucureşti, 1993, p. 335, 1016, 1051-1058, 1063

ACADEMY MEMBER DR. STEFAN ODOBLEJA (1902-1978) – ALWAYS OF TOPICAL INTEREST



NICOLAE POPESCU[1], GRIGORE - ALEXANDRU POPESCU[2]

1. **Craiova University, Drobeta Turnu-Severin University Centre, Faculty of Physical Education and Sport**
2. **Municipal Clinical Emergency Hospital Timisoara, Department of Surgery**

ABSTRACT

The Romanian scientist Ştefan Odobleja was the first thinker to offer a universal cybernetic explanation for all domains of human thinking. In fact, the entire scientific work of Ştefan Odobleja contains ideas which echoed in present and future scientific fields. In medicine, the work ,,Fonoscopia - o nouă metodă de explorare clinică"(1935) (Phonoscopy – A New Method of Clinical Investigation), makes him a precursor of ultrasonography, and with his work ,,Psihologia consonantistă"(1939) (Consonantist Psychology) he achieved a deep analysis on the influence of psychological upon somatic factors and by reversability, feed back, consonance, binarity he detects and reveals the phenomenon of adaptation of living organisms to environmental conditions, thus contributing to the development of the psycho-somatic concept.

Moreover, in this work, Stefan Odobleja intuitively states that the elements on which psychological processes rely are invisible, energetical, and thus he may also be regarded as a string theory pioneer.

***Key words**: diagnostic ultrasonography, psychosomatics, consonantist psychology, strings.*

Correspondence to:

Nicolae Popescu MD
Adress: 67, Carol I Boulevard, street code 220146, Mehedinți, Drobeta Turnu-Severin, Romania
Phone: +40722213910
E-mail address: npopescu_mf_kt@yahoo.com

INTRODUCTION

Seventyfive years after the publication of ,,*La Phonoscopie*" by the editors Gaston Doin et Cie, Paris, the engineer Stefan Odobleja jr., son of the Academy Member, saw fit to re-publish the Romanian translation of this work, thus maintaining the present attention on the scientific work of his distinguished predecessor. Concomitantly, by creating the ,,Stefan Odobleja Foundation" (1999), the study of the extensive scientific works kept by the Drobeta-Turnu Severin National Archives is intended, as by its ideas, theories and thesis an important contribution in various scientific fields may be offered with the possibility of generating revolutionary changes.

The idea of functionality based upon consonance, antagonism; binarity was predicted by Stefan Odobleja in the previously mentioned work, dedicated to the phonoscopic method, even before the publication of „*Consonantist Psychology*". We make reference to a series of articles such as: *Valoarea comparativa a transonanței sunetelor de plescăit si frecare* (Comparative Value of Transsonance in the Case of Splash and Friction Sounds), in ,,*Buletinul medico-terapeutic*", October-November 1930; *Transonanta sunetului de frecare* (Friction Sound Transsonance), in the journal ,,*Spitalul*", December 1930; *Percutia cordului* (Heart Percussion), in ,,*Buletinul medico-terapeutic*", December 1933; *Transonanta sunetelor de frecare si plescait la cord* (Cardiac Transsonance of Friction and Splash Sounds), in ,,*Buletinul medico-terapeutic*", May 1934; *Fonoscopia* (Phonoscopy), in ,,*Miscarea medicala romană*", no.1-2, 1934; *Semnul banului* (The Coin Sign), in ,,*Miscarea medicala romană*", no.4-5, 1934; *Fonoscopia în colectiile pericardice* (Phonoscopy in Pericardial Collections), in ,,*Miscarea medicala romana*", no.7-8, 1934; *Percutia si fonoscopia ficatului* (Percussion and Phonoscopy of the Liver), in ,,*Miscarea medicala romana*", no.1-2, 1935. The author percieves phonoscopy as a physical method combining the advantages of a practical, clinical and objective method meant to evaluate volume and consistency variations in internal organs by a simple procedure involving finger and ear, by rubbing or gently tapping the surface of the skin. In this way a sound is generated which crosses the organ and returns loaded with information. The gesture triggering the stimulus finds its equivalent in its oposite – the perception of sound reflected by the organism.

In fact, the entire scientific work of Stefan Odobleja, also included, of course, ideas which echoed in present and future scientific fields.

Thus, in medicine, profesor dr. Scheau from the Craiova Faculty of Medicine and dr. Al. Olaru, some of the main promoters of the works of the academy member and doctor Stefan Odobleja, who, after studying his works on phonoscopy and acustic semiology, in the lecture presented at the Symposium in Craiova in 1992, labeled him as a precursor of the ultrasonic investigation method in medicine.

The scientist's ideas and theories are of topical interest for the bio-psycho-social model closely connected with the ecological-existential side for which he may be considered as an initiator in a field so discussed nowadays, namely psychosomatics, a holistic approach in medicine which explains the source of diseases of the human body. The consonantist psychology applies to natural phenomena and, particularly, to those in biology and psychology, with the aid of the reversibility law, it detects and reveals by inversed connection the phenomenon

of adaptation of living organisms to the environment, thus anticipating the "general adaptation syndrome" elaborated by Hans Selye in 1946.

The relation between mind (psychic), body (soma) and the environment (ecological as well as existential), their balance and disbalance represented throughout previous periods, a constant preoccupation for scientists contributing to the developemnt of the psychosomatic concept. Stefan Odobleja, in his work Psihologia consonantistă (Consonantist Psychology), performed a deep análisis of the influences exerted by the psychological upon the somatic by reversibility, feedback, consonance, binarity. The following must be mentioned: 1912- A. Adler, 1922- F. Deutsch, 1943-F.L. Dumbar, 1946-H. Selye, 1950-F. Alexander, 1964-R. Tzanck, 1979-Von Eieff and others, as well as Romanian personalities: D. Danielopolu, A. Athanasiu, P. Derevenco, I.B. Iamandescu, etc. The work Matematizarea psihologiei consonantiste de Stefan Odobleja (Mathematical Approach of Consonantist Psychology by Stefan Odobleja), authored by Marcel Voica, president of the Foundation for Matriceal Interdisciplinary Studies and Ileana Voica, published by Matricea Editors, Bucureşti, October 2002, represents a guide for the traditional study of consonantist psychology and for its informatized use. The authors underline that the scientific value of consonantist psychology increases in time. It attracts attention not only by the author's extraordinary power of penetration and prediction but, especially by the fact that it increasingly becomes an action instrument with the expansion of electronic computing technology.

The transposition in a matriceal data base relaunches consonantist psychology into the present and projects it into the future on a novel informatic platform.

On the quantic structure of the mind, the present level of knowledge allows us to state that the matter has two main forms of manifestation: atomic and quantic, the latter being also known as energy. The hypothesis is circulated that the cellular anatomic structure is accompanied, interwaved and interactive with a stable quantic structure. The quantic structure of the brain is an overstructure and this seems to be the real site of the mind.

The fundamental components of the universe, identified by phyicists until the present are: electrons, neutrinos, quarks representing a true matter alphabet. The increasingly circulated strings theory states that there are fundamental matter particles which have been mathematically but not practically demonstrated by some physicists, such as: Gabriele Veneziano - Italian physicist (The European Centre for Nuclear Research), Leonard Susskid – American physicist, John Schwarz, Michel Green – University of Cambridge, Edduart Witten – the author of the „M" theory. These fundamental particles are not punctiform but composed of minute filaments (strings) which vibrate or oscillate within a single dimension, in the opinion of the above mentioned physicists.

Univeristy Professor Dr. Ion Manzat, the President of the Romanian Transpersonal Psychology Association states that the psychological resonance infered in 1938 by St. Odobleja is a transpersonal energy vibration explained by extended psychosynergy.

In this context we may undoubtedly state that Stefan Odobleja was also a precursor of the string theory intuitively discovering their presence. He states that the true elements on which psychological phenomena and processes are based are invisible – as are the elements or the material substrate of physical energies – and

analogical if not identical to the latter…The psychological process …is an extremely fine process, an energetical one. An energetical microscopy able to identify these invisible elements has not yet been invented.

In the chapter „*Division of psychological processes*" of the above mentioned work (pag. 130), Stefan Odobleja classifies them according to vital phases and energy in propagation and transformation processes, the former being further classified into nervous currents conducted through fibres and psychological waves wirelessly propagated thus exemplifying telepathy. This is yet another proof he intuitively discovered the possible existence of an invisible energetical element which could not be identified with the technological means available at that moment.

He versified his scientific reasonning as shown by the following poem:

Thinking inside the brain and in the air (thinking and telepathy)

It is proven fact that thinking propagates at long distances

This being already clearly and well demonstrated.

A proof that thought propagates in the shape of fine waves.

Similar to the herzian wave, similar to the light wave.

Since it freely, continuously spreads in the air,

Why do we wish to force it in the brain to go through wires?

If it would go through wires in the head, it would do the same outside,

As the thought could not jump off the wire.

Were the thought to travel on a wire, telepathy would be no more,

A thought could not fly over a thousand kilometers

If thinking was wire-based, as some people teach us,

Then television would also propagate on wire.

It is absurd to suppose that thinking would walk

On wire for morphologists, and differently for others.

It is more likely it evenly behaves

And walks as a beam and has no wire at all.

Returning to phonoscopy, a term introduced by Stefan Odobleja, this must be also considered a precursor of echography which is synonymous to ultrasonography and to diagnostic sonography, as he considered it was best suited for the acustic phenomena used in medicine in those days.

Stefan Odobleja defines phonoscopy as a new diagnostic method, an acustic semiology which must be supplemented and perfected together with technological processes, by introducing precision instruments waiting for their inventors, thus intuitively describing the modern echograph.

The Academy member systematically studies and experiments the way sound changes its characteristics as crosses the human body, thus allowing the identification of both shape and consistency of various organs and certain processes. He did not perform these experiments with high frequency sounds (ultrasounds) but with low frequencies produced by "tapping" or "splashing" on various body surfaces. The perception of the modified sound (echo) was achieved by listening with the ear on the opposite side. He states that between 40,000-100,000 vibrations / second, the human ear does not percieve the sounds he calls "ultrasonic". Ultrasound investigation was already being used in engineering and for military applications, long time before their use in medicine by the use of the echograph.

Regarding the continuation of research in the field in question we must state that ultrasonic energy was applied for the first time in medicine in

relation to the patient by Dr. George Ludwig in 1940 at the Naval Medical Research Institute, Bethesda, Maryland.

The first purely imagistic application is then achieved by the physician Karl Theodore Dussik (1942-1947) at the Vienna University, who used a method which he named „hyperphonography". Profesor John I. Wild at the University of Cambridge and John M. Reid at the Pennsylvania University bring important contributions to the type A working method (1945-1952).

Profesor Douglass Howary at the Colorado University, Joseph Holmes and others (1954-1957) invented the first type B echographic scanner, obtainning bidimensional images. Joseph Holmes is the one who will work for many years as a member in many research teams in order to improve the ecograph, bringing it closer to its present shape.

The most important contributions on the evolution to modern ultrasonography and implicitely to the modern echograph were brought during one century by Japanese scientists, but Europeans active in the field must not be forgotten.

The work ,,*La Phonoscopie*" is composed of four distinct chapters. In the first one, entitled Semiologia acustica (Acustic Semiology) the qualities of sound are systematically and thoroughly described, these being found in modern echography (ultrasonography, diagnostic sonography) as physical and acustic properties of ultrasounds such as: frequency, wavelength, period, propagation speed, intensity, reflexion, difraction, impedance, amplitude, rigidity or elasticity.

In the second chapter, entitled Fonoscopia (studiu general) (Phonoscopy – General Study), Stefan Odobleja describes a clinical investigation method by assessing the organism's permeability to sounds.

Ecography is presently defined as an imagistic method using ultrasounds to visualize subcutaneous body structures and internal organs, oriented to the identification of various pathologies or lesions. Both phonoscopy and echography are sound based clinical investigation methods, low frequencies being used in phonoscopy and high frequencies in echography.

Stefan Odobleja also describes a method for graphic representation on paper for various echoes which in echography are visualized on a screen. The third chapter, Fonoscopie comparata (valoare comparativă) (Compared Phonoscopy – Comparative Value), presents percussion techniques and procedures, sound producing modalities, perception of percussion induced sounds and the interpretation of symptoms in comparison with other methods, such as "the coin sign".

The fourth chapter entitled Applied Phonoscopy includes experiments and a detailed description of internal organs and the acustic topographical diagnosis of some liquid or pus collections, or tumors, which suit modern echography. As in echography, he describes abdominal air as an impediment to phonoscopic examination, suggestively describing it as the"great concealer".

At present, there are three synonyms defining acustic semiology: echography, ultrasonography and diagnostic sonography. Consecutively, phonoscopy which, in fact, also means the use of acustic phenomena in medicine, is synonymous to diagnostic sonography? This includes both the graphic record resulting from the patient's examination with the aid of a sound produced by the gentle tapping of the body surface and listening to the echo, as well as recording sounds produced by the transductor functioning as gene-

rator and receiver of ultrasounds on the monitor of the oscilloscope.

All these lead to assessing the presented work as a revolution in clinical semiology, in general, by defining and describing the acustic semiology resulting as a consequence of deep scientific research, method labeling the Academy Member Stefan Odobleja among the precursors of echography.

„*La Phonoscopie*" received the „Medic General dr. Papiu Alexandru" prize which is awarded to the most valuable works written by military physicians. The auscultation method proposed by dr. Stefan Odobleja was presented at the IX-th International Congress of Military Medicine held in Bucharest in 1937 with the title "Demonstration de phonoscopie" (Phonoscopy Demonstration). The communication was received with great interest by the foreign participants, especially Dr. W. S. Bainbridge, head of the American delegation, physician of the military navy.

The scientist Stefan Odobleja who by observation and meditation, tried to uncover realities of the human mind and the surrounding processes it reflects...had genious qualities and deserves a place in universal science, as revealed by the following opinions of scientific personalities:

…Applying his concepts to social life, economy, sociology, Stefan Odobleja states the universality of the cybernetic concept of closed loop reaction circuits. For this reason, the entire unique moment offered to Romanian and international history, science and culture by Stefan Odobleja in 1938-1939 receive the brilliance of great ideas of the human mind..."

Academy Member Mihai Draganescu: „Possibly one of the most gifted creators, the scientist from Drobeta worked alone as did the great solitary thinkers. He rediscovered a lot and invented even more, using a single instrument – his innate intelligence".

Academy Member Alexandru Surdu: „The value of Odobleja's system for the integration of sciences is similar to Einstein's system in physics."

Engineer Stelian Bajureanu: „Stefan Odobleja might be considered not only as a modern Socrates, but also as a second Columbus, for he had the same destiny of discovering the America of science and eventually this America did not receive his name but a different one".

Prof. Dr. Iosif Constantin Dragan: „You have a golden man deserving a golden statue ".

B.H.Rudall-University of Wells

REFERENCES

1. Odobleja St., Psihologia Consonantistă, Ed. Stiintifică şi enciclopedică, Bucuresti, 1982, pp. 14, 41-43, 84.
2. Odobleja St., La Phonoscopie – nouvelle methode d,exploration clinique, Ed. G. Doin & Cie, Paris, 1935, p. 6.
3. Odobleja St, Pagini inedite – editie în grijita si prefatată de C. Negreanu, Ed. Scrisul românesc, Craiova, 1986, p. 113.
4. Odobleja St, Jr., N. Popescu, Centenar Stefan Odo-bleja (13.10 1902 – 13.10.2002), in „ Medicina in evolutie", nr.4/2002, Ed. Fundaţia „Pentru Voi ",Arad, p. 9.
5. Popescu N, Psihosomatica şi psihologia consonantista, Zilele Spitalului Militar de Urgenta din Craiova, 16 – 18 octombrie, 2002.
6. Popescu N, M. Cenea M, Factorii de stres si patologia psihosomatică – teorii si cercetari la nivel organizational, Ed. Universitatii Craiova, 2006, p.p. 38 – 39.
7. Vissarion M, Structura cuantică a psihicului, în „ Viata Medicala " , nr. 31, 31 iulie 2009, p. 2.

INSTRUCTIONS FOR AUTHORS

The journal publishes general reviews, studies and clinical, epidemiological, experimental and laboratory research, clinical case presentation, papers from the history of medicine, reviews, scientific and technical state-of-the-art articles, medical informations and opinions. Only papers which have not been published or sent for publishing in other journals are accepted. The authors are responsable for the opinions expressed in the papers. ***The paper must be edited both in Romanian and in English; the English version will be supervised by our collaborator Dana Brehar-Cioflec, MD, PhD; typed on white A_4 paper (fonts - Times New Roman 12, Romanian characters, line spacing 1.5, upper and lower margins 2cm, left border 3cm, right border 2cm) and on CD, DVD or Memory Stick***.

Manuscripts will not exceed:

- general reviews: 6-8 pages
- studies and researches: 5-7 pages
- case presentations: 2-4 pages
- reviews, scientific and technical state-of-the-art articles, medical informations and opinions: 1-2 pages.

The paper will be edited according to international editing rules for manuscripts. The title will be written in capital characters and it will be followed by the name and surname of the author (authors), followed by their place of work (place where the paper has been elaborated). Studies and researches will be followed by a brief abstract, followed by 3-4 key-words.

The body of the paper will be structured on the following chapters: introduction, aim, objectives, material and method, results and discussions, conclusions. The references will be presented alphabetically and in conformity to the Vancouver Convention, including:

- for articles: name of the authors and surname initials, title of the article in the original language, title of the journal according to the international abreviation system, year of issue, volume, number, pages;
- for books: name of the authors and surname initials, volume, publisher (editors), city of publishing, year of issue.

Citation of references inside the body of the paper will be put between brackets, Harward style (author, year) or Vancouver style (number in square brackets or superscript). Cited reference titles will be selected, maximum 6 for studies and case presentations and 12 for general reviews. Acceptance, rejection or the need of alterations in sent materials, or in inconography, will be comunicated to the authors in due time. For this, the authors will indicate the person and address for corespondence (phone number, e-mail address). Given the less pleasant experience of the editorial board with some articles being rejected because they did not meet publishing criteria, we decided to support those who intend to publish in this journal by detailing the way such a paper should be elaborated, as well as our requirements.

Except some particular aspects concerning this journal, the following details are general requirements asked or imposed by other journals as well. Conditions to be met in order to propose a paper for publishing. The main author has the responsability to make sure the article has been approved by all the other authors. The journal will have copyright for papers accepted for publishing. The editorial board reservs the right to change the style and dimensions of an article (major changes will be discussed with the main author) and to decide the date of issue.

2. FIRST PUBLICATION

The editorial board will not consider a paper already reported in a published general review or described in a paper proposed to or accepted by another journal. This does not exclude papers which have been rejected by other journals. Also, papers which have been presented at a scientific meeting will be accepted for discussion if they have not been entirely or partially published in a similar publication. „Multiple" publishing of the same study is seldom justified. One of the possible justifications is publishing in a second language but only if the following conditions are met:

- Editors of both journals involved are fully informed;
- Priority of the initial publication will be respected by a minimum publishing interval of two weeks;
- For the second publication, a shortened version will suffice;
- The second version strictly reflects data and interpretations in the first;
- A footnote may state: „This article is based upon a study initially published in [title of the journal]".

3. PATERNITY

Paternity must reflect the common decision of the coauthors. Each author must have participated enough to take public responsability for the content. A paper with collective paternity must have a key person responsable for the article.

4. COPYRIGHT

In order to reproduce materials from other sources, written agreement from the copyright owner must be obtained:

- photographer – for unpublished photographs;
- hospital where the photographer (physician) is employed – for unpublished photographs performed during the employment period;
- initial publisher – for a table, picture or text which have previously been published elsewhere.

5. ETHICAL ASPECTS

Do not use name of patients, initials or hospital observation charts numbers. If a photograph of a body part which could allow direct or deductive recognition of the

patient needs publishing, then the paper must be accompanied by the written consent of the patient and clinician, as well.

6. PRESENTING THE MANUSCRIPT

For the journal *„Medicine in evolution"*, the manuscript must be typed double spaced, on white A_4 paper – 210 x 297mm, on one side (2.5cm upper and lower borders, 3cm left and 2cm right border, respectively), in clear characters, no further corrections or addings. It is advisable that articles are presented on CD or other data transfer methods, in Word format, 12 Times New Roman fonts - using Romanian characters – respecting the same page order, accompanied by a printed version. Graphs – black and white or coloured – may be generated in MS Excel or MS Graph, inserted in the body of the paper or presented in a different file.
Infected materials will not be used.

6.1. FIRST PAGE (TITLE PAGE)

Together with the title and names of the authors, the first page must include the affiliation, professional and university degree (if applicable), marked by asterisc for every author; it is advisable to give at least a phone and/or fax number or e-mail address of the first author who may be contacted by the editors for additional recommendations or explanations.

6.2. ABSTARCT OF THE PAPER

6.2.1 Recommendations for original studies

Original studies must include a structured abstarct of maximum 150 words, containing the following titles and informations:

- Aim and objectives;
- Material and methods;
- Results;
- Conclusions;
- Key words: give 3-5 key words;
- The abstract will be translated into an international circulation language.

6.3 CONTENT OF THE PAPER

6.3.1 For original articles

The text will usually be divided into sections:

- Introduction – presentation of general aspects, in the context of the approached theme
- Aim and objectives – Define the aim of the article. Briefly expose the rationale of the presented study or observation. Make strictly pertinent referals and do not exhaustively review the subject. Do not include data or conclusions from the paper.

- Material and methods – Describe the selection of observations or subjects for the experiment (including controls). Identify methods, equipments (with the name and address of the manufacturer in brackets) and give sufficient details on procedures. Give references for the selected methods, including statistical methods; offer details and brief descriptions for previously published methods which are not well known; describe new or substantially modified methods, justify their use and assess their limitations. Precisely identify all used drugs and chemicals, including generic names, dosage and administration ways. Describe statistical methods with sufficient details for reported results to be verified. Whenever possible, quantify discovered aspects and present them with appropriate measurement indicators for the uncertainty or error of measurement (such as confidence intervals).
- Results – Present results in a logical succession as text, tables and illustrations. Emphasize or briefly describe only important observations.
- Discussions – Underline new, important aspects of the study. Do not repeat in detail data which have been presented in previous sections. Include implications of revealed aspects and their limitations, including implications for future studies. Connect your observations to other relevant studies. Relate the results to the aim proposed for the study.
- Conclusions – organize conclusions which emerge from the study. In the end state: a) contributions to be acknowledged but which do not justify paternity right; b) thanks for technical support; c) thanks for financial or material support.

6.3.2 Indications for case reports

Themes may be selected from all medical fields. Manuscripts which offer a special gain for daily activity will have priority. The title must be clearly, precisely stated. It may be completed by a subtitle. It is advisable to include in the key words of the title the main message, the special element which may be observed from the case evolution. The content of a case report must be divided into three parts:

- Introduction – It must include a maximum of 15 typed rows (half page). Here, the main medical problem is summarized in order to place the case in a specific domain.
- Case report – It contains essential specific information on the case.
- In order to make a logical, chronological and didactical case report the following 5 chapters are needed:

  I. Anamnesis;
  II. Clinical examination data;
  III. Laboratory data;
  IV. Additional paraclinical investigations;
  V. Treatment and evolution.
- Discussions – The reason for the case report must be stated. The report must be patient-centered. Occasional deviations from typical (characteristic) evolutions, nosologically important facts must be presented in such a manner to expose the clinical picture as completely as

possible. The case report must not appear as an appendix of a general review. Dimensions of a case report: maximum 6-8 typed pages, 30 rows of 60 characters/page.

6.4. MEASUREMENT UNITS, SYMBOLS, ABREVIATIONS

All measurements must be expressed in International System (IS) units. Abreviations must be fully explained when first used.

6.5. TABLES

Tables are noted with Roman figures and they will have a brief and concise title, concordant with their content.

6.6. ILLUSTRATIONS

Number all illustrations in Arabic figures in a single succession. Apply a label on the back side of every illustration, containing its number and an arrow indicating the upper side. Coloured illustrations may be accepted but it is the choice of the editors, according to particular technical abilities of each journal issue, or it may involve a fee in special cases.

6.7. EXPLANATIONS FOR DRAWINGS AND GRAPHS

Explanation for drawings and graphs must be clear and in readable dimensions, considering the necessary publishing shrinkage.

6.8. PHOTOGRAPHS

Offer glossy, good quality photographs. Any annotation, inscription, etc. must contrast with the ground. Microphotographs must include a scale marker.

6.9. ILLUSTRATION LEGENDS

Include explanations for each used symbol, etc. Identify the printing method for microphotographs.

6.10. REFERENCES

A numbered list of references must be provided at the end of the paper. The list should be arranged in the order of citation in the text of the publication, assignment or essay, not in alphabetical order(according to the Vancouver rules). List only one reference per reference number. It is very important that you use the correct punctuation and that the order of details in the references is also correct.

- Books - Standard format - #. Author of Part, AA. Title of chapter or part. In: Editor A, Editor B, editors. Title: subtitle of Book. Edition(if not the first). Place of publication: Publisher; Year. p. page numbers.
- Journal Articles - Standard format - #. Author of article AA, Author of article BB, Author of article CC. Title of article. Abbreviated Title of Journal. year; vol(issue):page number(s).
- E-Books - Standard format - #. Author A, Author B. Title of e-book [format]. Place: Publisher; Date of original publication [cited year abbreviated month day]. Available from : Source. URL.
- E-Journals - Standard format - #. Author A, Author B. Title of article. Abbreviated Title of Journal [format]. year [cited year abbreviated month day];vol(no):page numbers[estimated if necessary]. Available from: Database Name (if appropriate). URL.
- Internet Documents - Standard format - #. Author A, Author B. Document title. Webpage name [format]. Source/production information; Date of internet publication [cited year month day]. Available from: URL.

7. COPIES FOR PUBLISHING

In order to accelerate publishing, the main author will send a set of printed sheets presenting the final version of the paper, as it will appear in the journal. It is really helpful that texts to be also sent on electronic support, diacritic characters mandatory.

8. REJECTION OF PAPERS

If a paper does not meet publishing conditions, whatever these may be, the editors will notify the first author on this fact, without the obligation of returning the material. Original photographs or the whole material will be returned only if the author comes to the editor and takes them.

Papers submitted for publishing will be addressed to:

Prof. Angela Codruta Podariu, DMD, PhD

Journal Medicine in evolution
Department of Preventive, Community Dental Medicine and Oral Health
Splaiul Tudor Vladimirescu no. 14 A
300041, Timişoara
Phone: 0256-204950
Email: proiectetm@yahoo.com

Dana Brehar-Cioflec, MD, PhD

Institute of Public Health *"Prof. Dr. Leonida Georgescu"* Timişoara
Bd. Victor Babeş no. 16
300226, Timişoara
Phone: 0256-492101
Email: dcioflec@yahoo.com

Order form

☐ **Rate 38 Euro (Outside Romania)**

☐ **Rate 160 Ron(Inside Romania)**

The money will be pay in the account: RO80 ABNA 3600 264 1000 89 288 opened at RBS BANK, Timisoara

Name_____

Speciality _____

Adress _____

Phone _____**Fax** _____**Email** _____

……

Order form

☐ **Rate 38 Euro (Outside Romania)**

☐ **Rate 160 Ron(Inside Romania)**

The money will be pay in the account: RO80 ABNA 3600 264 1000 89 288 opened at RBS BANK, Timisoara

Name_____

Speciality _____

Adress _____

Phone _____**Fax** _____**Email** _____