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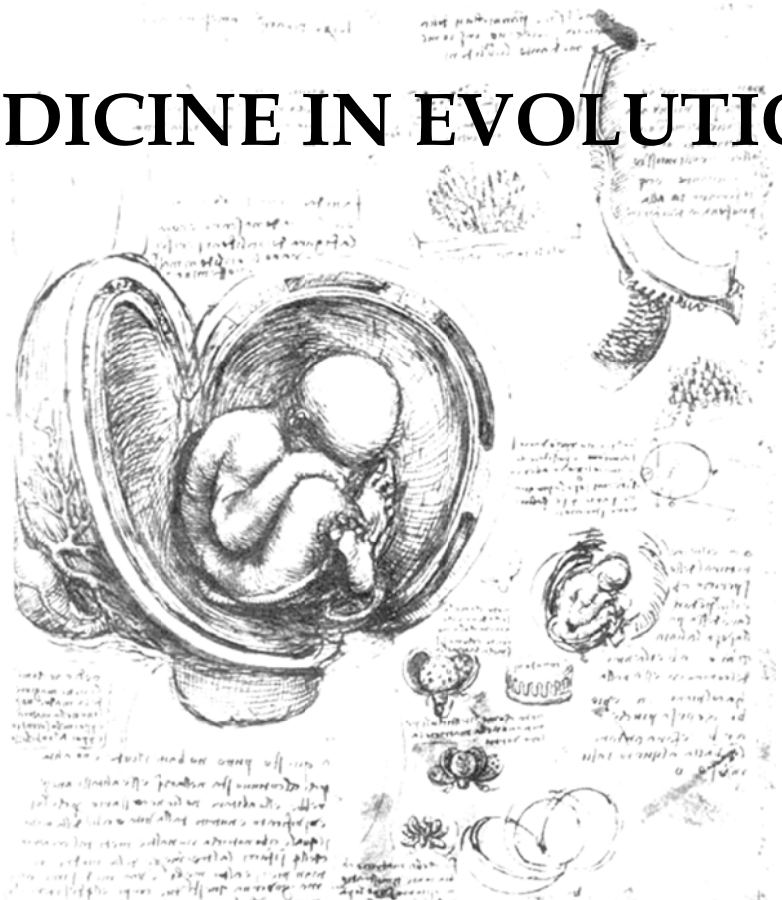

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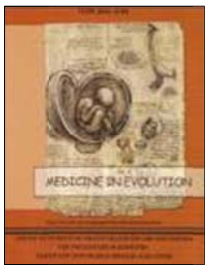
CONTENTS

ARTICLES

LDL-CHOLESTEROL AND C REACTIVE PROTEIN (HS-PCR) ROLE IN ASSESSING THE CARDIOVASCULAR RISK AT PATIENTS WITH CORONARY ARTERY



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ABSTRACT

***Aim and objectives**: The present study tries to demonstrate a possible correlation between hs-CRP, LDL cholesterol and cardiovascular risk at patients with coronary angiography revealing significant coronary arthery disease.*

***Methods**: We included in our retrospective study a number of 87 patients with coronary artery disease. There was made a clinical evaluation; EKG, echocardiography, fasting glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides and hs-CRP were determined.. Based on LDL-cholesterol and hs-CRP level we formed 4 groups: group A (LDL-cholesterol>70mg% and hs-CRP>2mg/l; group B (LDL-cholesterol <70mg% and hs-CRP<2mg/l), group C(LDL-cholesterol>70mg% and hs-CRP<2mg/l) and group D(LDL-cholesterol <70mg% and hs-CRP<2mg/l).*

***Results**: There were reported 5 cardiovascular events. The percent of cardiovascular events reported was 10% for group A, 7.62% for group B, 7.14% for group C and 4% for group D.*

Conclusions: CRP was a strong predictor of cardiovascular events even in case of patients with normal LDL-cholesterol levels. There is a strong correlation between hs-CRP and LDL-cholesterol level on the hand and the cardiovascular events on the other hand.

***Key words**: coronary artery disease, hs-CRP, LDL-cholesterol, secondary prevention.*

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INTRODUCTION

LDL-cholesterol role in cardiovascular disease is well documented by the study of atherosclerosis being supported by the clinical studies (WOSCOPS, ASCOT-LLA, 4S, LIPID, HPS) that demonstrated that lowering LDL values determined the reduction of cardiovascular events. LDL-cholesterol is one of the most important fractions of the lipid profile. In 50% of patients cardiovascular events (myocardial infarction, stroke) succeed at apparent healthy persons with normal LADLE-cholesterol [4].

Proving the role of inflammation in acute coronary syndromes made the inflammation markers determination an important step of cardiovascular risk assessment and efficient secondary prevention. Inflammation determines the erosion and rupture of the atherosclerotic plaque. C reactive protein is the most studied of the various inflammation markers involved in the process of atherosclerosis. Studies had proved the strong predictive role of has-CRP for cardiovascular events at patients with unstable angina or myocardial infarction, higher levels of has-CRP being associated with a lower percent of survival at these patients [1]. The prognostic role was also observed in patients with stroke or peripheral artery disease.

AIM AND OBJECTIVES

The present study tries to demonstrate a possible correlation between hs-CRP, LDL cholesterol and cardiovascular risk at patients with coronary angiography revealing significant coronary artery disease.

MATERIAL AND METHODS:

We included in our retrospective study a number of 87 patients with a coronary angiography revealing significant CAD (lumen reduction ≥ 70%) aged 40 to 78 years with statin treatment (atorvastatin 80mg/day).

The group included 39 women (44.82%) and 48 men (55.17%). They have been followed up for a period of 3 years being reported the cardiovascular events (unstable angina, myocardial infarction or the need of reperfusion) or cardio-vascular death that occurred. Clinical evaluation, EKG, echocardiography, fasting glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides were determined. Hs-CRP, a more sensitive CRP test, was determined at hospital admission day, with values that varies between 0.2-8mg/l (0.02-0.8 mg/dl). Hs-CRP was determined by latex-turbidimetry assay.

The study did not include patients with diabetes mellitus and patients that had any sign of infection, systemic inflammation or trauma. Hs-CRP values varied from 0.3 mg/l to 5.1 mg/l.

We divided our patients in 4 groups depending on LDL-cholesterol and hs-CRP values. Group A that included patients with LDL-cholesterol > 70mg% and hs-CRP > 2mg/l (10 patients); group B with LDL-cholesterol < 70mg% and hs-PCR > 2mg/l (13 patients); group C with LDL-cholesterol > 70mg% and hs-PCR < 2mg/l (14 patients) and group D with LDL-cholesterol < 70mg% and hs-PCR < 2mg/l (50 patients).

After adjustment for age, smoking status and presence of arterial hypertension we used Pearson correlation and Cox proportional hazards models to examine the association between hs-CRP, LDL-cholesterol values and cardiovascular events. Study limits are the relatively small number of patients and not reporting the strokes that occurred.

Table 1 *Group repartition of patients based on LDL-cholesterol and hs-CRP*

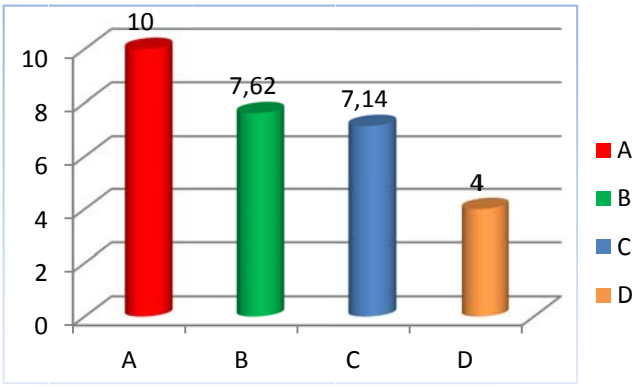
GROUP	LDL CHOLESTEROL (MG/DL)	PCR (MG/L)
A	> 70	> 2
B	< 70	> 2
C	> 70	< 2
D	< 70	< 2

RESULTS

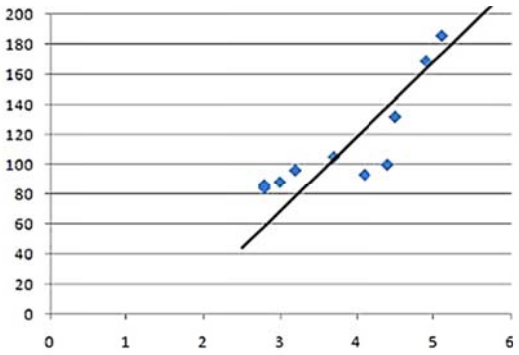
The median age was 59 years. There have been reported 5 cardiovascular events (1 cardiovascular death and 4 patients needed percutaneous transluminal coronary angioplasty and stent implantation).

In each group B, C and D was reported one cardiovascular event and in group A there were 2 cardiovascular events. In group A the percent of cardiovascular events was 10%, 7.62% in group B, 7.14 % in group C and 4% in group D.

There was observed a high percentage of cardiovascular events in group A, followed by group B.



There was observed a strong linear correlation between higher level of hs-CRP and LDL-cholesterol (r=0.84) (group A).



The majority of patient with hs-CRP levels higher that 2mg/l were women.

DISCUSSION

C reactive protein is a stronger predictor of cardiovascular events that LDL-cholesterol. Screening for both biological markers could offer more pro-

gnostic information that each one considered separately. Besides exploring lipid profile during statin treatment we should consider evaluating C reactive protein for superior results in secondary prevention.

CONCLUSIONS

C reactive protein (hs-CRP) is a strong predictor of cardiovascular events even in patients with normal LDL-cholesterol level. There is a strong linear correlation between significant high levels of hs-CRP and LDL-cholesterol on a hand, and acute coronary syndromes on the other hand. Evaluating both parameters had a significant powerful predictive value for coronary artery diseased patients compared to each of them taken separately.

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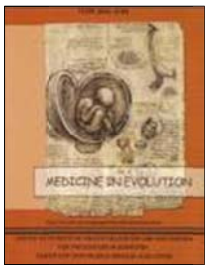
ANALYSIS OF TRACHEAL SOUND LEVEL AS A POTENTIAL DIAGNOSTIC TOOL FOR PULMONARY OBSTRUCTIVE SYNDROMES



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ABSTRACT

Aim and objectives: *to asses the possibility of developing a tracheal sound analysis tool capable of diagnosing obstructive syndromes*

Material and methods: *33 subjects were stratified to the obstructive or control group. Tracheal sound was recorded during a forced expiratory maneuver; acquired signals were analyzed in terms of sound level vs time and a linear regression model was computed. The obstructive subgroup included 21 vs 13 controls. We found statistically significant between group differences for expiratory duration and for the linear regression and negative significant correlations between slope and expiratory duration, FEV1 and FEV1/VC. Building the ROC curve a threshold value of -19.67 for the slope of the linear regression model will associate a sensitivity of 95% and a specificity of 84.6% for this test.*

Conclusion: *Available data suggests that tracheal sound level analysis could be developed into a diagnostic and monitoring tool; additional mathematical approaches are probably necessary.*

Key words*: COPD, bronchial asthma, sound.*

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INTRODUCTION

Although computerized pulmonary sound analysis is at least 30 years old and undergone significant evolution lately its exact role in clinical setting is far from clear. Present technical improvements create the possibility of significant developments; this approach could possibly be introduced as a regular diagnostic and monitoring approach for pulmonary diseases patients[2].

Lately, respiratory sound analysis saw some use for bronchial asthma monitoring[1, 9], newborn and intensive care unit patients respiratory patterns, sleep apnea detection[7, 10] but there are developing perspectives such as sound based imagistics[3].

This potential developement is supported by scientific media in terms of standardizing capturing, filtering, digitizing and analyzing pulmonary sounds. The European project Computerized Respiratory Sound Analysis (CORSA) generated some guidelines relevant to method standardization for pulmonary acoustic research.

AIM AND OBJECTIVES

Aim: to asses the possibility of developing a tracheal sound analysis tool capable of diagnosing obstructtive syndromes.

Objective: to identify significant differences between obstructive and non-obstructive subject in terms of tracheal sound level variations.

MATERIAL AND METHODS:

Our study group included 33 subjects which agreed to participate in our study by signing an informed consent form. Each subject was asked to undergo a standard spirometry test complying to ERS/ATS standards. The results were used to assign subjects to obstructive or control (normal values) study subgroups.

An electret monodirectional microphone was then placed on the suprasternal notch and the subject was asked to perform three forced respiretory maneuvers. The microphone was connected to a standard computer soundcard and commercial recording software was used to obtain a 48000 kHz sample rate wav file.

The sound file was analyzed using a virtual instrument created using the National Instruments LabView 8.2 development system. Expiratory sounds were identified using a semi-automatic approach – our virtual instrument detected sharp rises and falls on the signal spectrogram and a human operator sorted out non-expiratory events (inspiratory and artifacts). Once obtained, only the longest expiratory effort was further analyzed – the signal was filtered using a band pass (100-5000 Hz) 5th order Butterworth filter and then was fed to a sound level measurement module. A fast mode exponential averaging approach was used and a sound level vs time chart was produced. (Fig. 1) This curve was truncated and only the time of maximum – expiratory end (minus 0.2 seconds) interval was used to generate a linear regression model.

We used this approach to eliminate effort-dependent expiratory phases – we assumed that maximum tracheal sound intensity is concurrent with PEF + time. The following para-

meters were recorded in the final database: demographic data (age, sex), morphometric data (height), spirometry values (FEV1, FVC, FEV1/VC, PEF as % of redicted values NHANES III), expiratory effort duration, median sound level and regression model slope. The data was analyzed using SPSS 17.0.

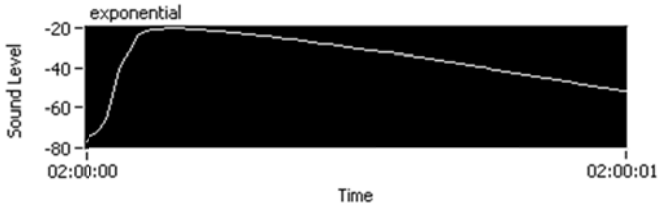

Fig.1 Sound level vs time curve – from a healthy subject.

RESULTS

The obstructive subgroup included 21 subjects (7 females) with a mean age of 57.1 +/- 11 years and the control group 13 subjects (6 females) – mean age 42.5 +/- 15 years. The obstructive syndrome subjects had a significant history of chronic obstructive pulmonary disease or bronchial asthma (4 subjects).

Main descripttive statistics of spirometry and acoustic data are shown in table I.

Table 1 *Mai descriptive statistics of spirometry and acoustic data*

GROUP		FEV1 (%)	FVC (%)	FEV/VC (%)	PEF (%)	Expiratory duration (s)	Median sound level (dB)	Linear regression slope
CONTROL	Mean	102,8	99,3	85,8	89,3	2,0	-35,9	-24,5
	Std. Deviation	14,8	11,4	4,6	16,3	0,4	9,1	4,8
	Range	54	38	18	50	1,6	28,9	14,1
OBSTRUCTIVE	Mean	58,4	74,5	61,8	44,5	4,3	-36,3	-9,1
	Std. Deviation	20,0	22,1	8,9	20,4	2,3	11,4	6,0
	Range	66	78	37,7	78	9,1	39,4	22,5
TOTAL	Mean	75,9	84,2	71,2	62,1	3,4	-36,1	-15,1
	Std. Deviation	28,3	22,1	14,0	29,0	2,1	10,4	9,4
	Range	107	82	47	92	9,7	39,4	29,0

We found statistically significant between group differences for expiratory duration – mean difference 2.2 s (95% Confidence Interval 1.1 – 3.4 s) for p=0.002 (two tailed independent samples t-test equal variances not assumed) and for the linear regression slope – mean difference 15.4 (95% Confidence Interval 11.4 – 19.3). There were no between groups' significant differences for median sound level.

There were negative significant correlations between slope and expiratory duration (Pearson - 0.643, two tailed significance > 0.0001), between slope and FEV1 (Pearson - 0.621, two

tailed significance > 0.0001) and between slope and FEV1/VC (Pearson - 0.703, two tailed significance > 0.0001) (Fig. 2).

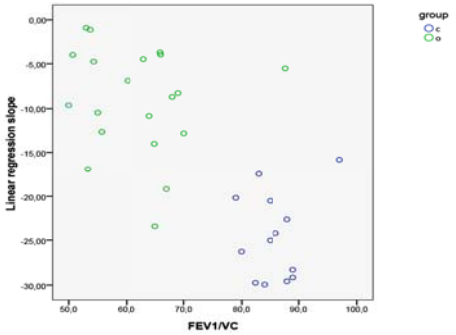

Fig.2 Scatter dot diagram of slope vs FEV1/VC values for the two subgroups.

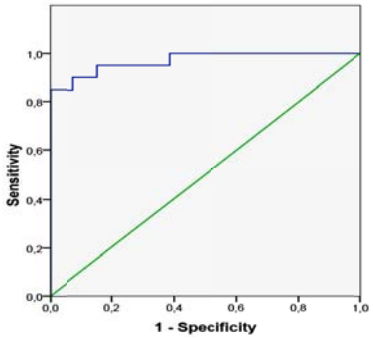

Fig.3 ROC curve for the slope of the regression used a diagnostic test for obstructive syndromes (Area under curve 0.969, asymptotic sig. > 0.0001)

Assuming the role of a diagnostic tool for the slope of the regression the following ROC curve was constructed (Fig. 3) – using a threshold value of 19.67 thi test should have a sensitivity of 95% and a specificity of 84.6%.

DISCUSSION

Study limits

The results need to be cautiously interpreted. The group under study had a relatively small size and results may need further validation. The subgroups were also unbalanced in terms of age – this was mainly due to avoid the grey area of normal subjects' showing some age related decrease in FEV1 and FEV1/VC.

Furthermore there was little control on the quality of the expiratory maneuvers we recorded – in order to minimize the chance of a sound artifact the recording took place after spirometry. The sound level regression slope values were tackled using three hypotheses relevant to obstructive disorders subjects:

- a significant larger maximum expiratory sound level;
- a significant longer expiratory maneuver;
- a significant higher end-expiratory sound level.

There where no significant between group differences as far as maximum expiratory sound level was concerned.

These data is particularly difficult to interpret due to some individual confusion factors (such as fatty tissue) but we assumed that from a theoretical point of view there is a low probability for obstructive subjects to associate high values. There are data suggesting the main tracheal sound component is due to local turbulent airflow. The Reynolds number may be calculated to characterize flow as turbulent or laminar using the formula $Re = (2ru\rho)/\eta$ (r – radius, u – gas velocity, ρ – gas density, η – gas viscosity) – tracheal value (r ~ 15 mm) is over 2000 for air suggesting turbulent airflow for high velocity reached at PEF time. It seems reasonable to assume a lower velocity for obstructive subjects whom may lead to lower turbulence and hence to a lower sound level at least during the first phase of the expiratory maneuver.

Our data show a significant between group's difference in terms of expiratory maneuver duration as it was expected considering the COPD and bronchial asthma dynamic obstruction phenomena which limit the airflow irrespective of the lung volumes [6, 8].

It is worth mentioning that a prolonged expiratory maneuver is not the sole explanation for the slope differences – the correlation is strong and negative but not perfect. Given this seems reasonable to assume a higher end expiratory sound level with a non-tracheal source. Considering the low tracheal flow this source lies probably within the small airway dynamic obstruction turbulence. There are data from studies on in vivo and post mortem airflow resistance suggesting that an important obstruction component may be identified within the bronchi of the 4th-14th order characterized by a diameter of less than 2 mm [5, 11]. This hypothesis is also supported by imagistic data suggesting a strong correlation between overall obstruction intensity expressed as FEV1 value and distal airway diameter [4]. Furthermore common clinical experience mentions forced end-expiratory wheezes even for subjects with mild obstructive syndromes. Even so our method has the advantage of a lower detection threshold and an objective approach. Different mathematical developments such as spectral analysis may be useful to fully characterize these phenomena.

The degree of correlation between the slope of the linear regression used and the intensity of the obstruction may confer our method o potential role as a monitoring tool (particularly useful for bronchial asthma patients), should further research estimate the magnitude of individual variability.

DISCUSSION

Available data suggests that tracheal sound level analysis could be developed into a diagnostic and monitoring tool; additional mathematical approaches are probably necessary.

List of abreviations

COPD – Chronic obstructive pulmonary disease
FEV1 – Forced expiratory volume at 1 second
FVC – forced vital capacity
VC – vital capacity

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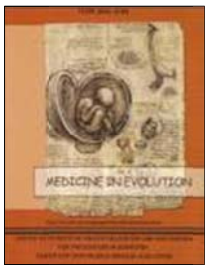
A SEVERE NON-SYNDROMIC OLIGODONTIA IN PERMANENT DENTITION. A CASE REPORT



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ABSTRACT

Disturbance in tooth development leads to various dental anomalies, including tooth agenesis, or congenital absence of primary and / or permanent teeth. Oligodontia is a rare condition that can occur in association with genetic syndromes, or as a non-syndromic isolated trait [2]. Several studies have shown that gene MSX1 and PAX9 play a role in early teeth development [7, 10].

We present a case where the clinical and radiographic examination determined that 14 permanent teeth are present, while 14 permanent teeth are absent; except for third molars. There was no history of previous extractions, a systemic disease or syndrome. In our case, cytogenetic analysis exhibits the fragile site at 1p36 chromosome.

A multidisciplinary approach is essential to achieve better aesthetics and function in such cases.

***Key words**: oligodontia, nonsyndromic, genetics, cytogenetic, chromosome, multidisciplinary approach.*

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INTRODUCTION

Tooth development is under strict genetic control. Human teeth agenesis is caused by several independent defective genes, acting alone or in combination with other genes, leading to a specific phenotypic pattern [10].

Agenesis of one or more teeth is one of the most common human development anomalies. The prevalence of permanent teeth agenesis ranges between 1.6-9.6% depending on the population studied and it reaches 20% if third molars are considered [2].

Oligodontia is defined as the congenital absence of 6 or more permanent teeth, excluding the third molar and has a prevalence of 0.3% in the permanent dentition [1]. It occurs more frequently at girls, at a ratio of 3:2 [1].

Oligodontia is a rare condition that can occur in association with genetic syndromes, or as a non-syndromic isolated familial trait [2].

In the literature, patients with syndromic and non-syndromic oligodontia are differentiated using the criteria of extra-oral or non extra-oral symptoms [2].

The occurrence of non-syndromic oligodontia is poorly understood, but in recent years several cases have been described where a single gene mutation is associated with oligodontia. Several studies have shown that gene MSX1 and PAX9 play a role in early teeth development [10].

According to recent investigations in the majority cases oligodontia is genetically conditioned, although this does not exclude the presence of external and internal factors. In most cases anomalies in the number of teeth (hypodontia, oligodontia) are connected with anomalies in the shape and size of the teeth. Microdontia often occurs at persons with oligodontia [3, 8].

Characteristic dental features are reduced number of teeth, reduction in teeth size and form, an delayed eruption.

MATERIAL AND METHODS:

Patient S.M.D, 8 years, applied to the Department of Pedodontics and Orthodontics with the chief complaint of missing teeth, aesthetic and functional problems.

There was no history of previous extractions, a systemic disease or syndrome. Diagnostic records included orthopantomogram (OPG), lateral cephalogram and study models. Clinical and radiographic examination determined more missing teeth (14 teeth), mixed dentition, persistence of inferior incisors uncommon with pacient age, interdental spaces and diastema associated with microdontia. In our case, except for third molars, 14 permanent teeth are present, while 14 permanent teeth are absent. The missing permanent teeth numbers are: 1.7, 1.6, 1.5, 2.4, 2.5, 2.6, 2.7, 3.6, 3.5, 3.1, 4.1, 4.5, 4.6, and 4.7.

RESULTS

Genetic investigation was made and the results are: Chromosomal fragile sites are specific loci that preferentially exhibit gaps and breaks on metaphase chromosomes following partial inhibition of DNA synthesis. In our case, cytogenetic analysis exhibits the fragile site at 1p36 chromosome. This

region [1p36(FRA1A)], can be associated with dominant negative and dominant positive mutations. No clear phenotype-genotype relationship between phenotypes of dental agenesis and fragile site 1p36 has been described, but instability of the region was reported [6].

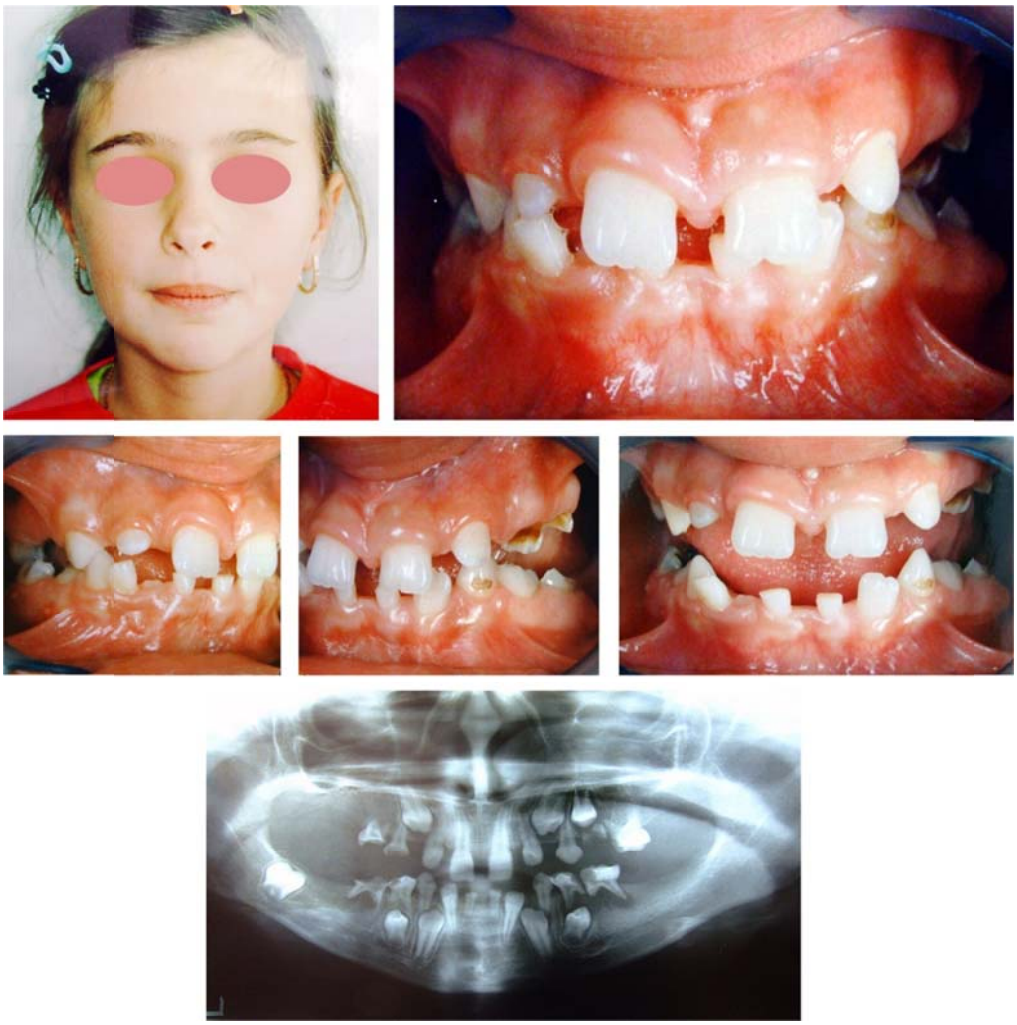

Fig.1 Patient S.M.D, 8 years.

Fig.2 Cariotype and metaphase.

DISCUSSION and CONCLUSION

Isolated or nonsyndromic oligodontia is inherited in an autosomal dominant form with reduced penetration. In the case of patients with oligodontia careful planning treatment is necessary. This requires a multidisciplinary approach consisted in a combination of orthodontic therapy, restorative implants and prosthetic procedures [1]. Objectives of orthodontic treatment could be opening or closing spaces, correcting the occlusal relation with removable or fixed appliance.

Patients with severe oligodontia and partial edentulism frequently exhibit a reduced potential for orthodontic treatment due to intraoral anchorage limitation. Implants may be placed into alveolar bone to provide anchorage strictly for teeth movement, to provide initial orthodontic anchorage, or to support a prosthetic restoration [1]. The prosthetic rehabilitation includes occlusal stability, establishing of correct vertical dimension, and presserving the health of the soft and hard tissue as well that of temporomandibular joint.

The masticatory efficiency and ability are important components of the oral functionality. A shortened dental arch (SDA) concept can be discussed as a temporary prosthetic rehabilitation [4, 9]. A shortened dental arch is defined as having an intact anterior region but a reduced number of occluding pairs of posterior teeth [4, 9].

If the premolar region is intact and there is at least one pair of occluding molars, the SDA may not impair the masticatory efficiency [9].

An impared masticatory ability and associated changes manifest only when there are less then ten pairs of occluding teeth. In this case the future possible contacts are in region 21, 22, 13, 23, 43, 33, 14, 44 after orthodontic treatment.

Treatment of this case could consist of fixed partial denture from the first right premolar to the left canine in the maxilla and from the right first premolar to the left first premolar in the mandible. The rest of the dental arch could be completed with the dental implants but only at the age of 18.

Treatment in severely compromised oligodontia patients not only improves phonetic, nutritional and aesthetic problems but also has pshychological implications.

Majority of oligodontia patients seek orthodontic care because of unaesthetic malocclusion. Familial teeth genesis is transmitted as an autosomal dominant, recessive, or x-linked condition [5]. Affected members within a family often exhibit significant variability with regard to the location symmetry and number of teeth involved. Permanent dentition is more affected than the primary dentition. Several studies have shown that MSX1 and PAX9 play a role in early teeth development [3]. PAX 9 is a paired domain transcription factor that plays a critical role in odontogenesis. All mutations of PAX 9 identified to data have been associated with nonsyndromic form of teeth agenesis [5, 7]. A multidisciplinary approach is essential to achieve better aesthetics and function in such cases.

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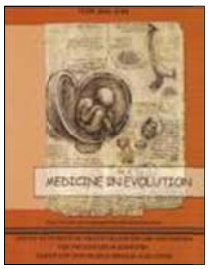
THE ERGOGENIC AND PAIN REDUCTION ROLES OF CAFFEINE



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ABSTRACT

Caffeine (1,3,7-trimethyl xantine) is the most widely used behavior influencing substance consumed on earth. It has been used to enhance the physical fitness component (ergogenic aid) in sports. Caffeine enhances performance during both low-intensity endurance events (which last several hours) and high intensity exercise (mainly of short-duration). Possible mechanisms involved include increased fat oxidation, stimulation of the central nervous system activity and/or energy metabolism, decreased perception of pain and attenuation of the elevation in potassium concentration. Also, caffeine delays fatigue and improves the contractile strength of cardiac and skeletal muscles.

The hypoalgesic effect of caffeine might stem from its action on peripheral or central adenosine A1 and A2A receptors, involved in the nociceptive system.

***Key words**: caffeine, performance, ergogenic aid, hypoalgesic effect, adenosine*

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INTRODUCTION

Caffeine (1, 3, 7-trimethyl xantine) is a widespread stimulant of the central nervous system; it is the most widely used behavior influencing substance consumed on earth [10]. Caffeine is easily found in our diet or in some medicateons and used to increase athletic performance.

The caffeine ergogenic effect

Both caffeine and xanthine-based substances have been used as ergogenic aids in many sports and are presently very popular with soccer players [6, 10].

Ergogenic aids can be defined as drugs, nutritional strategies or physiological procedures that cause enhancement of a physical fitness component [3].

Caffeine is a well-established ergogenic aid which, soon after ingestion, can prolong time to exhaustion during exercise [3]. Its ergogenic potential has attracted renewed scientific interest since its removal in 2004 from the World Anti-Doping Agency's (WADA) prohibitted list. This removal occurred even though there is general consensus from research findings that caffeine improves prolonged continuous exercise performance [6, 10].

For sports caffeine has many favorable effects, its ergogenic effects on endurance exercise being well documentted.

Numerous studies have established that ingestion of caffeine improves performance in low-intensity endurance events lasting several hours. It would appear that caffeine ingestion also enhances performance during single efforts of short-duration, high-intensity exercise and during repeated sprint exercise [1, 10].

In a recent study, Bridge C.A. and Jones M.A. [4] reported that caffeine improved performance in an 8-Km run by ~ 1.34%. Since a number of athletes competing in 5-Km racing regularly con-sume caffeine (with a little scientific rationale for its ergogenic benefits at this distance), O'Rourke M.P. at al. [23] have investigated the action of caffeine ingestion, one hour before a 5 Km-race. They revealed that caffeine ingestion is likely to improve 5-Km performance. It is debatable whether or not a 1% improvement in completion time would be considered a worthwhile enhancement.

A previous study performed by Hopkins W.G. and Hewson D.J. [11] suggests that the smallest worthwhile gain in performance time is ~ 1% for well-trained runners and therefore caffeine offers a worthwhile advantage for these athletes. The possible suggested mechanism (s) by witch caffeine elicits its performance-enhancing properties in short-duration high-intensity events include (s) increased fat oxidetion, enhanced central nervous system activity and decreased perception of pain and attenuation of the elevation in extracellular potassium (K+) concentration [13].

The studies which have documented caffeine ergogenic effects have been done primarily with subjects with a high maximal aerobic power and with performance tests that usually, but not always, lasted longer than 30 minutes. The extent of improvement appears to be dose-related until a dose of 5-6 mg. Kg-1, above which no further enhancement occurs [9].

Skinner T. at al. [28] investigated the ergogenic effects of small caffeine doses (2 mg/Kg, 4 mg/Kg and 6 mg/Kg) on high intensity exercise performance in well-trained rowers. They found there was a small but statistically significant ($p<0.05$) improvement in performance with increasing plasma caffeine concentration. There were also significant differrences in heart rate and plasma lac-

tate, glucose and caffeine ($p<0.05$) following caffeine intake, demonstrating a physiological effect of caffeine. However, it is difficult to determine whether caffeine is ergogenic during short duration, high intensity rowing due to the considerable variation in absorption of caffeine between subjects [28].

A number of studies have investigated the influence of caffeine on muscle metabolism and exercise performance. It has been shown that acute caffeine ingestion before exercise can lead to enhanced endurance exercise performance increases in plasma epinephrine and serum free fatty acids (FFA) and sparing of muscle glycogen [8, 9]. Greater increases in plasma FFA concentration and lipid oxidation have not been consistently observed with caffeine during exercise as compared to control conditions. An acute increase in the circulating concentration of epinephrine during sub-maximal exercise has been shown to increase hepatic glucose production, but had variable effects on skeletal muscle glycogenolysis [5]. However, increases in epinephrine associated with caffeine ingestion do not appear to influence muscle glycolgenolysis. Therefore, an increase in epinephrine mediated by caffeine administration could lead to an increase in plasma glucose. Such an increase in glucose availability could contribute to glycogen sparing also observed with caffeine administration, since the increase in epinephrine with caffeine alone does not appear to influence muscle glycogenolysis. Caffeine acts as an antagonist to the adenosine A1 and A2 receptors. Adenosine stimulation of hepatocytes has been found to increase the activity of glycogen synthase phosphatase and phosphorylase phosphatese.

Such alterations should theoretically lead to an increase in glycogen synthesis; however, increases in adenosine are also associated with increases in cAMP, which would lead to a stimulation of glycogen breakdown.

Roy B.D. at al [25] investigated the possible influence of oral caffeine administration on endogenous glucose production and energy substrate metabolism during prolonged endurance exercise. Their study demonstrated that acute oral caffeine administration did not influence plasma glucose rate of appearance or plasma glucose rate of disappearance in trained endurance athletes during prolonged exercise. They also demonstrated that caffeine did lead to increased concentrations of blood lactate during the exercise. These findings suggest that endogenous glucose production and glucose disposal in trained endurance athletes are not influenced by oral caffeine administration.

The ergogenic effects of caffeine ingestion have been attributed to a wide range of physiological factors leading to stimulation of the central nervous system and / or energy metabolism in peripheral tissues, including adenosine receptor blockade, improved neuromuscular transmission, increased muscle contractility and increased catecholamine levels, although the link between the latter and improved performance has recently been questioned [9].

By Graham T.E. [10] the ergogenic effects of caffeine are attributed to its action to delay fatigue and via an improvement in contractile strength of cardiac and skeletal muscles. The role of caffeine on the oxidative stress during exercise is not well known: prooxidative and antioxidant substance. One of the theories about caffeine actuation mechanism suggests that caffeine increases adrenaline concentrations during physical efforts [12]. From this point of view, a greater plasma concentration may induce an oxidative stress because catecholamine metabolic inactivation is a recognized source of

free radicals, as well as higher aerobic metabolism, therefore the antioxidant system may be unable to offset completely this proposed increase in oxidative stress. In this case, caffeine could act as a pro-oxidant substance.

On the other hand, some research may indicate that caffeine could itself conduct, under certain conditions, as an antioxidant substance [15]. As a result, it is possible that caffeine's ergogenic benefit may be related to its antioxidant properties.

In addition to the above-noted effects, caffeine appears to influence the immune system response to exercise [2]. Specifically, some research has demonstrated increased levels of leukocytosis and lymphocytosis after an exercise stress involving caffeine administration.

It is also important to note that caffeine use, along with exercise, leads to an enhanced activation of both the hypothalamic - pituitary - adrenal axis and the autonomic nervous system [14, 10], which can also affect immune responses to exercise. By Maridakis V. et al. [18] it may be the case that the ergogenic effects of caffeine occur by acting on the nervous system rather than directly on skeletal muscle excitation-contraction mechanisms.

The caffeine pain reduction role

Muscle pain is commonly experienced during exercise. Moderate-to-high intensity exercise results in transient, naturally occurring muscle pain located within the activated musculature. Muscle contractions are thought to cause pain by placing mechanical pressure on pressure-sensitive nociceptors and by generating a host of algesic biochemical by-products within the muscle, including bradykinin, serotonin, potassium, histamine, P substance, hydrogen ions, prostaglandins and adenosine. The relative contribution of those algesic substances to naturally occurring muscle pain during exercise has not been fully explained.

Prostaglandins alone do not appear to play a major role in naturally occurring muscle pain during exercise. Ingestion of a large dose of aspirin prior to exercise, which presumably reduced the concentration of prostaglandins, did not have a significant effect on muscle pain intensity ratings.

Adenosine does appear to play a primary role in naturally occurring muscle pain during exercise. It is an algesic known to be involved in inflammation; it has receptors in numerous neural tissues involved in nociception and pain processing, including peripheral afferent nerves, the dorsal horn of the cord, and higher brain areas [18].

Caffeine has documented exercise-related hypoalgesic effects; it reduces experimentally induced ischemic forearm muscle pain as well as quadriceps muscle pain that naturally occur during cycling exercise [19, 21]. The magnitude of the hypoalgesic caffeine effect may be dependent on the caffeine dose.

Supplementation with caffeine decreases muscular pain perception, effort perception and neuromuscular reaction time, which can further facilitate exercise performance [27, 13].

Several possible mechanisms for the caffeine-induced reduction in muscle pain during exercise exist. The hypoalgesia might stem from caffeine acting on peripheral or central adenosine A1 and/or A2A receptors involved in the nociceptive system [26].

The precise pain reduction mechanism is uncertain because caffeine is a competitive nonselective adenosine receptor antagonist with high affinity for both A1 and A2A receptors. Orally ingested caffeine by humans has effects on both peripheral and central nervous system because caffeine crosses the blood brain barrier. The locus within

the nervous system for the antinociceptive effect is uncertain because of the widespread location of adenosine receptors and the complexity of adenosine effects. Adenosine receptors are present in, and caffeine has acces to, nociceptive afferent fibers, the dorsal horn of the spinal cord, and brain areas involved in pain, such as primary and secondary somatosensory, insular, anterior cingulated and prefrontal cortices. Also, caffeine might indirectly influence the nociceptive system, for instance, by altering muscle sensory processes [24] or by acting on neurons involved in regulating the cardiovascular system. Multiple lines of evidence link hypoalgesia to elevated systolic blood pressure. For example, hypertensives, relatives of hypertensives and normotensives with "high normal" resting systolic pressure all exhibit reduced sensitivity to noxious stimuli.

The acute consumption of ≥ 200 mg of caffeine consistently raises systolic blood pressure by 3-14 mmHg and caffeine-induced increases in blood pressure are associated with hypoalgesia in response to the cold pressor test [16].

CONCLUSIONS

In summary, the caffeine supplementation has ergogenic effects and also decreases muscular pain perception, effort perception and neuromascular reaction time, which can further facilitate exercise performance.

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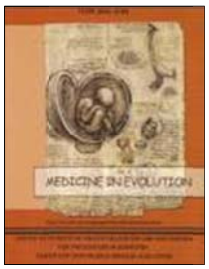
THE IMPORTANCE OF THE AUDIOGRAM IN PATIENTS WITH CLEFT PALATE



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ABSTRACT

The cleft lip and palate cannot be viewed in isolation and attention has to be given to associated pathology. The present study investigates the results and interpretation of hearing screening in treated or untreated patients with cleft palates. It was conducted on 28 subjects, with cleft palate with or without cleft lip operated in the Hospital of Cranio-Maxillo-Facial Surgery Timisoara between 1998 and 2010. Hearing screening outcomes were collected and audiogram was performed. Among the 28 patients with cleft palate with or without cleft lip, 21 (75 %) had positive otological findings although they had already attended different stages of treatment. The cranio-maxillo-facial surgeon is the principal specialist in treating these anomalies. It is his responsibility to ask for otolaryngologic consulting and audiogram.

***Key words**: hearing, audiogram, cleft palate.*

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INTRODUCTION

The cleft lip and palate cannot be viewed in isolation and attention has to be given to associated pathology. Although these patients are treated mainly by the cranio-maxillo-facial surgeon, treatment is interdisciplinary and implies monitoring of the somatic development, finding the hearing loss, learning the pronunciation, orthodontic treatment, etc.

When there is a cleft in the palate, hearing problems can arise due to the muscles in the soft palate not functionning correctly. These muscles move when we yawn, eat or swallow and they pull open the Eustachian tube to allow passage of air into the middle ear. If this does not happen then fluid accumulates in the middle ear, the Eustachian tube's function is impaired and hearing problems can occur.

Studies confirm that between 95 and 100% of infants born with cleft palate have fluid present in the middle ear space at birth. This condition, known as middle ear effusion, continues to exist for several years if left untreated but unfortunately can continue also in operated patients usually from birth until eight or nine years of age. Rarely the problem may persist through adulthood.

AIM AND OBJECTIVES

The present study was undertaken to explore the correlations between surgical treatment in patients with cleft lip and palate and hearing in order to provide a more comprehensive picture of associated pathology in such cases. It investigates the results and interpretation of hearing screening in treated or untreated patients with cleft palates.

MATERIAL AND METHODS

The present study was conducted on 28 subjects, comprising of 19 males and 9 females, age between 4 and 12 years, with cleft palate with or without cleft lip as follows: 4 patients not yet treated; 24 patients in different stages of treatment, operated in the Hospital of Cranio – Maxillo - Facial Surgery Timisoara between 1998 and 2010.

Hearing screening outcomes were collected. We recorded: age, sex and weight, the presence of hearing loss and the presence of associated syndromes. These patients were subjected to an exhaustive clinical and audiological examination with particular attention to the incidence of ear pathology and deafness, discharge or any other finding relevant to the middle ear.

Otorhinolgic consulting was performed by means of rhinoscopy and otoscopy and it showed: chronic otitis media, adenoid vegetation, abundant secretion, rhinitis, nasal obstructive syndrome. The hearing assessment was done by pure-tone audiometry. We also asked parents at what age they felt hearing-related problems had ceased.

RESULTS

Among the 28 patients with cleft palate with or without cleft lip, 21 (75 %) had positive otological findings (Table I). Significant rhinoscopic fin-

dings were seen in 16 patients (Table-II) in the form of deviated nasal septum 11 (39, 3 %) cases, inflamed mucosa in 5 cases (17, 8 %).

Table I: Otological findings:

TYMPANIC MEMBRANE FINDINGS	NO. OF CASES	PERCENTAGE
Normal Ears	7	25,0
Retraction	12	42,8
Diffuse Cone Of Light	6	21,5
Bulging	3	10,7

Table II: Rhinoscopic findings:

RHINOLOGICAL FINDINGS	NO. OF CASES	PERCENTAGE
Normal	12	42,8
Deviated septum	11	39,2
Rhinitis	5	18,0

Table III: Preoperative Audiometric findings inpatient with diminished hearing (mean value) n=28

FREQUENCY (HZ)	LEFT EAR (DB)		RIGHT EAR (DB)	
	Air Conduction	Bone Conduction	Air Conduction	Bone Conduction
125	35		30	
250	30		35	
500	30	15	30	15
1000	20	20	20	20
2000	15	15	20	15
4000	20	10	15	10

12 patients (42, 8 %) revealed hearing loss in both ears. Bone conduction tests on these patients with hearing loss established that this loss was purely conductive in nature. Bilateral conductive hearing loss of mean intensity 30 dB for the low frequency range (125 Hz to 1000 Hz) was seen in patients of cleft palate with or without cleft lip with no evidence of sensorineural deafness. (Table III).

Audiometric evaluation done postoperatively in these patients with hearing loss on pure tone audiogram showed (Table IV and Figure 1):

Table IV: Post-operative audiometric findings and comparison with pre-operative air conduction in patients with diminished hearing (mean value) n=28

FREQUENCY (HZ)	LEFT EAR (DB)		RIGHT EAR (DB)	
	Preoperative	Postoperative	Preoperative	Postoperative
125	35	30	30	25
250	30	20	35	25
500	30	20	30	30
1000	20	20	20	20
2000	15	15	20	20
4000	20	20	15	15

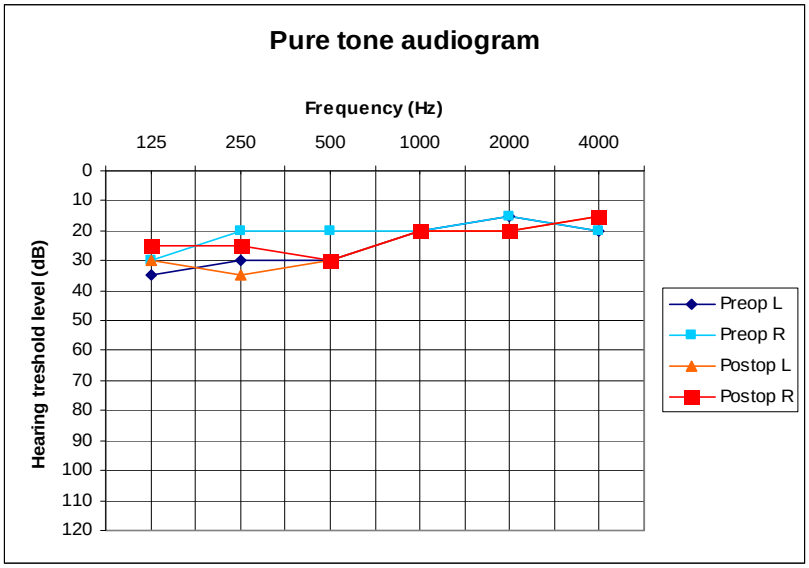

Fig.1. Audiogram means values before and after surgery. About the age they felt hearing-related problems had ceased, asked parents answered as follows:

20,3% felt their child's ear problems ceased at age 2-4 years;
39,5% felt their child's ear problems ceased at age 4-6 years;
26,9% felt their child's ear problems ceased at age 6-8 years;
13, 3% felt their child's ear problems ceased at age 8-10 years.

DISCUSSIONS

It has been revealed a high incidence of pathological changes in the ear despite the absence of subjective symptoms of otitis media. Pure tone audiogram is not a difficult test that can be performed as a screening method in children with cleft palate, with or without cleft lip in order to detect hearing loss. After the fluid is drained surgically and the minute tubes are inserted in the drums to aerate the middle ear space, parents frequently report an increase in baby's responsiveness and language acquisition.

Learning language is one of the most difficult tasks for an infant; an infant with even a mild hearing loss (15-25 dB) is probably at some disadvantage in trying to decipher our complex verbal system.

CONCLUSION

While such clinical attention is being focused on the cosmetic and speech habilitation of the child born with cleft palate, little attention is sometimes paid to the implications of the otologic histories of these patients. One cannot separate the problems caused by cosmetic, speech, and hearing concerns, but the latter should not be overlooked in developing analyses of behavior and psychological development of the patient with cleft palate. The cranio-maxillo-facial surgeon is the principal specialist in treating these anomalies. It is his responsibility to ask for otolaryngologic consult.

Parents are the guides for much of this development; parents and patients need to understand how the various aspects of the cleft affect the individual's perception of him. How much of that perception is formed in the mother's and father's arms, how much in the pre-school years, how much in the elementary grades, and how much during adolescence and young adulthood are individual. We as parents can only provide the strongest support and love as possible during each stage of development.

Obviously then the importance of an otologist in the team managing the cleft child cannot be ignored. The parent of an infant born with a cleft palate should have the infant's ears checked regularly by an otolaryngologist.

Newborns with cleft palate are at higher risk of failing their newborn hearing screen compared with healthy neonates.

Detection of sensorineural or conductive hearing loss unrelated to middle ear effusions is more difficult in this at-risk population with cleft palate because of the high prevalence of serous otitis media.

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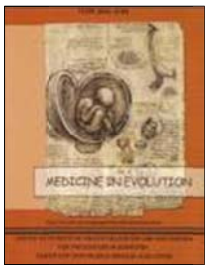
THE STUDY OF ANTIBIOTIC RESISTANCE OF SOME BACTERIAL STRAINS AND THE ANTIBIOTICS NEED



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ABSTRACT

***Aim**: The main goals of our study are: evaluation of the bacterial strains' resistance to anti infective chemotherapy in 2009 and analyzing the correlation between this resistance and the antibiotics need in the Intensive Care room of Emergency Clinical Hospital from Timisoara County.*

***Material and method**: The present study included 1408 patients. The classes of antibiotics considered were: cephalosporins, fluoroquinolones, aminoglycosides and carbapenems, drugs also prescribed in bacterial infection therapy and prophylaxis. For each patient there has been made up a personal file which included: age, diagnosis, the pathological probe gathered, the identified germ, the antibiogram, the antibiotic that was used (dosage form, dose, administration frequence / day, therapy duration). The antibiotic use was appreciated using the DDD/1000 patients/day.*

***Results**: The carbapenems, IVth generation cephalosporins, fluoroquinolones and imidoazole derivatives were more used than other classes. For three important bacterial species: Klebsiella pneumoniae, Escherichia coli and Staphylococcus aureus, can be noted a direct, strong and statistically significant correlation between the overall antibiotic use and the evolving trend of the identified strains' resistance. The strongest correlation could be seen for the S. aureus strains, where the resistant germs percentage's evolution was in accord with the antibiotic consumption.*

***Conclusions**: The analyze of global consuming, reported on monitored antibiotics classes, reveals the direct, positive correlation between the overall antibiotic preparations' use and the evolving trend of the identified strains' resistance. The degree the correlation varied from low to very strong, based on the bacterial species.*

***Key words**: antibio-therapy, bacterial species, resistance, correlation.*

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INTRODUCTION

One of the major problems of contemporaneous medicine is the huge prevalence of pathogen bacterial germs resistant to antibiotics. The nosocomial infections caused by resistant germs represent an important source of morbidity, mortality and also an economical load for medical care units, with a major impact for entire society. Most of the efforts in the field of antibiotherapy are directed to dose modifying, tolerance and also to increase the antibiotics classes existing on market at this time [1, 2].

AIM AND OBJECTIVES

The main goals of our study are: evaluation of the bacterial strains'resistance to anti infective chemotherapy in 2009 and analyzing the correlation between this resistance and the antibiotics need in the Intensive Care Unit of Emergency Clinical Hospital from Timisoara County (SCJUT).

MATERIAL AND METHODS:

The present study included 1408 hospitalized patients in the Intensive Care Unit of Emergency Clinical Hospital from Timis County during 2009.

Tabel 1 A classification of the registered diagnostics in the Intensive Care Unit during 2009

DIAGNOSTIC AT HOSPITALIZATION	NUMBER/PERCENT - PATIENTS 2009
Polytrauma	465 (33,05%)
Stroke	410 (29,14%)
Cerebral aneurysms	110 (7,83%)
Cancers	87 (6,21%)
Acute cardiovascular events	80 (5,67%)
Chronic obstructive pulmonary disease	44 (3,15%)
Bronchial pneumonia	42 (2,96%)
Acute intoxications	41 (2,94%)
Diabetes mellitus - complications	26 (1,87%)
Meningioma	26 (1,87%)
Miastenia gravis	14 (1,03%)
Hydrocephaly	16 (1,14%)
Hepatic cirrhosis	20 (1,42%)
Polyradiculoneuritis	7 (0,52%)
Arteriovenous malformations	7 (0,52%)
Chronic renal failure	7 (0,52%)
Osteitis	7 (0,52%)
Burns	7 (0,52%)
Total	1408 (100%)

The classes of antibiotics considered were: cephalosporins, fluoroquinolones, aminoglycosides and carbapenems, drugs also prescribed in bacterial infection therapy and prophylaxis. For each patient there has been made up a

personal file which included: age, diagnosis, the pathological probe gathered, the identified germ, the antibiogram, the antibiotic that was used (dosage form, dose, frequency of administration/day, therapy duration). The antibiotic use was appreciated using the DDD/1000 patients/day [3, 4, 5].

Tabel 2 Pathological products taken from patients hospitalized in the Intensive Care Unit of SCJUT, during 2009

PATHOLOGICAL PRODUCTS	POSITIVE SAMPLES		STERILE SAMPLES		TOTAL	
Bronchial aspirate	592	92,81%	31	7,19%	623	100%
Blood	191	37,93%	162	62,07%	353	100%
Ulcer secretion	154	92,48%	11	7,52%	165	100%
Urine	135	31,14%	192	68,86%	327	100%
Central catheter	39	35,74%	62	64,26%	101	100%
Sputum	23	100%	0	0	23	100%
Intraperitoneal fluid	17	100%	0	0	17	100%
Drainage fluid	11	100%	0	0	11	100%
Cerebrospinal fluid	7	11,56%	65	88,44%	72	100%
Pleural fluid	7	8,64%	72	91,36%	79	100%
Intra-articular fluid	6	100%	0	0	6	100%
Hypopharingeal aspirate	6	100%	0	0	6	100%
Pharyngeal exudate	5	22,41%	11	77,59%	16	100%
Pericardial fluid	0	0	14	100%	14	100%
Total	**1193**	**61,18%**	**620**	**38,82%**	**1813**	**100%**

The following table is listing a classification of the diagnostics that were registered at hospitalization in the Intensive Care Unit during 2009. A correlation between these diagnostics and the number of patients presenting them was also made.

Pathological samples were taken from all hospitalized patients during the studied period.

Microorganism-cultures were carried out using the samples in the Microbiology Laboratory of SCJUT and the results were confirmed at the Microbiology Department from "Victor Babes" University of Medicine and Pharmacy in Timisoara. At this point, the strains were isolated on growth mediums and antibiograms were determined by the Kirby-Bauer disk diffusion antibiotic sensitivity testing on extensive antibiograms with manual and electronic reading (using Osiris Evolution - Bio Rad analyser).

The diversity of the samples was big enough, taking into account that they were taken in the hospital. In the next tables, we enumerate the pathological samples collected from the patients hospitalized in the Intensive Care Unit during 2009 and also there are shown the numbers and percents for the sterile probes and for the positive ones.

For every patient was set up a personal record containing information about age, diagnosis, sampled pathological product, the identified germ, antibiogram, administered antibiotic (pharmaceutical forms, dose, number of administrations/day, duration of therapy). Antibiotics specific consumption was calculated according DDD / 1000 patients/day. Defined Daily Dose (DDD) is a universal recognized measuring unit, representing daily antibiotics dose administered to a 70 kg adult person. For the antibiotics we are refe-

rring to, in conformity with international regulations, the DDD utilized for our calculus is represented in table nr. III. The statistic analysis was performed using the dedicated software EPI-INFO, version 6.04. All the statistic tests were calculated with two extremities, and the value of p considered statistically significant was ≤ 0.05. For percentages comparing was utilized χ^2 test.

Tabel 3 Defined Daily Dose for the antibiotics utilized in the trial (World Health Organization Collaborating Centre for Drug Statistics Methodology, Oslo, Norway - www.whocc.no/atcddd/)

ANTIBIOTIC CLASS	ANTIBIOTIC	DDD	UNIT OF MEASURE	ROUTE OF ADMINISTRATION
CEPHALOSPORINS	Ceftriaxone	2	g	parenteral
	Cefuroxim	3	g	parenteral
	Cefotaxim	4	g	parenteral
	Cefamandol	6	g	parenteral
	Cefpirom	4	g	parenteral
	Cefepime	2	g	parenteral
	Cefoperazone	4	g	parenteral
FLUOROQUINOLONES	Ciprofloxacin	0,5 1	g g	parenteral oral
	Norfloxacin	0,8	g	oral
	Ofloxacin	0,4	g	parenteral oral
	Levofloxacin	0,5	g	parenteral oral
AMINOGLYCOSIDES	Gentamicin	0,24	g	parenteral
	Amikacin	1	g	parenteral
CARBAPENEME	Imipenem+cilastatin	2	g	parenteral
	Meronem	2	g	parenteral
	Ertapenem	1	g	parenteral
GLYCOPEPTIDES	Vancomycin	2	g	parenteral
PENICILLINS	Ampicillin	2	g	parenteral oral
	Oxacillin	2	g	parenteral oral
PENICILLINS + B-LACTAMASE INHIBTORS	Amoxicillin+clavulanic acid	3	g	parenteral
	Ticarcilina+clavulanic acid	15	g	parenteral
	Piperacilina+tazobactam	14	g	parenteral
	Cefoperazona+sulbactam	4	g	parenteral

RESULTS

Afterwards the analysis of data found in the registry of the Microbiology Department of SCJUT, respectively from the hospitalized patients' files in the Intensive Care Unit of the hospital, there could be identified 418 bacterial strains in 2009, with the following distribution:

Tabel 4 Bacterial species isolated during the study

SPECIES	YEAR 2009	
	Nr. strains	**%**
Klebsiella pneumoniae	130	31,1
Escherichia coli	56	13,4
Proteus mirabilis	28	6,7
Enterobacter cloacae	8	1,91
Pseudomonas aeruginosa	52	12,44
Acinetobacter baumannii	52	12,44
Serratia marcescens	/	/
Providencia stuartii	/	/
Citrobacter freundii	/	/
Enterobacter aerogenes	/	/
Serratia odorifera	/	/
Proteus penneri	/	/
Morganella morganii	/	/
Stenotrophomonas maltophilia	/	/
Flavimonas oryzihabitans	/	/
Staphylococcus aureus	92	22,01
Coagulaso-negative Staphylococcus sp.	/	/
Group D Streptococcus sp.	/	/
Total	418	100

The integration of the circulant strains in resistance phenotypes emphasizes the following aspects:

In the case of Klebsiella pneumoniae strains, the secretion of extended spectrum β-lactamases prevailed, beside the Kanamycin-Gentamicin-Tobramycin-Netilmicin (KTGN). Concerning the fluoroquinolones, most of the strains in 2009 had presented cross-resistance.

Most of the E. coli strains had secreted high-level penicillinase, the entire period of two years, while their res-

ponse to aminoglycosides and fluoroquinolones had varied. If in 2009 the majority had cross-resistance to fluoroquinolones and in equal percentages to KTGN or sensitivity to aminoglycosides.

During the entired studied period, most of the Staphylococcus aureus strains were meticillin-resistant, KTG resistant and sensitive to fluoroquinolones. Concerning the macrolides, the lincosamydes and streptogramins, in 2009, there could be identified MSLB-inductible and MSLB-constitutible staphylococci.

β-lactam resistance had decreased in al bacterial species found during the studied period.

DISCUSSION

The most important measures suggested by European Union countries researchers for lowering the bacterial multiple drug resistance consist in establishing a monitoring system of the bacterial resistance and assuring a feedback over the antibioresistance setting up and impact; paying attention to the rules of hygiene, for limiting the clonal spread of a strain and assuring an antibiotherapy control, for limiting the policlonal spread of the resistant strains; creating the antibiotherapy committees in the hospitals, for working with the national professional societies [6].

CONCLUSIONS

The carbapenems, IVth generateon cephalosporins, fluoroquinolones and imidoazole derivatives were more used than other classes.

For three important bacterial species: *Klebsiella pneumoniae, Escherichia coli* and *Staphylococcus aureus,* can be noted a direct, strong and statistically significant correlation between the overall antibiotic use and the evolving trend of the identified strains' resistance.

The strongest correlation could be seen for the *S. aureus* strains, where the resistant germs percentage's evolution was in accord with the antibiotic consumption, even for the fluoroquinolone use.

The obtained results demonstrate the direct, positive correlation between the overall antibiotic preparations' use. The degree the correlation varied from low to very strong, based on the bacterial species.

The study demonstrates the necessity of a rigorous policy for the antibacterial chemotherapy prescribing in the hospitals, together with a microbiological survey of the existing resistant phenotypes, for decreasing the multiple drugs – resistant germs infective pathology risks.

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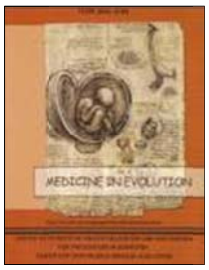
CONNECTING POSSIBILITIES BETWEEN MESO - AND SUPERSTRUCTURE OF IMPLANT SUPPORTED PROSTHESIS IN THE TOTALLY EDENTULOUS PATIENTS



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ABSTRACT

Prosthetic rehabilitation of totally edentulous patients, especially on the mandibular jaw, using implant overdentures, has entered a new period after the McGill University Consensus in 2002, adopted in Canada. According to that, the standard of this type of prosthetic restoration begins with inserting at least two interforaminal implants followed by the achievement of the overdenture. In time, this treatment concept became viable and the treatment solutions have diversified a lot. New concepts have emerged (Spiekermann, Bret, and Misch). At the same time, the concepts of infra-, mezo- and superstructure and their connections have appeared.

In the present work, our team presents its three years time experience regarding the variety of connection systems between meso- and superstructure. Thus, 60 mandibular and 22 maxilar overdentures have been manufactured, using several types of connections (bars with metal/plastic riders, galvanoformed riders, double mechanical retention riders).

The patients have been kept under observation and recalled every 6 months (up to 2 years) and observations have been made regarding the integrity of the connections. Generally, the patients were satisfied with this type of prosthetic restorations and their quality of life had risen. Among connections, the retention of metallic riders began to diminish after 2 years, at the plastic riders we noticed a slight improvement of retention and the galvanoformed riders group being the most satified with the worn restaurations (the retention through friction, specific for these systems, is replaced with hydraulic adhesion, which is a benefit for the implants' life as well).

***Key words**: galvanoformed rider, hydraulic retention, zirconium milled bar.*

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INTRODUCTION

Total edentation is characterized by the absence of all dental-periodontal units on one or both maxillaries, with consecutive disturbance of the essential functions of the stomatognate system. In most cases, due to the alteration of phonetic function and physiognomy, the total edentulous patient is socially disinserted, quickly becoming"socially handicaped" [6, 7, 20].

A series of studies show that the prevalence of total edentation decreesed in some developed countries by around 10% in each decade during the last 30 years (USA) due to the increase of over 65 year olds [24].

The etiology of total edentation includes, beside carious processes and periodontal disease, other factors such as: age, gender, education, income, individual general health, psychosocial factors, place of residence (urban or rural), the economic status of the citizens of each country, availability of and addressability to preventive and social services, etc.[18].

Thus, patients with low incomes and those comming from rural areas are more prone to becoming edentulous [31]. These socioeconomic determinants may only partially explain inequalities between countries or regions. Cultural and psychosocial factors also play an important role. Traditionally, women are considered to be more predisposed to dental loss then men [9]. Total edentation is more frequently encountered among diseased and debilitated elderly patients [30].

Clinical experience confirms a considerable variability among individuals regarding the adaptation to traditional total dentures, despite the fact that total dentures proved to be a deficitary functional masticatory replacement of complete natural dentition [20, 33].

Most elderly patients are not content with their dentures. The main reasons for this are: discomfort, instability and decreased retention, frequent decubitus lesions, occuring with increased frequency in the mandible [5, 26]. Their ability to chew solid and hard food is decreased, with negative consequences on the nutritional status and general health.

The multiple unsolved problems raised by the traditional restoration of total edentation, especially in the mandible, were the subject of an international congress (Canada, 2002). Thus, on this occasion, the McGill University Consensus was adopted concluding that restoration of total mandibular edentation by a conventional denture is no longer considered as the most appropriate treatment method [12]. The participants formulated a consensus declaration according to which the first choice assistance standard for edentulous mandibles is the Overdenture supported by 2 implants [2, 3, 32].

The 2 implant supported overdenture proved to be better than the traditional total denture in clinical randomized and nonrandomized studies performed over periods ranging from 6 months to 9 years, the patients being significantly more content, regardless of the type of connection used [17]. The patients found the denture to be more stable, and with a significantly improved mastication capacity for various foods. This has consequences upon the nutritional status as well, and a strong positive impact on the general health status, especially in senior patients who are vulnerable to malnutrition. They feel more comfortable and may speak more easily. The life quality of these patients is increased as compared to those having conventional dentures [17].

In restoring total edentations with implant supported Overdentures three interconnected levels may be detected: the implants (the infrastructure), the connecting bar (the mesostructure) and the Overdenture representing the superstructure [23, 8].

Between the meso and the superstructure a certain type of conjunction is mandatory. In modern prosthetic restoration of total edentulous patients, other infrastructure designs are also used apart from the one with 2 interforaminal implants [18]. We often insert 4 bar connected implants in total mandibular edentulous patients. Mesostructures may be differently connected to superstructures. The purpose of the present paper was to present various connecting systems between meso and superstructure, as well as to assess their effectiveness.

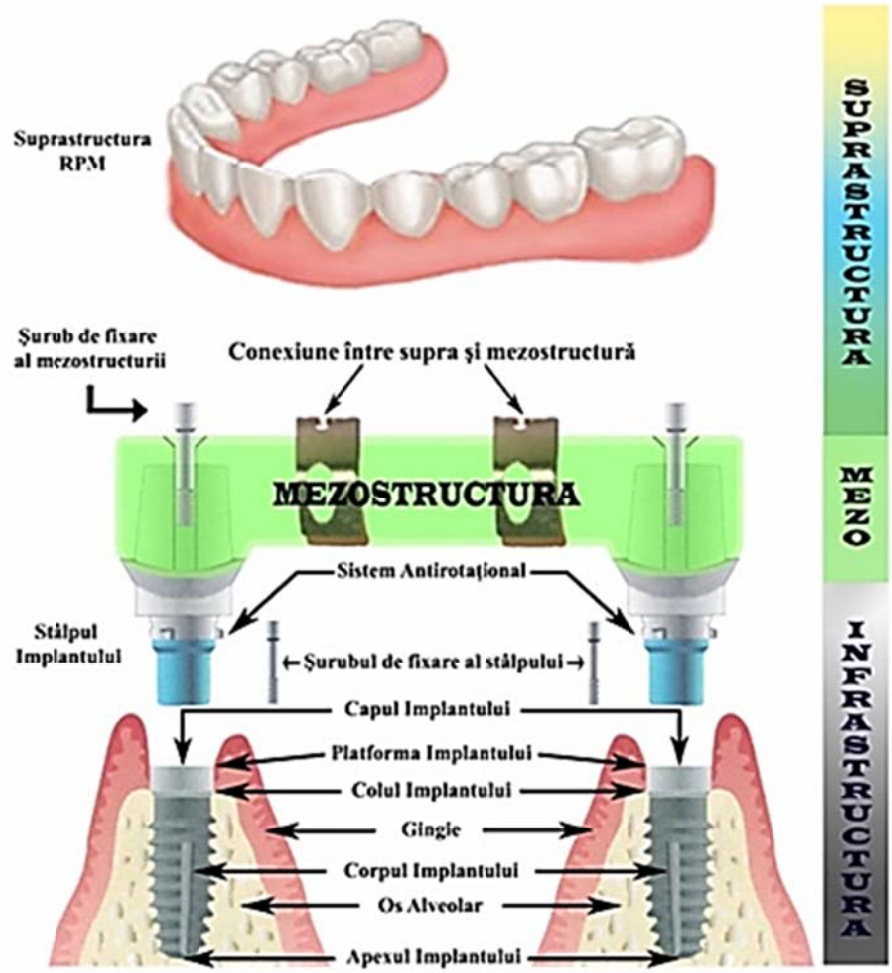

Fig.1. Infra, meso and superstructure of implant supported prosthesis in total edentation.

FRICTION RETAINED RIDERS	**METALLIC** **NON-METALLIC**
HYDRAULIC RETENTION RIDERS	**CONTINUOUS** **DISCONTINUOUS**
DOUBLE MECHANICAL RETENTION RIDERS	**RIDERS + BALL RETENTION** **RIDERS + MAGNET RETENTION** **RIDERS + PRESSURE BUTTON**

MATERIAL AND METHODS

During the period between 2006-2009, 86 patients (30 ♀ and 56 ♂) aged between 56-75 years were restored by implant-supported overdentures in the Clinic for Implant-Supported Prosthetic Restorations and in "Prof. Dr. Bratu" Clinic. Most patients had total mandibular edentations (66 patients) and only 20 had total maxillary edentations.

All patients received overdentures with a total prosthesis design (including a tertiary Co-Cr framework) in the mandible and a metallic folded basis in the maxilla. The infrastructures in our patients were represented by Seven (MIS) and XIVE (Dentsply, Friadent) dental implants.

The mesostructures were represented by 72 friction-stabilized riders, 82 hydraulic retention riders (60 with continuous and 22 with discontinuous retention), and 18 with double mechanical retention (riders + ball retention).

Galvanoformed riders (35) were achieved using the AGC micro (Wieland) and Preciano CL-GF (Heraeus-Kulzer) equipments, and the 18 mecanical double-retention ones were friction retention riders combined with ball retention devices (RHEIN, MIS).

Patients were monitored and examined at certain time intervals, during a 2 year period, with particular focus on riders' retention. Together with clinical observation on the behaviour of overdentures, performed in the dental practice, the degree of patient satisfaction with this type of prosthetic restorations was also verified.

The degree of patient satisfaction was tested by questionnaires which they filled in during periodic control visits at the Clinic for Dental Prosthesis: upon insertion of restoration; at 6 months; after 1 year; after 2 years.

Questionnaires included the following types of questions:

A) Is the denture stable during masti-cation?
B) Do you experience pain/pressure from the denture on the oral mucosa during eating?
C) Are you able to eat hard foods? What about sticky ones?
D) Is the denture stable while you speak?
E) Is the denture difficult to disinsert for cleaning purposes (1=min, 5=max)?
F) What would be the score measuring its oral reinsertion (1=min, 5=max)?

Answers were gathered and entered in a table:

Nr	Nam First Name	Gender	Age	DIAGNOSTIC	Type of restauration	Satisfaction DEGREE					
						A	B	C	D	E	F
1.	M.N.	♂	56 years	Total ed. m	Overdenture with friction retained riders	Yes	No	Yes	Yes	5	5
2.	M.S.	♂	71 years	Total ed. m	Overdenture with friction retained riders	Yes	No	Yes	Yes	5	5
3.	A.B.	♀	60 years	Total ed. m	Overdenture with galvanoformed riders	Yes	No	Yes	Yes	5	5
4.	E.R.	♂	69 years	Total ed. m	Overdenture with friction retained riders	Yes	No	Yes	Yes	5	5
5.	S.W.	♀	65 years	Total ed. m	Overdenture with double mechanical retention	Yes	No	Yes	Yes	5	5
6.	J.H.	♂	71 years	Total ed. m	Overdenture with galvanoformed riders	Yes	No	Yes	Yes	5	5
7.	L.R.	♂	61 years	Total ed. m	Overdenture with galvanoformed riders	Yes	No	Yes	Yes	5	5
8.	V.B.	♀	56 years	Total ed. m	Overdenture with friction retained riders	Yes	No	Yes	Yes	4	4
9.	T.H.	♂	63 years	Total ed. m	Overdenture with friction retained riders	Yes	No	Yes	Yes	5	5
10.	L.G.	♀	64 years	Total ed. m	Overdenture with double mechanical retention	Yes	No	Yes	Yes	5	5
11.	D.N.	♀	67 years	Total ed. m	Overdenture with galvanoformed riders	Yes	No	Yes	Yes	5	5

12.	S.P.	♂	72 years	Total ed. m	Overdenture with friction retained riders	Yes	No	Yes	Yes	5	5
13.	G.H.	♂	61 years	Total ed. m	Overdenture with galvanoformed riders	Yes	No	Yes	Yes	5	5
14.	L.I.	♀	56 years	Total ed. m	Overdenture with friction retained riders	Yes	No	Yes	Yes	5	5
15.	F.T.	♀	65 years	Total ed. m	Overdenture with double mechanical retention	Yes	No	Yes	Yes	4	5
16.	A.C.	♂	68 years	Total ed. m	Overdenture with galvanoformed riders	Yes	No	Yes	Yes	5	5
17.	S.A.	♂	71 years	Total ed. m	Overdenture with friction retained riders	Yes	No	Yes	Yes	5	4
18.	D.B.	♂	60 years	Total ed. m	Overdenture with galvanoformed riders	Yes	No	Yes	Yes	5	5
19.	D.J.	♂	73 years	Total ed. m	Overdenture with double mechanical retention	Yes	No	Yes	Yes	5	5
20.	L.G.	♂	64 years	Total ed. m	Overdenture with friction retained riders	Yes	No	Yes	Yes	4	5
21.	A.J.	♂	75 years	Total ed. m	Overdenture with galvanoformed riders	Yes	No	Yes	Yes	5	5
22.	M.N.	♂	61 years	Total ed. m	Overdenture with friction retained riders	Yes	No	Yes	Yes	5	5
23.	P.P	♂	74 years	Total ed. m	Overdenture with friction retained riders	Yes	No	Yes	Yes	5	5
24.	M.T	♂	57 years	Total ed. m	Overdenture with double mechanical retention	Yes	No	Yes	Yes	5	5
25.	D.M.	♂	67 years	Total ed. m	Overdenture with friction retained riders	Yes	No	Yes	Yes	5	5
26.	P.C.	♂	62 years	Total ed. m	Overdenture with galvanoformed riders	Yes	No	Yes	Yes	5	5
27.	N.C.	♂	68 years	Total ed. m	Overdenture with friction retained riders	Yes	No	Yes	Yes	5	5
28.	R.J.	♀	70 years	Total ed. m	Overdenture with galvanoformed riders	Yes	No	Yes	Yes	4	4
29.	A.S.	♂	63 years	Total ed. m	Overdenture with double mechanical retention	Yes	No	Yes	Yes	4	5
30.	A.M.	♀	71 years	Total ed. m	Overdenture with friction retained riders	Yes	No	Yes	Yes	5	5
31.	C.K.	♂	57 years	Total ed. m	Overdenture with galvanoformed riders	Yes	No	Yes	Yes	5	5
32.	C.D.	♀	62 years	Total ed. m	Overdenture with friction retained riders	Yes	No	Yes	Yes	5	5
33.	A.E.	♂	59 years	Total ed. m	Overdenture with double mechanical retention	Yes	No	Yes	Yes	5	5
34.	A.B.	♀	72 years	Total ed. m	Overdenture with friction retained riders	Yes	No	Yes	Yes	5	5
35.	F.G.	♂	63 years	Total ed. m	Overdenture with galvanoformed riders	Yes	No	Yes	Yes	5	5
36.	L.U.	♂	56 years	Total ed. m	Overdenture with galvanoformed riders	Yes	No	Yes	Yes	5	4
37.	H.M.	♀	74 years	Total ed. m	Overdenture with friction retained riders	Yes	No	Yes	Yes	3	4
38.	P.I.	♂	65 years	Total ed. m	Overdenture with double mechanical retention	Yes	No	Yes	Yes	5	5
39.	G.E.	♂	57 years	Total ed. m	Overdenture with friction retained riders	Yes	No	Yes	Yes	5	5
40.	L.N.	♂	69 years	Total ed. m	Overdenture with galvanoformed riders	Yes	No	Yes	Yes	5	5
41.	C.I.	♂	70 years	Total ed. m	Overdenture with friction retained riders	Yes	No	Yes	Yes	5	5
42.	S.T.	♀	60 years	Total ed. m	Overdenture with galvanoformed riders	Yes	No	Yes	Yes	5	5
43.	C.P.	♂	72 years	Total ed. m	Overdenture with friction retained riders	Yes	No	Yes	Yes	4	5
44.	M.C.	♀	56 years	Total ed. m	Overdenture with galvanoformed riders	Yes	No	Yes	Yes	5	5
45.	F.G.	♂	64 years	Total ed. m	Overdenture with friction retained riders	Yes	No	Yes	Yes	5	5
46.	D.P.	♀	75 years	Total ed. m	Overdenture with double mechanical retention	Yes	No	Yes	Yes	5	4
47.	M.F.	♀	58 years	Total ed. m	Overdenture with galvanoformed riders	Yes	No	Yes	Yes	5	5
48.	A.V.	♂	62 years	Total ed. m	Overdenture with friction retained riders	Yes	No	Yes	Yes	5	5
49.	R.M.	♀	70 years	Total ed. m	Overdenture with friction retained riders	Yes	No	Yes	Yes	5	5

50.	R.G.	♀	58 years	Total ed. m	Overdenture with galvanoformed riders	Yes	No	Yes	Yes	5	5
51.	O.J.	♂	61 years	Total ed. m	Overdenture with friction retained riders	Yes	No	Yes	Yes	3	5
52.	G.P.	♀	65 years	Total ed. m	Overdenture with double mechanical retention	Yes	No	Yes	Yes	5	5
53.	D.H.	♀	74 years	Total ed. m	Overdenture with galvanoformed riders	Yes	No	Yes	Yes	5	5
54.	N.N.	♂	66 years	Total ed. m	Overdenture with friction retained riders	Yes	No	Yes	Yes	5	5
55.	A.B.	♂	58 years	Total ed. m	Overdenture with double mechanical retention	Yes	No	Yes	Yes	5	5
56.	P.B.	♂	64 years	Total ed. m	Overdenture with friction retained riders	Yes	No	Yes	Yes	5	5
57.	R.E.	♂	69 years	Total ed. m	Overdenture with galvanoformed riders	Yes	No	Yes	Yes	5	5
58.	V.C.	♂	74 years	Total ed. m	Overdenture with friction retained riders	Yes	No	Yes	Yes	5	5
59.	E.B.	♀	62 years	Total ed. m	Overdenture with double mechanical retention	Yes	No	Yes	Yes	4	5
60.	R.R.	♂	72 years	Total ed. m	Overdenture with galvanoformed riders	Yes	No	Yes	Yes	5	5
61.	M.C.	♂	67 years	Total ed. m	Overdenture with friction retained riders	Yes	No	Yes	Yes	5	5
62.	J.W.	♂	58 years	Total ed. m	Overdenture with galvanoformed riders	Yes	No	Yes	Yes	5	5
63.	E.D.	♂	66 years	Total ed. m	Overdenture with friction retained riders	Yes	No	Yes	Yes	4	5
64.	U.H.	♀	70 years	Total ed. m	Overdenture with galvanoformed riders	Yes	No	Yes	Yes	5	4
65.	K.M.	♀	64 years	Total ed. m	Overdenture with friction retained riders	Yes	No	Yes	Yes	5	5
66.	S.U.	♂	75 years	Total ed. m	Overdenture with galvanoformed riders	Yes	No	Yes	Yes	5	5
67.	P.I.	♀	68 years	Total ed. M	Overdenture with double mechanical retention	Yes	No	Yes	Yes	5	5
68.	H.L.	♂	59 years	Total ed. M	Overdenture with friction retained riders	Yes	No	Yes	Yes	4	3
69.	P.O.	♀	74 years	Total ed. M	Overdenture with galvanoformed riders	Yes	No	Yes	Yes	5	5
70.	D.L.	♂	62 years	Total ed. M	Overdenture with double mechanical retention	Yes	No	Yes	Yes	5	5
71.	F.H.	♂	67 years	Total ed. M	Overdenture with galvanoformed riders	Yes	No	Yes	Yes	5	5
72.	I.R.	♀	59 years	Total ed. M	Overdenture with friction retained riders	Yes	No	Yes	Yes	3	3
73.	O.F.	♂	65 years	Total ed. M	Overdenture with double mechanical retention	Yes	No	Yes	Yes	5	5
74.	R.S.	♂	73 years	Total ed. M	Overdenture with friction retained riders	Yes	No	Yes	Yes	5	5
75.	P.A.	♂	61 years	Total ed. M	Overdenture with galvanoformed riders	Yes	No	Yes	Yes	5	5
76.	E.K.	♂	75 years	Total ed. M	Overdenture with friction retained riders	Yes	No	Yes	Yes	4	5
77.	M.L.	♀	66 years	Total ed. M	Overdenture with friction retained riders	Yes	No	Yes	Yes	5	5
78.	R.F.	♀	59 years	Total ed. M	Overdenture with galvanoformed riders	Yes	No	Yes	Yes	5	5
79.	P.E.	♂	63 years	Total ed. M	Overdenture with double mechanical retention	Yes	No	Yes	Yes	5	4
80.	G.A.	♀	75 years	Total ed. M	Overdenture with double mechanical retention	Yes	No	Yes	Yes	5	5
81.	B.P.	♂	65 years	Total ed. M	Overdenture with friction retained riders	Yes	No	Yes	Yes	5	5
82.	H.J.	♂	73 years	Total ed. M	Overdenture with galvanoformed riders	Yes	No	Yes	Yes	4	5
83.	C.C	♀	60 years	Total ed. M	Overdenture with friction retained riders	Yes	No	Yes	Yes	5	5
84.	V.O.	♂	67 years	Total ed. M	Overdenture with double mechanical retention	Yes	No	Yes	Yes	5	5
85.	P.R.	♂	74 years	Total ed. M	Overdenture with galvanoformed riders	Yes	No	Yes	Yes	5	5
86.	S.T.	♂	63 years	Total ed. M	Overdenture with friction retained riders	Yes	No	Yes	Yes	4	4

RESULTS

In Fig. 2 and 3 we illustrate the variety of connections between meso- and superstructures we performed in the 86 patients.

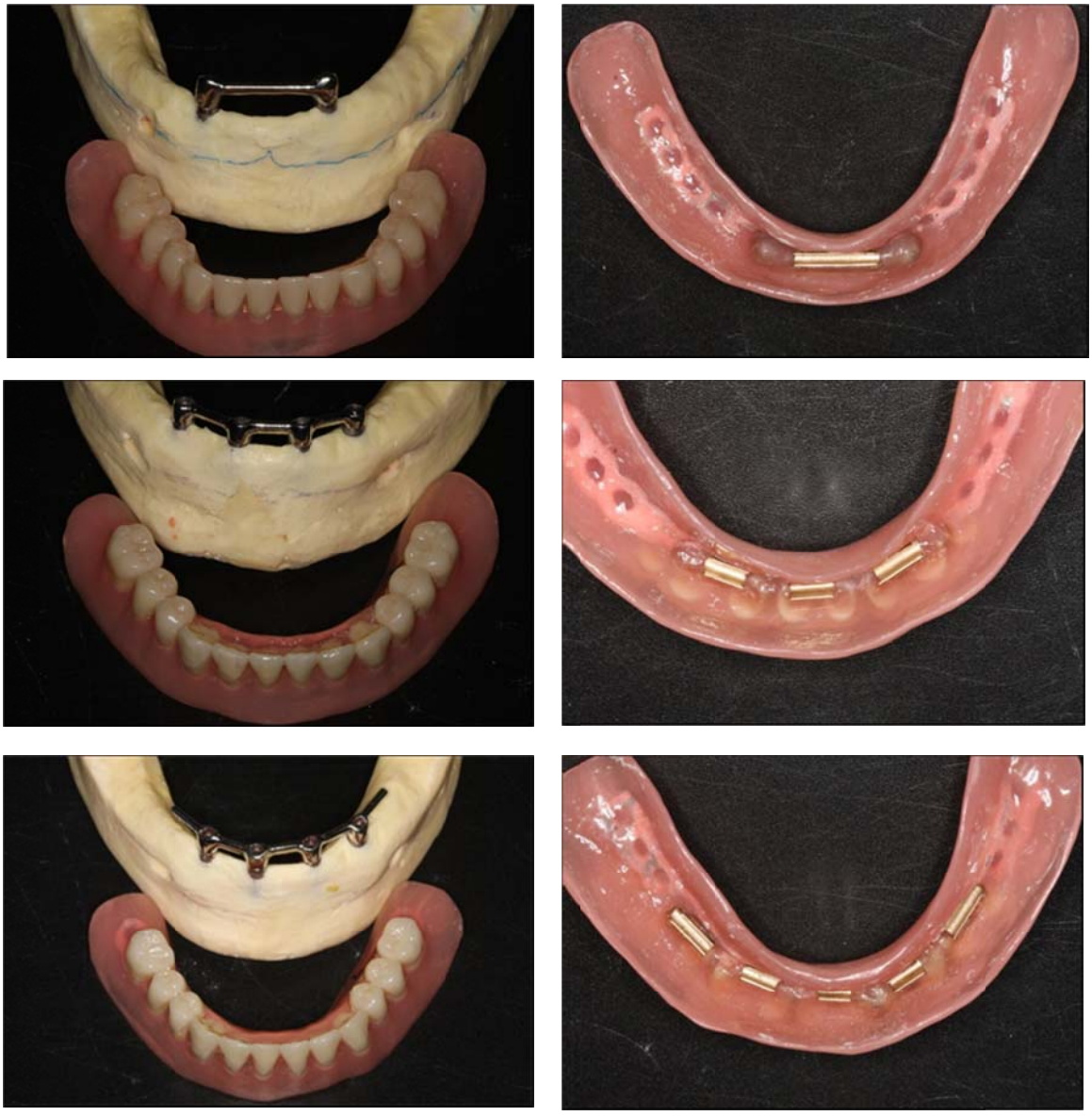

Fig. 2 Totally edentulous mandible restored through implant supported prosthesis:
a, b – 2 implants splinted with a cemented bar with one rider;
c, d – 4 implants splinted with a short bar with 3 riders;
e, f – 4 implants splinted with a long bar with 5 riders;

Most patients constantly attended control visits for 2 years (15 patients needed rescheduling by phone), period during which the retention of overdentures and the behaviour of meso- and superstructure connections were especiallly monitored.

During this time interval no fractures of denture basis were recorded, nor did fractures and detachments of riders, respectively. After the first year, rebasing of some dentures and activetion of some metallic riders were needed.

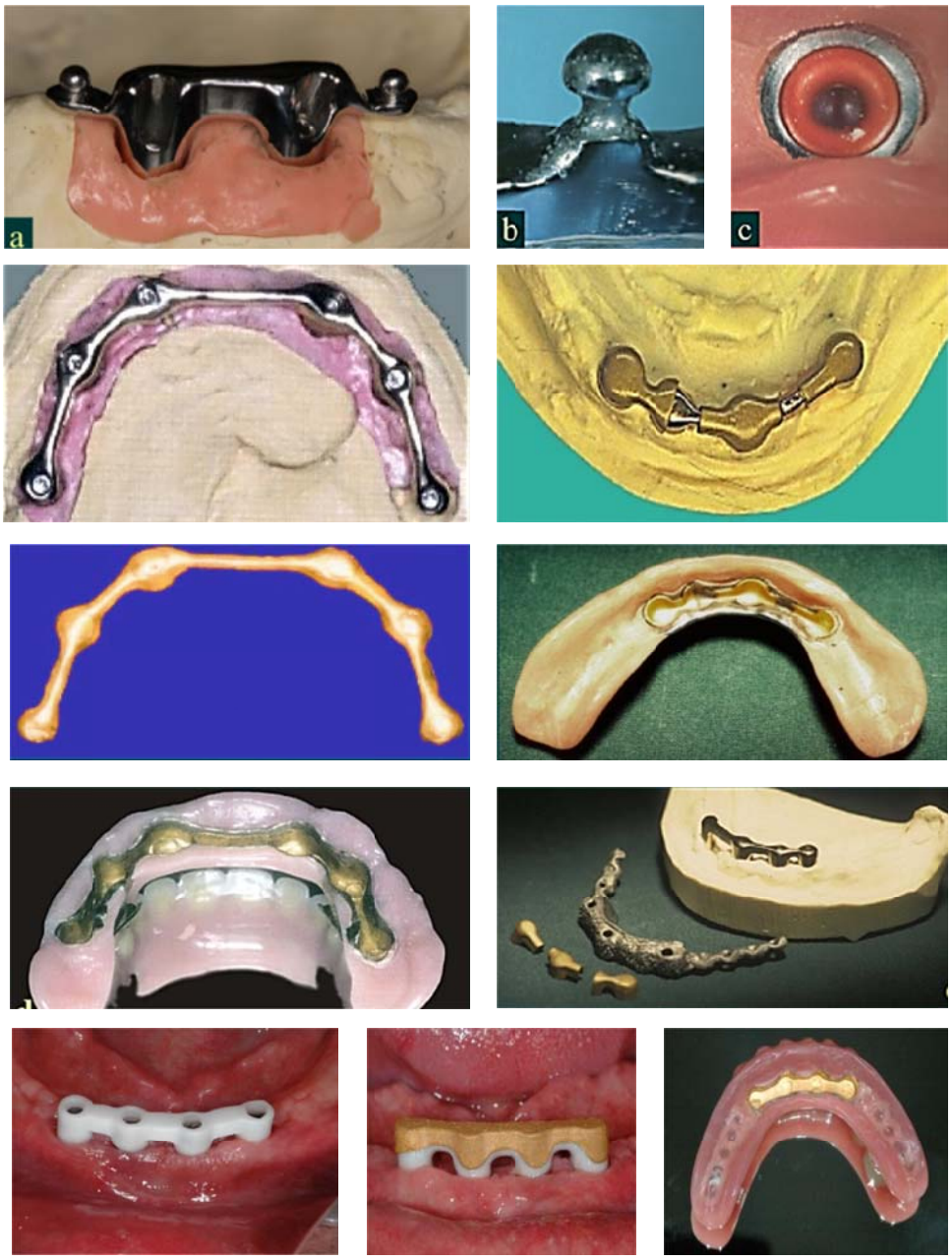

Fig. 3 Totally edentulous mandible; 2 interforaminal implants splinted with a bar; atypical position of insertion (implants are very close), does not allow to place one rider; at the bar end were made two pressure butons (in distal extension);
d, e, f – bar and continuous galvanoformed riders with hydraulic retention;
g, h, i – bar and discontinuous galvanoformed riders;
j, k, l – zirconium milled bar and galvanoformed continuous rider.

The analysis of Questionnaire data lead to the following results:

1) After inserting the restoration, initially there were no complaints, patients being content with the retention of the overdenture, and stability being maintained at predictable levels. Nevertheless, the daily wearing of these overdentures, the oral environment as well as the insertion/disinsertion after each meal for cleaning purposes may lead to a decrease of denture retention, within functional limits.

2) After 6 months and 1 year, respectively, no high differences in the retention degree of overdentures were recorded in none of the 3 situations, as shown by the satisfaction described by patients in questionnaires.

3) After 2 years, some patients started to observe a decrease of the retention degree of overdentures with metallic riders. Oppositely, in overdentures with polymeric riders, some patients observed an improvement of retention (probabily due to plastic surface deformation of riders or thermic expansion of the material which is also clinically observable upon attentive examination). The group of patients with galvanoformed riders was the most content with the overdentures throughout the entire examination period, both regarding behaviour during mastication and phonation, comfort and esthetic aspect, as well as with respect to the retention degree (hydraulic retention of overdentures with more than 3 riders, and of those supported by long bar segments may be transformed into friction retention in certain areas).

Retention differrences between overdentures are also dependant on individual rider characteristics: the length (the longer the rider, the larger friction surface, and the higher retention) and distance between the retention portions of the rider (the smaller the free space between retentive arms, the stronger the resistence to disinsertion), the thickness (the thicker the rider, the less flexible and, consequently, more retentive), the number of riders or the diameter of the bar.

DISCUSSIONS

Implant supported overdentures are in use for over 30 years. During this interval, connexions evolved depending on progress in the field of mesostructures and connectors. During the first period, friction connections were predominant (metallic bars and metallic riders/metallic bars and plastic riders) [29, 19], and later, in Europe, galvanoformed riders became increasingly used [28, 35]. During recent years, metallic bars were replaced by zirconium oxyde ones [1], associated to galvanoformed continuous/discontinuous riders [16, 4].

Our cases also reflect this diversity of restorations, including mesostructures and connections belonging to the three mentioned cathegories. As for the value of denture ***retention*** and the ***satisfaction degree*** of the patient, these were obtained by analysing questionnaire data. Thus, the following were observed:

- ***Retention*** of friction metallic riders decreases in time;
- ***Retention*** of plastic riders is maintained or improved during the 2 year interval, due to plastic surface changes, or to thermal expansion of the material [13].

The same conclusions resulted in the study conducted by Mesquita & Henriques, in which they compared the retention capacity of metallic and plastic riders, after 5,500 insertion / disinsertion cycles (corresponding to 5 years oral use of dentures) [27].

Walton & Ruse also performed laboratory tests for retention differences between plastic and metallic riders, after 5,500 cycles. They record significant differences both in metallic and in polymeric riders, comparing the initial situation and the situation at the end of the test. No fracture or sleeve loss is observed, suggesting that functional overloading or parafunction and not repeated insertions/disinsertions lead to this type of complications [19].

Cune & Van der Bilt performed a comparative study between the initial retention force and retention loss after 3 months, in the 3 connexion systems: bar-rider, ball and magnet [34]. No differences were observed between initial and final retention forces for any of the connecting systems (it is a known fact that the bar/rider connecting type offers the best retention of all existing connecting modalities) [11]. Other studies [25] describe retention loss in the case of bar-rider, ball and magnet connections after longer time periods, over 5 years. This retention loss was more evident in the bar-rider group. We intend to continue the observations regarding this time period in another study.

Dixon and Breeding compared the in vitro retention of one sleeve overdentures with that of two riders overdentures, before and after function simulation. The authors [21] discover that the use of two instead of one rider significantly improves the overdenture retention. Function simulation does not cause a significant change of retention in none of the two situations.

As Anglo-Saxon literature is poor in describing galvanoformed riders, we limit to the conclusions extracted from our own experience.

Retention in galvanic riders, the higher the number of implants and the amplitude of bars the more ***hydraulic retention*** may be transformed in ***friction*** on certain areas. This particular retention type, hydraulic retention or retention by adhesion, is based upon intermolecular adhesion forces mediated by the saliva layer which comes between the bar and the galvanoformed rider. The advantages are supperior to the traditional friction retention: firstly, its longevity is higher. Friction disappears in time, and soone or later, prosthetic restorations must be reoptimized in order to recover friction. In the case of galvanoformed riders, retention is initially based upon adhesion. The fact that between bar and rider there is no direct contact but only one mediated by the saliva layer, guarantees the "passive fit" and the longevity of the retention [28].

Less information is reported on the patient perception after insertion of implant supported maxilla overedentures [22].

Chane & col. [10] solved maxilla edentations with atrophic field by implant supported overdentures following previous bone augmentation treatment and they observed improvements when evaluating patients regarding comfort, esthetics, mastication and phonation.

CONCLUSION

- All patients are content with the retention degree of overdentures during the 2 years period;
- Out of the 86 patients we treated, 31 initially wore traditional total dentures. All these patients state that they increased their life quality and their mastication significantly improved;
- The retention degree was maintained in all overdentures (a slight decrease was observed only in metallic riders);
- No significant differences were recorded between the 3 cathegories of riders;
- No rider fracture or loss occured, probably also due to an attentive case selection (patients with parafunctions were excluded from the study).

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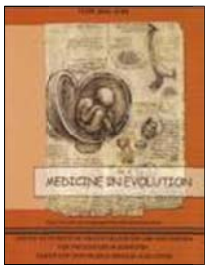
NICKEL ALLERGIC CONTACT DERMATITIS. GENERAL CONSIDERATIONS



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ABSTRACT

Contact dermatitis (CD) is an inflammatory skin disease occurring after skin contact with different allergens. Only 80% of these reactions are irritant, the rest are allergic. Inflammatory skin lesions ranged from average (mild erythema) to severe (cracks, erythemato-squamous lesions and lichenification), depending on the irritant, the body part affected and the individual reactivity. Allergic contact dermatitis may occur at any age, but there was a higher incidence in middle-aged people. Nickel, chromium and mercury are the most allergic metals and are found in various metal alloys, caps, jewelry, watches, eyeglasses frames and contact lens solutions, mobile phones, toys. Cobalt also fits in the list of potentially allergenic metals for CD.

Nickel (Ni) is the most frequent cause of contact allergy among the female population. This makes it interesting to examine thresholds for elicitation under different conditions.

Type IV sensitization to nickel sulfate is common in the general population. Nickel can be found in different metals leading to occupational exposition in industrial professions. Symptoms such as itching, redness and local heat should lead the patient direct to the dermatologist who will determine the cause of suffering and will assess the therapeutic conduct after the determination of the incriminated agent. There is no effective treatment for allergy to nickel. Avoiding substances responsible for contact dermatitis remains the only effective measure for the preventive treatment.

***Key words**: nickel sulfate, dermatitis, patch testing, fillagrins, and sensitization.*

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INTRODUCTION

Allergic contact dermatitis (ACD) is described for the first time in 1895 by Jadassohn. He is also the one who invented patch skin test to determine if there is an allergic component of the contact reaction. Contact dermatitis is an inflammatory skin disease occurring after skin contact with certain allergens. Only 80% of these reactions are irritant, the rest are allergic.

The nickel element was discovered in 1751 by Cronstead in Sweden. The elements name comes from the fact that he was obtained from an ore called "*kupfernickel*". Nickel occurs in the earths crust at a level of 80 ppm [3, 4].

Nickel is the most frequently incriminated metal allergen on contact eczema. Nickel is found in different metal composition, including gold jewelry, and it is used in metal alloys. Many chrome plated objects contain enough nickel to produce an allergic reaction in sensitized individuals. Stainless steel also contains nickel, but strong chemical bonds do not allow the dissolution of nickel atoms on the alloy, so that it is safe for most susceptible individuals. The earrings that contained nickel can cause earlobe dermatitis, a very frequent issue. Nickel allergy can begin drilling needle time, continuing and in the initial period of wearing earrings. Sterile stainless steel needles mustbe used for piercing and the patients must wear only nickel-free earrings, at least in the first three weeks. Clothing accessories made of nickel such as zippers, buttons, eyelets, metal buckles, can cause dermatitis. Sensitized individuals to nickel may substitute items quoted with nylon accessories [18, 20, 32]. Nickel is found in many alloys used in dental treatment to provide improved physical and chemical properties, such as strength and durability, as well as to reduce the cost of using precious alloys such as gold. The amount of nickel in any dental alloy can vary from a few percent to over 60%. Nickel is a common allergen and the use of nickel containing materials in dentistry has sometimes been reported as a source of adverse reactions. This advisory note aims to provide reassurance and advice about the safe use of nickel as part of an alloy in dental materials [10, 20].

Nickel is contained in cigarette smoke and even in tap water. A common area where nickel allergy appears is at the ear lobes, from earrings, eyeglasses frames (or periocular) or mobile phones [22], joints of hands from the bracelet or watch, at the lower abdomen from pants metal buttons, belt buckle. But nickel is not only a component of fashion accessories, but it is also found in everyday household objects, from coins to domestic objects [31, 18].

Nickel contact dermatitis may occur at any age. Once a person has developed an allergic reaction to nickel objects, it will persist for years or even for lifetime. Nickel allergy is more common at the females because they come more in contact with nickel containing objects such as earrings, bracelets, and necklaces. The allergy severity varies from person to person. Some people develop dermatitis even from a short contact with such objects, while in others cases the eczema begins after years of using products with nickel [18, 21].

The sweating worsens nickel dermatitis in susceptible individuals. Such objects that contain nickel can cause itching in 15 to 20 minutes after skin contact with a sweaty skin, even if the rash appears within a day or two. The same items can be worn several hours without any problem if the person is not sweating. Although it seems stran-

ge, antiperspirants may exacerbate symptoms of allergy to metals. It was found that a healthy person sweat in a sauna may contain a level 20 times higher of nickel in blood plasma, this may be important in order to know what can improve the health. Antiperspirants can reduce the body's natural ability to remove heavy metals [3, 4].

In accordance with the above point, it must be stressed that only free Ni+++ ions induce contact allergy. These ions obviously derive from soluble nickel compounds and complexes. On the contrary, insoluble or poorly soluble complexes are generally unable to cause dermatitis, which only develops after close, prolonged contact with objects releasing Ni++ ions.

The minimal concentration of nickel able to elicit allergic contact dermatitis in a sensitized subject is 1=10 ppm, measured by a patch test with scaled aqueous solutions of NiSO4 on healthy skin of the back [7].

Analysis of the table shows that it is not usually alloys with a high content of nickel that cause contact allergy, which is more often induced by alloys with low nickel content. This demonstrates that there is no relation between the nickel content in a metal object and its ability to cause allergy, whereas there is a relation with the objects ability to release nickel ions (Ni ++) [6].

In the past, various industrial sectors posed a particular risk (metallurgic, metal mechanical, nickel-plating, electronic), whereas nowadays these processing cycles involving contact with nickel are sealed-off. The same affliction is observed in 30-60% of nickel-sensitized subjects working in "wet" conditions (housewives, health workers, hairdressers, workers in the food industry) [6].

Table 1 *The metal alloys with a nickel base most commonly used in industry*

Alloy	Ni%	Contact dermatitis
Ni-Cu	10-30	+ + +
Ni-Ag	15	+ + +
Ni-Cr	79	+
Ni-Cr-Mo	54	+
Ni-Fe	36	+ + +
Ni-Si	85	+ +
Ni-Sn	35	+ +
Ni-Zn	12	+ +
Stainless steel	9	+
White gold	2 – 12	+ +

SOURCE OF EXPOSURE

Nickel is ubiquitous in the nature. The exposition sources are multiple.

Domestic: the epicutaneous contact are multiple through jewelry (plated and/or alloys semi-precious), through cloths (jeans buttons, belt loops, garters, bra locks), through kitchen materials (stainless steel), through couture material, coins.

Professional: the eczema of contact with nickel as well as the asthma to nickel is a professional disease compensable for patients working in electrolytic metals plating. The professions exposable to contacts with nickel are nu-

merous, but because of the ubiquitous character/nature of the allergen, the professional responsibility in a dermatosis is difficult to prove. Thus a study shows that the hairdressing students have a clearly higher rate of sensitization to nickel than the general population before any work of experience.

Iatrogenic: the bio-implants, often containing based nickel alloy, are sometimes the location of corrosion phenomena causing the release of nickel ions: orthopedic material of prosthesis or osteosynthesis, pacemaker, dental prosthesis. The allergic dermatoses to nickel from prosthesis material or from osteosynthesis were dominated by a metal-metal prosthesis that is hardly ever used. Rare observations were connected to the presence of metal-plastic prosthesis or to static implants. A recent study prospectively followed patients previously sensitized to nickel; after placing an orthopedic implant containing nickel there was no dermatologic or orthopedic complication. A single case of sensitization to a nickel frame from stainless steel of the pacemaker was described. Some cases of dermatoses were related to wearing dental repairing prosthesis containing nickel as the orthopedic devices to the child. Meanwhile, no perspective study was made to determine their exact frequency. Some allergic reactions to nickel introduced in the organism by the parents were reported: released by needles, infusion sets, dermo Jet tanks, acupunctural needles. Allergic reactions to surgical retractors, to surgical suture staples are also reported.

Accidental: rare cases of secondary dermatoses from war projectiles or secondary dermatoses from accidenttally swallowing coins have been described.

Food related: the role of food nickel for sustaining an allergic dermatose to nickel was presented by Christensen in 1875 and was consolidated later by many works.

The determination of food nickel was made by many teams due to some modern analytic techniques. Essentially the plants are impregnated. The richest foods in nickel are: the cocoa, soybeans, dry vegetables, dry fruits, out four and the black wheat. The proteins that have animal origin contain a little out of shells and salmon. The tap water can be contaminated by the valves especially the first liters drained in the morning. The foods are sometimes contaminated during processing of canning and cooking (especially foods rich in acid which entertain the release of nickel). The nickel content in margarine may be higher because of the use of nickel as catalyst in commercial hydrogenation [23].

DEFINITIONS AND CLASSIFICATIONS

The eczemas represent a common and diverse group of inflammatory skin diseases whose definitions and pathogenic mechanisms have often been confused and controversial, but fresh approaches are providing better insight. Research has focused much more upon the epidermis and the very relevant signaling pathways that contribute to spongiosis, proliferation, generation of proinflammatory factors, and differentiation to form an effective stratum corneum barrier.

Animal models are beginning to predict mechanisms in which such direct perturbation of keratinocytes may initiate inflammation and condition immune responses in irritant contact dermatitis and atopic dermatitis. These conceptual shifts are nurturing more balanced approaches to understanding eczema and hold the hope for better

prevention efforts and more specific molecular targeting for therapy [17, 26].

Contact dermatitis is an inflammatory skin condition characterized by successive polymorphic type lesions erythema, papulous, gallbladder, squamous type, with associated itching symptomatology. The evolution of this disease is most often recurrent and the causes are multiple, both external (exogenous) and internal (endogenous). From an epidemiologically point of view, the eczema is one of the most common dermatological diseases, according to several authors, representing between 20% and 30% of all hospitalizations. On the other hand, the socio-economic cost caused by the eczema is reflected in long term hospitalizations, complex and often difficult treatment, as well as very frequent recurrences.

The word "*eczema*" comes from the Greek word "*ekzein*", which means downcast, which underscores the clinical nature of the eczema lesions. There are authors who made a certain difference between the terms "eczema" and "dermatitis", saying that dermatitis is an acute reaction, while eczema is a chronic illness. In terms of classification of the eczemas, most authors classify them into eczema endogenous, exogenous and mixed. Exogenous eczemas are the contact eczemas (allergic and irritation) and allergic contact eczemas by systemic means, endogenous eczemas have atopic dermatitis as an example, while mixed eczemas exo / endogenous consist in nummular eczema, seborrheic eczema, infection eczema, gravitational eczema, dishydrosis, fissure eczema, eczema of intestinal malabsorption, eczema associated with internal diseases. Irritative dermatitis is the most common, it does not require previous sensitizing contacts, at the patch removal tends to disappear, no skin reactions beyond the edges of the patch, allergic contact dermatitis requires previous sensitizing contact, late occurrence of patch testing with progressive worsening, often the reactions appears beyond the edges of the contact patch and may be even more rare in atopic individuals.

On the other hand, there are many genetic disorders that may present eczematous reaction: Wiskott-Aldrich syndrome, ataxia telangiectasia syndrome, Leiner's disease associated with deficiencies of C3, mucopolysaccharidosis, Hartnup disease, histiocytosis X, and enteropathic acrodermatitis. The atopy term refers to the IgE hypersensitivity, representing a predisposition to allergic diseases and the tendency to exaggerated IgE antibody responses, while allergy refers to clinical expression of atopic IgE-mediated diseases [5, 33].

EPIDEMIOLOGY

The prevalence of metal allergy is high in the general population, and it is estimated that up to 17% of women and 3% of men are allergic to nickel. Among dermatitis patients, the prevalence of metal allergy is even higher. Metal allergy is mainly an environmental disorder although null mutations in the filaggrin gene complex were recently found to be associated with nickel allergy and dermatitis [11, 26]. Although consumer exposure is responsible for most cases of metal allergy, the importance of occupational metal exposure remains present and should always be taken into consideration when one interprets allergic patch test reactions to metals [1]. The epidemiology of metal allergy has recently changed in Europe as nickel allergy among ear-

pierced Danish women has decreased following regulatory intervention on nickel release from consumer products. In the United States, the prevalence of nickel allergy is still increasing, which may be explained by the absence of regulation. A study in Denmark since 1990 regulated about the use of nickel in certain consumer goods. The aim of this study was to reveal the clinical characteristics of nickel-allergic patients seen in seven private dermatology clinics and to identify current sources of nickel that may elicit nickel dermatitis. 634 patients with dermatitis aged 17-91 years were patch-tested and completed a questionnaire including a question about the occurrence of dermatitis following skin contact with earrings or ear-pins, watches, buttons or metal clasps. Chi2 tests were applied to test for statistical significant differrences. The conclusion was that nickel allergy has decreased among young females with dermatitis due to the nickel regulation. Nickel allergic subjects are at risk factor of acquiring hand eczema. In 1990 and 1994, respectively, Denmark and member states in the EU regulated nickel release from selected consumer products. The intention was that the nickel dermatitis could be controlled and prevented if the general population was protected from high cutaneous nickel concentrations. Despite a decrease, the prevalence of nickel allergy remains high as nearly 10% of young women are nickel allergic. Inexpensive jewelry and hair clasps were purchased from 36 stores and street vendors in Copenhagen and were later tested for nickel release using the DMG (dimethylglyoxime) test. The study showed that 19.3% hair clasps, 14.8% earrings, and 12.9% necklaces intended for adult women released an excessive amount of nickel. Of 25 stores visited, 36.0% sold DMG positive jewelry. For items designed for children, excessive nickel release was identified in hair clasps (79.4%) and in finger rings (20%). Four (50.0%) of 8 children clothing stores sold jewelry that released too much nickel. However, 1/5 of purchased items released nickel in concentrations that may lead to nickel allergy. Especially hair clasps intended for children released an excessive amount of nickel [12, 30].

Metal allergy may result in allergic contact dermatitis and systemic allergic (contact) dermatitis. Furthermore, metal allergy has been associated with device failure following insertion of intracoronary stents, hip and knee prostheses, as well as other implants. There is evidence that stimulants such as alcohol and tobacco have an effect on the immune system, but little is known about how these lifestyle factors affect the prevalence of contact sensitization. A study effectuated in Copenhagen on 3460 subjects from general population confirmed that smoking is associated with nickel sensitization, but rejected an association with alcohol consumption [31, 34].

CLINICAL MANIFESTATIONS

Allergic contact dermatitis is by far the most prevalent clinical picture. Acute toxic events involving several organs have been reported in the industrial environment. A carcinogenetic action has been shown in the working environment, especially due to the inhalation of insoluble or poorly soluble nickel compounds, including gassy nickel carbonyl, responsible for tumours of the upper airways and lungs. Vasculitis and asthma are very rare pictures [6]. Inflammatory skin lesions vary from average (mild erythema) to severe

(fissures, erythemato-squamous lesions and lichenification), depending on the irritant, the body affected and the individual's reactivity.

Clinical manifestations of eczema are polymorphic and pass through successive stages, namely erythematosus, micropapulo-vesicular lesions, desquamation and lichenification (in chronic cases). In terms of basic lesion, it is gallbladder, without which there can not be talking about eczema, but has a passenger evolution being quickly replaced with other types of lesions.

As it is, acute or chronic eczema, dresses very different forms. Acute eczema is manifested by the appearance of bright red boards' poorly demarcated, which is accompanied by pruritus, then appearing as blisters that break in, causing a leak, finally, it is forming scabs more or less thick, which are falling after one-two weeks and leave pink scars.

Table 2 *The different clinical pictures induced by nickel, in order of incidence*

1.	Allergic contact dermatitis
2.	Irritant contact dermatitis
3.	Black dermographism
4.	Urticaria
5.	Acute toxic systemic events
6.	Vasculitis
7.	Asthma
8.	Carcingenesis

Chronic eczema, more varied, are classified into three main categories: dried forms, which is the form with red placards and with crusts, ill-defined, with a scaling sometimes fine, sometimes in large flaps; lichenification forms, which is characterized by beaches of thick skin, violet-purple, mapped of ditches with drawing rhombuses; dishydrosis forms, which are expressed by the appearance of blisters on the lateral side of the fingers, which can break and form scabs or fissures, particularly on the palms and plants surfaces.

The clinical aspect of contact dermatitis is that of a vulgar eczema reaching all its evolution phases: the acute phase of erythematosus plaque with vesicles and madidation, subacute phase with erythematous and forming squamous-crusts scabs, the chronic phase with diffuse erythematous plaque covered by an abundant deposit of dry scales, white, multilayered and adherent, which gradually are lost in the normal skin and the lichenification hyper-chronic phase, in which the skin physiological ribs becomes obvious to the naked eye [6, 33].

The nummular clinical picture (rounded lesions with clear-cut margins, the size of various coins) is relatively common, as is dermatitis of the fingertips of the hands. Airborne skin contact, resulting in dermatitis of the face and in particular of the upper eyelids, can occur in the metal mechanical industry, while dyshidrosiform eczema is more commonly present in systemic ACD. Nickel "scabies", or eczema-prurigo, war sometimes observed in the professional context in the past but is no longer seen because the working environment is "cleaner" and most nickel compound processing cycles are now sealed-off [6].

Table 3 *Clinical forms of allergic dermatitis due to the contact with nickel*

1	Erythemato-micropapulo-vesicular dermatitis
2	Dry contact dermatitis of the fingertips
3	Nummular eczema
4	Airborne contact dermatitis
5	Dyshidrosiform contact dermatitis
6	Lichenoid contact dermatitis
7	Nickel "scabies"
8	Lymphomatoid contact dermatitis
9	Burning mouth syndrome
10	Chemical lymphangitis

PATHOGENIC MECHANISM AND IMMUNOLOGICAL CONSIDERATIONS ON NICKEL CONTACT DERMATITIS

Nickel may have unique antigenic properties through differing associations with a variety of cells during induction of sensitization. Nickel ions, too small for antigenic recognition, are highly reactive in combining with extracellular proteins and altering spatial conformation to become allergenic. The nickel-induced changes in tertiary structure allow for dendritic cell (DC) presentation of haptenic complexes in the context of major histocompatibility complex (MHC) class II molecules to T-cell receptors (TCRs) on CD4+ lymphocytes. Alternatively, nickel's reactivity with intracellular proteins can provide for processing and presentation, in association with MHC class I molecules, to CD8+ T lymphocytes. A third, metabolism- independent pathway allows direct linkage of nickel to dendritic cell MHC and to TCRs, analogous to superantigen T-cell activation. Nickel may also have direct effects on DCs to enhance maturation, trigger signaling pathways, and increase expression of chemokines and costimulatory molecules [23, 26].

Attempts have been made to elucidate the immunopathogenesis of contact allergy; yet, the exact mechanism by which nickel-induced allergic contact dermatitis (NACD) occurs is far from clear. It seems to suggest that a direct nickel-MHC (major histocompatibility complex) class II molecule binding on the skin antigen presenting cells such as Langerhans cells (LCs) would result in Th1 cell activation. Substances such as serotonin and cytokines such as TNF-alpha produced by activated mast cells may increase adhesion molecule expression and thus, enhance T cell trafficking in the skin. Cytokines such as IFN-gamma and IL-1 and perhaps IL-12 certainly play a crucial role in the activation of Th1 cells. Along with possible function of CD8 cells, down-regulation of NACD may be mediated by suppressed function of LCs via the action of activated keratinocytes, derived IL-10. Inhibition of NACD can also be generated by feeding with nickel, suggesting that the induction of oral tolerance to nickel may be beneficial for an alternative immunotherapy of nickel allergy. Nevertheless, this testable model provides a direction for further investigation.

IL-17-producing Th (helper) and Th 17 cells are key mediators of chronic inflammation in mice. Recent studies have implicated Th 17-mediated inflammation in the pathogenesis of allergic

contact dermatitis: human keratinocytes were stimulated with nickel in vitro followed by measurements of IL-23 and IL-12 production by quantitative PCR and ELISA. Allergen-specific memory T cells from the blood of individuals with nickel allergy and healthy controls were identified and characterized by using a short-term ex vivo assay. Nickel patch test lesions and normal skin were analyzed for the expression of Th 17-related cells and molecules by using immunohistochemistry. Keratinocytes were found to produce IL-23, but no detectable IL-12, in a response to nickel stimulation. Memory T cells isolated from peripheral blood of individuals with nickel allergy, but not healthy controls, contained Th 17 and Th 1 cells proliferating in response to nickel-pulsed DCs. Inflamed skin of nickel-challenged allergic individuals contained infiltrating neutrophils and cells expressing IL-17, IL-22, CCR6, and IL-22R. The results demonstrate the involvement of Th 17-mediated immunopathology in human allergic contact dermatitis, including both innate and adaptive immune responses to contact allergens.

Allergic contact dermatitis is a common disease caused by an exaggerated T cell-mediated immune response to skin-applied haptens. It was demonstrated that NK cells affect skin immune responses to haptens by releasing type 1 cytokines and inducing keratinocytes apoptosis. Keratinocytes play a key role in the pathogenesis of allergic contact dermatitis (ADC) induced by the sensitizing agent nickel. It exist a study that has analyzed the effects of treatment with nickel and of the pretreatment with zinc on HaCaT cells and primary human keratinocytes. Cell counting, 5-bromo-2'-deoxyuridine incorporation assay and adenosine triphosphate (ATP) bioluminescence detection showed that treatment with NiSO4 induced DNA synthesis and cell proliferation and that pretreatment with ZnSO4 was able to abrogate this proliferative effect. This nickel-induced cell growth appeared enhanced when primary human keratinocytes were co-cultured with fibroblasts. Western blot analysis demonstrated that nickel ions induced up-modulation of the expression of the keratinocyte growth factor receptors (KGFR) without affecting the keratinocyte differentiation, whereas the protein levels of the epidermal growth factor receptor (EGFR) and of its ligand transforming growth factor-alpha (TGF-alpha) appeared unmodified by the treatment. Double immunofluorescence showed that the effect of nickel on DNA synthesis was mainly exerted on KGFR expressing cells, suggesting that KGFR up-modulation could be required for the nickel-induced cell proliferation. These results indicate that KGFR and its ligands may play a role in the mechanism of action of nickel ions and in the protective effect of zinc pretreat-ment [8, 13, 17].

The phosphoglycoprotein osteopontin (OPN) has chemotactic and Th1 cytokine functions and in various models is essential for robust T cell-mediated immunity. It was demonstrated that OPN is abundantly expressed by both effector T cells and keratinocytes in allergic contact dermatitis lesions. T cells from nickel-allergic donors secrete high levels of OPN following antigen-specific stimulation. OPN may substitute for missing IFN-gamma secretion in T effector cells because low IFN-gamma-producing T cell clones secrete high levels of OPN, and OPN down-modulates their interleukin-4 expression. Furthermore, interferon-gamma from T effector cells augments OPN in allergic contact dermatitis by inducing OPN in keratinocytes, which in turn polarizes dendritic cells and attracts inflammatory cells. In the mu-

rine contact hypersensitivity (CHS) model for allergic contact dermatitis, OPN is strongly induced in antigen-specific proliferating T cells, and OPN null mice display a reduced chronic CHS (contact hypersensitivity) inflammatory response due to a decreased influx of effector T cells. It is very important because of its function for chronic allergic contact dermatitis; OPN may well be a therapeutic target, because anti-OPN antibody treatment in part suppresses established chronic CHS [8, 24].

It was recently shown that filaggrin gene (FLG) null mutations are positively associated with nickel sensitization. It was hypothesized that histidine-rich filaggrin proteins in the epidermis chelate nickel ions and prevent their skin penetration and exposure to Langerhans cells. Allergic contact dermatitis (ACD) is of the the most common and widely subject of the studied of the eczemas. In 2006 a revealing new light focused on AD (atopic dermatitis), firmly associating that disease with ichthyosis vulgaris and loss-of-function mutations in the filaggrin (FLG) gene, and subsequent studies have confirmed that finding. This insight gave molecular support to long-standing predictions that AD might be caused by an epidermal barrier defect allowing penetration of irritants, microbes, and protein antigens. Those revelations led naturally to the question of whether FLG barrier defects might also predispose to ACD by allowing greater penetration of chemical haptens [11].

POSITIVE DIAGNOSIS

The positive diagnosis is established based on the history, clinical examination and patch testing. From the history we must retain the daily conduct of the patient, especially professional route, number of episodes, frequency and intensity during the patient's life.

Patch test is performed by applying on the skin a patch above which is placed a low dose of allergen and has to be kept on the skin for 24-72 hours, following local skin reactions (late hypersensitivity). Patch testing represents a diagnostic method in vivo and the diagnosis in vitro consists of: increased of total IgE (200 ng / ml), IgE - specific (ELISA), eosinophilia (in the serum) leukotrienes (LT-C4, LT-D4) in serum, urine and histamine release. IgE determination also represents an important step in diagnosing allergies. IgE is synthesized by B cells, induced by IL-4 and IL-13, shows high affinity for mast cells, basophils, eosinophils and CD23. The allergen triggers the degranulation of the mast cells [15, 29, 33].

TREATMENT

The most important prophylactic therapy to be followed is to remove allergens from the patient's occupational and domestic environment.

Medical treatment is mainly pathogenic (NSAIDs) plus topical corticotherapy (local) for small lesions. There are four classes: class I containing Clobetasol propionate (Dermovate, Cormax, 2 times per day, in the morning and in the evening), do not apply on the face, class II: Elocom, Advantan, Cutivate: they have a promptly effect and are not addictive as Dermovate,

Betaderm, class III: Flumethasone pivalate, Triamcinolone, Fluocinolone not to be applied on the face and class IV: Hydrocortisone acetate (a weaker product, not addictive).

In the dry eczema (chronic) cases is recommended ointments (contains only fat excipients) and in the sub-acute and acute cases of eczema is recommend creams (contains fat excipients and water), the treatment begins with class I or II and after the phenomenon is improving classes III and IV are recommend, administered for maximum 3 weeks. They have the mechanism: reducing vascular permeability; the resins (Ichtiol) decrease the epidermopoesis, PUVA therapy, climatetherapy. Systemic therapy is applied in case of very extensive lesions, where topical treatment failure is also known, Prednisone 30-40 mg / day, for 2-3 weeks plus topical cortisone therapy.

Table 4 *Aliments rich in nickel*

1	Acidic foods cooked in stainless steel vessels
2	Conserved fruits and vegetable
3	Cocoa and chocolate
4	Dried fruits
5	Liquorice
6	Nuts
7	Scallops
8	Tomatoes
9	Flour or seeds bread
10	Green beans

Table 5 *Distribution of daily intake of nickel in a normal alimentation (23)*

1	Vegetables	29,5 p. 100
2	Cereals	24.5 p. 100
3	Fat	13,4 p. 100
4	Milk products	8,7 p. 100
5	Fruits	5,1 p. 100
6	Roe	4,9 p. 100
7	Beverages	4,8 p. 100
8	Sugar	4,5 p. 100

In addition to these treatments are antihistamines with sedation effects (diphenhydramina or hydroxyzine), or antihistamines without sedation effects: cetirizine (Zyrtec) or loratadine (Claritin) and as appropriate, antibiotics for secondary bacterial infection.

Palliative treatments include washing the area with warm water and soap, wet and cold compresses with milk or aluminum subacetate, calamine lotions or oily lotions and skin moisturizers to restore the skin balance: eucerin, aquafor cetaphil.

For highly sensitized individuals is recommend during active skin lesions a poor diet in nickel. In this way the patients will be instructed to further avoid the aliments rich in nickel [1, 14, 19].

Nickel is widespread in its occurrence and is distributed widely in foods. Estimates of daily human intake are from about 0, 24 to 1 mg Ni/day. Vegetable material contains much more nickel than material from animal origin. Low levels of nickel were observed in muscle meats, eggs, and milk [4].

DISCUSSIONS

Since the term allergy was introduced in the early years of this century a very diverse range of agents eliciting an allergic response has been identified. Among them nickel was identified in 1925 and since then other metals, notably cobalt and chromium, have been shown to be implicated. Nickel (Ni) is the most frequent cause of contact allergy among the female population. This makes it interesting to examine thresholds for elicitation under different conditions. Even though Ni exposure may be open, occluded, penetrating or oral, most dose-response studies in the literature concern single occluded application. Comparisons of different kind of exposures across studies are difficult, because of differences in the studies, although a comparison could be made by a study that compares the different exposures within the same individuals at the same time.

Human exposure to metals and their compounds may give rise to a number of adverse effects. Of particular interest are such effects that can be elucidated by chronic low-level exposure, since such exposures may occur in human populations both in the general environment and occupationally [1, 2, 16]. The adverse effect that occurs at the lowest exposure has been termed the "critical effect" since it is critical in relation to preventive action aiming at limiting exposures to a safe level. When reviewing the present evidence on the toxicology of metals it becomes evident that "critical effects" may be related to various organ systems, disease categories and biochemical mechanisms of damage. A category of effects, which is of particular interest in relation to critical effects, is hypersensitivity. Other important considerations are whether effects display threshold or non-threshold type dose-response relationships, the latter category obviously being more likely to display a low incidence response at low dose exposure [3, 11].

Type IV sensitization to nickel sulfate is common in the general population. Nickel can be found in different metals leading to occupational exposition in industrial professions. The individual clinical relevance of nickel allergy can easily be identified but it can be difficult to assess if nickel allergy was acquired privately or occupationally. Important conditions for acquiring nickel allergy are contact with nickel-plated and nickel-releasing materials, the kind of skin contact, the status of the epidermal barrier, and the individual working conditions with an increased bioavailability of nickel. In cases of type IV sensitization to nickel, the affected person cannot continue to work in metal plating or with contact to nickel-plated metals. The causal relationship between the type IV sensitization to nickel and the occupation needs to be clarified in each individual case. In general, occupationally caused nickel allergy is rare and the occupational relevance of nickel allergy is often overestimated [28].

CONCLUSIONS

Body-piercing fashion that grew in recent years is in part responsible for the increased incidence of this type of contact allergy. In 1980 the incidence of nickel allergy was 10% and now is found to be 15% at women and 0.5% at men. Higher incidence at women can be explained by using a greater proportion of earrings from an early age [5, 18].

Genetic predisposition is important in terms of allergies, which are more common at people who come from families with allergic disease. Risk of developing allergies in the general population is 15%, 50% increasing in those with an allergic parent and 70-80% in those with both parents allergic. The individuals with fillagrins mutateons seem to have an increased risk of sensitization to nickel [11].

There is no effective treatment for nickel allergy. Once appeared the sensitization to this element, the symptoms will occur whenever the skin comes into contact with objects that contain nickel. Avoiding the substances responsible for contact dermatitis remains the only effective preventive measure of treatment. In cases where sensitive agents were found, the doctor will have to give detailed instructions on avoiding them. In cases where the allergen can not be avoided will be used creams and protective clothing [1, 4].

Allergic contact dermatitis represents a potentially severe pathology which must be considered important in terms of impact on the quality of life.

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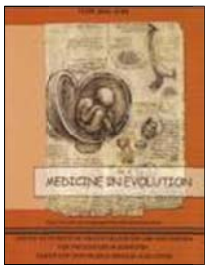
GLOBAL BURDEN OF COPD – REVIEW –



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ABSTRACT

At present COPD represents the 5 most prevalent cause of mortality in the world. The symptoms usually reported are dyspnea, cough and fatigue. Smoking represents the main factor in COPD. The age of 35 year (for smokers people) represents the start of pulmonary decline, these patients losing on average 25-30 ml of FEV_1 yearly. Approximately 50% of the patients with COPD present between 6-10 comorbidities. COPD represents in the European Union the most important cause for absenteeism from work.

***Key words**: COPD, risk factors, inflammation, comorbidities*

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is characterized by the obstruction of the airways. This obstruction is irreversible and usually progressive and is associated with an abnormal inflammatory response to toxic particles and inhaled gas in a pulmonary system. The obstruction of the airways is due to a combination of inflammatory leziunes of the airways and pulmonary parenchyma (fig.1).[1]

The symptoms usually reported are dyspnea, cough, fatigue. Based on the study by Rennard et. al 67% of the patients reported dyspnea symptoms (45% on a daily basis), 70% of the patients reported cough and 65% showed variable quantaies of sputum.

COPD represent in this moment one of the most widespread disease in the world, viewed no longer as a diseases of the pulmonary system, as it was done in the past, but as a true syndrome with multi-systemic implications at presents.

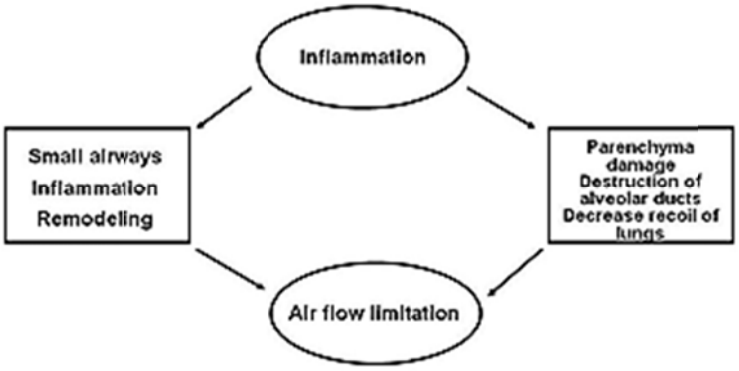

Fig. 1 Basis mechanism of airflow limitation in COPD1.

INCIDENCE AND PREVALENCE

COPD prevalence worldwide is continuously rising, it is estimated that over 600 million patients suffer from this disease;[3] in Europa alone it affects 2,5% of the population over a span of 30 years.[4] Although, COPD was considered in the past a disease occurring generally in the older population (> 50-55 year) recent data shows a prevalence among a younger group of people (20-44 year).[5,6] WHO estimates that the global recurrence of COPD is 0,8% of the world population, with an equal distribution between men and women (including all the age distributions).[7]

In the USA at least 16 million people present symptoms of COPD and an other 16 millions have undiagnosed symptomless COPD; the disease affecting in total 30-35 million people. Germany showed that more than 50% of the people aged 70 or more, suffered from COPD after undergoing a spirometry (VEMS/CV<70% of the predicted or VEMS<80% predicted); and this statistics seem to be confirmed in most western countries.[8,9]

Although, in Romania there aren't at present moment any rigorously implemented studies, Dutu St et al. showed that the prevalence of COPD in rural environment is of 4,6% in men and 2,3% in women over 40 years, this rate being higher in smokers people.[10]

MORTALITY

The evolution tendency in time of COPD mortality indicates that disease is the only disease with growing mortality. At present COPD represents the 5 most prevalent cause of mortality worldwide, estimates that by the end of the year 2020 the disease will be at 3rd place.[11,12]

In Europe (with remarkable geographical differences) approximately 300,000 people die yearly of COPD, the mortality rate being 2-3 times higher in men than in women.

The European countries with the highest mortality rate in men(>80%ooo) are Ukraine, Kazakhstan, Ireland and Romania, and in women (<30%ooo) are in Romania, Ireland, Kazakhstan and Denmark.[13-15] Global Burden of Disease Study estimates that by the year 2020, COPD will be responsible for over 6 million deaths annually.[16]

RISK FACTORS

Smoking represents the main factor in COPD, the WHO estimates that 75% of global COPD is triggered by this vice, the percentage reaching 90% in developing countries.[15-18] Although, smoking is very harmful for the body (especially the lungs), the percentage of smokers continues to rise, especially in developing countries in women and youth.[18,19]

The damage on the pulmonary structures manifests through:

- stimulation of mucus
- inhibition of the movement of bronchial cilia
- favoring the accumulation of macrophages and neutrophils around the distal airways, the stimulation of irritation receptors in the bronchial submucosa.

Air pollution is another important factor in the evolution and development of COPD. The in-door type pollution with gass's produced by the combustion of solid fuel for warming and food preparation is the main reason for COPD onset especially in women.[20,21]

Air pollution of the out-door type (for example: occupational dust, volatile chemical substances, photo-oxidant particles,etc) also contribute to the risk of COPD.[22,23] This risk is directly proportional to the duration and intensity of the exposer and depending on the immunologic mosaic of each patient.

Other favoring factors for COPD are: cotton dust, wheat dust, oil and cadmium smoke. A study done by Vasilieva O. et al. in Russia in 2004 among 880 workers in the rubber industry showed a level of incidence for COPD of 21,5%.[24]

The age of 35 year (for smokers people) represents the start of pulmonary decline, these patients losing on average 25-30 ml of FEV1 yearly.[25]

Determing the genetic factor in the onset of COPD is very difficult. The hereditary α1 – antitripsin deficiency is a major risk factor in the premature development of emphysema and in some people the remodeling of the airways in chronic asthma could lead to a fix obstruction and development of COPD.

Epidemiological studies identified additional factors that lead to the onset of the disease:

- male sex, caucasian race and high socio-economic statutes are all associated to a high COPD prevalence.[26]
- recurrent respiratory infections (mucus hypersecretion or/and infections that

might lead to a significant decline of pulmonary function).[27-30]
- the number of exacerbation (often the patient doesn't recover totally to the pre- exacerbation level before another exacerbation appears).
- nutritional status (high intake of antioxidants, vitamin C and E, is a protection factor against oxidative stress).[31-34]

COPD MULTI-SYSTEMIC INFLAMMATION

Oxidative stress represents an imbalance in the fragile balance between oxidants vs anti-antioxidants elevated in patients with BPOC (this fact is corelated to the reduced anti-oxidant capacity) especially during the periods of exacerbation. Oxidative stress could play an important role in accelerating the process of lung senescence through the inhibitive effect over the anti-aging molecules (SIRT molecules) trigging a more accelerated decline of pulmonary function. The pulmonary function submits to the physiological process of senescence of the body, but in patients with COPD this process is more accelerated. Oxidative stress is mostly linked to skeletal muscle decline (increase level of carbonyl-protein in the quadriceps muscles to patients with COPD). The increase of oxidative stress in COPD could be due to reduced endogen antioxidants concentrations as well as to coding genes of this anti-oxidants set by the transcription factor Nrf2 (key factor in anty-oxidant gene control).[33]

COPD –COMORBIDITIES

As shown in the diagram below (fig.2), approximately 50% of the patients present between 6-10 comorbidities. Analyzing the diagram we can observe that this complex syndrome (COPD) affects the entire population group through the multitude and variety of comorbidities [35].

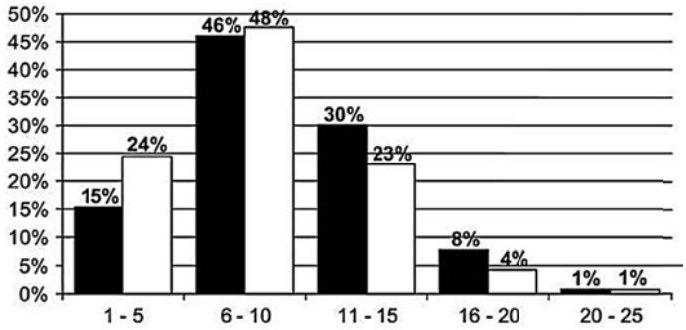

Fig. 2 Number of comorbidities (black bars represents women, white bars represent men)

1. Muscle dysfunction

One of the most important effect caused by COPD is muscle damages. Muscle dysfunction contributes to the limitation of physical tolerance and increases the degree of disability in patients with COPD. It has been proven recently that patients with COPD have a lower capacity of sustaining repetitive muscle contractions and that their

muscles grow tired faster. The following factors may induce muscular dysfunction: periods of relative inactivity, the use of systemic glucocorticoides, malnutrition, systemic inflammation and oxidative stress.[15] There is a loss of 1-1,5% per day of muscular strength during prolonged bed rest. It is remarkably that until now nutritional supplement or hormonal therapy haven't given validated results.

2. Cardiovascular

Curkendall et al. observed an increase of prevalence and risk of mortality in cardiovascular patients with COPD comparative with control subjects.[36] Antonelli et al. in a study involving 270 hospitalized patients with COPD observed that the frequency of the arterial hypertension is on the first place with a 28% incidence, followed by the mellitus diabetes (14%) and ischemic cardiopathy with 10% of the cases.[37] In a canadian study performed by Huiart et al. analyzing 2553 deaths caused by COPD they observed as cardiovascular diseases produced more deaths in comparison with COPD alone.[38] Sidney et al. discovered in a study spanning over a period of 3 years that the patients with COPD and cardiovascular diseases associated are more inclined to mortality risk than patients with only cardiovascular disease.[39]

3. Lung cancer

Patients with COPD have 3-4 times more chances of developing lung cancer. There is a higher risk of cancer with small cells and with scuamoas cells than adenocharcynoms. The increase prevalence of pulmonary cancer in patients with COPD is probably linked to the high inflammation and the oxidative stress. Activating NF-kB may offer a link between inflammation and lung cancer. The pro-inflammatory cytokines can promote the process of tumoral angiogenesis that accelerates the cellular malign proliferation and the development of methastasis. The transcription factor Nrf2 that regulates multiple antioxidants and detoxifying genes is deficient in lungs affected by COPD and may contribute to the high sensibility to lung cancer. Patients with COPD present in the epithelium of the respiratory airways a increased expression for the receptors of the epidermic growth factor (mechanism of mucus hypersecretion) correlated with a cellular proliferation and inducing cancer.[15]

4.Osteoporosis

Patients with COPD present a higher risk of osteoporosis due to their old age, limited physical activity, lower BMI,smoking, hypergonadism, bad nutrition and long therm use of corticotherapy. Men around the age of 60 years have a prevalence rate of vertebral fractures equal or higher than women in post menopause with an age ≥65 years. Limited studies suggest a semnificative association between CO PD and osteoporosis independent of corticosteroids treatment. The prevalence rate in patients with COPD was 29% for vertebral fractures, 68% presents osteopenia or osteoporosis and 24% presents fractures through compresion. Systemic corticotherapy remains the most frequent cause corellated with osteoporosis and a meta-analysis concluded that the use of more than 6,25 mg of prednisone daily increases the risk of fractures.[40-51] Also half of the patients with COPD recruited for TORCH study have been diagnosed with osteoporosis or osteopenia processes (determinate by DEXA method).[52]

5. Endocrin /metabolic system

Metabolic syndrome represents a complex disturbance manifested clinically through: abdominal obesity, high level of triglycerides, atherogen dislipidemia, arterial hypertension, high

glycemic levels and/or insulin resistance. Type II mellitus diabetes is an important risk factor in the prevalence of mortality of COPD (it is uncertain if strict control of glycemia could improve the results).[53] Patients with CO PD frequently present one or more components of metabolic syndrome. Even when a specific comorbidity goes unnoticed according to present criterion, COPD is frequently associated to an marker of chronic diseases (for example: lower glucose tolerance, arterial hypertension, decrease of bone density). Anemia frequently appears in patients with COPD and the altered level of hemoglobin aggravate tissue hypoxia having negative prognosis impact. The prevalence of anemia in CO PD can vary between 13-23%.[54]

6. Gastro esophageal reflux

Mokhlesi et al.[55-57] reported an increase of prevalence of gastroesophageal reflux disease (GER) and other disorders of the esophagus in patients with COPD compared to healthy patients. It has been noticed that at a FEV_1 cut-off of 50% the GER symptom are more frequent in patients with a higher FEV. The helicobacter pylori infection can amplify the inflammation of the airways and increases the rate of exacerbation of COPD in patients with GER.[58]

7. Senescence and COPD

Alveolar cells through the process of senescence induce a continuous inflammation through the production of inflammatory cytokines in different tissues. Limphocytes and Clara cells have a faster aging cycle in patients with COPD. The lymphocytes senescence can induce a auto-immune reaction and increase the infection sensibility, while the senescence of Clara cells could maintain a continuous inflammation of the respiratory airways affecting the cellular regeneration.[59] Thelomeres represent a complex structure of DNA proteins situated at the end of the chromosomes. It was demonstrated that the length of thelomeres (determined by fluorescence) is shorter with age. In a study published by Morla thelomeres was measured in smokers and nonsmokers people. The result of the study showing that in smokers people the length of the thelomeres shortens significantly and it`s correlated with age. Thereby it has been confirmed that the exposure to cigarette smoke accelerates the shortening of thelomeres in the circular lymphocytes.[60]

8.Cognitive function

The cognitive dysfunction is associated with a increasing level of mortality and the degree of handicap. Smoking could influence the cognitive function trough exacerbation of the cerebral hypoxia induced by the increase of carbon monoxide concentration that leads to a left deviation of the oxyhemoglobin dissociation curve. Studies have shown a correlation between Pa CO_2 and cognitive function. In hypercapnic patients $PaCO_2$ is correlated to memory, attention and information processing speed. As a result of the systemic decondition, patients with COPD are frequently isolated and incapable of engaging in social activities. Anxiety and depression are very frequent in these patients (between 10-80%of the cases).[6]

ECONOMIC BURDEN

In the European Union, the direct total costs generated by respiratory pathology represents appreciatively 6% of the total budget assigned to health,

which is more than a half (56% appreciatively 40 billion euro).[62] Also, COPD represents the most important cause for absenteeism from work, the financial losses on european level are estimated to be over 30 billion euro annualy. In total the budget allocated for the treatment and the socio-economic losses caused by the disease are estimated over 70 billion Euro per year.

In our country the patients with COPD present at least 2 exacerbation per year that need hospitalising. The costs regarding the COPD comorbidities are very high. Over 60% of the patients present pathological cardiovascular associations, the percentage being directly proportional with the stage of the disease. Invalidity due to COPD burden is in a continuous increase all over the world, by 2020 (according to statistic estimations) it`s estimated that it will be on 5th place in the world (as disability level).[63]

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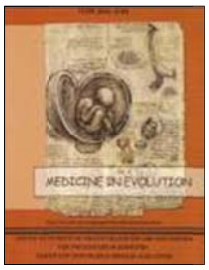
BONE MORPHOGENIC PROTEINS – REGULATING FACTORS FOR STEM CELLS DIFFERENTIATION DURING DENTAL TISSUE DEVELOPMENT – REVIEW-



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ABSTRACT

Bone morphogenetic proteins (BMPs) are multi-functional growth factors that belong to the transforming growth factor beta (TGF beta) superfamily. The roles of BMPs in embryonic development and cellular functions in postnatal and adult animals have been extensively studied in recent years. Signal transduction studies have revealed that Smad1, 5 and 8 are the immediate downstream molecules of BMP receptors and play a central role in BMP signal transduction. Studies from transgenic and knockout mice and from animals and humans with naturally occurring mutations in BMPs and related genes have shown that BMP signaling plays critical roles in heart, neural and cartilage development. BMPs also play an important role in postnatal bone formation. BMP activities are regulated at different molecular levels. Tissue-specific knockout of a specific BMP ligand, a subtype of BMP receptors or a specific signaling molecule is required to further determine the specific role of a BMP ligand, receptor or signaling molecule in a particular tissue. This review article will focus on recent progress in identifying and characterizing BMP receptors, with emphasis on their possible roles in tooth development. Preclinical and clinical studies have shown that BMP-2 can be utilized in various therapeutic interventions such as bone defects, non-union fractures, spinal fusion, osteoporosis and root canal surgery. The mRNA expression patterns of several of the BMPs as well as the results from in vitro studies suggest that these proteins may be involved in regulating both tooth morphogenesis and differentiation of specialized cellular structures in the tooth. In the developing tooth, complex spatial and temporal expression patterns have been revealed for the receptors as well as the BMPs.

***Key words**: BMP, stem cells, dental tissues, odontoblasts, osteoblasts*

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INTRODUCTION

As early as 1889, Senn noticed that decalcified bone can induce healing of bone defects. He was treating osteomyelitic defects in the bone by using decalcified residue of ox bone with iodoform.

The seminal discovery of the ability of the bone matrix to induce bone was made by Urist in 1965. Urist was director of the bone research laboratory at the University of California, Los Angeles School of Medicine, and was a practicing orthopedic surgeon. He showed that crude bone extracts induced new bone in an ectopic site (in a muscle pouch) in a rat model. He coined the term "bone morphogenetic protein" or "osteogenic protein" which was the active ingredient contained in this extract. His research, however, was hampered by the fact that there was no reproducible assay for the protein. Additionally, it was not conclusively determined that this putative protein was responsible for the induction of new bone in an ectopic site. That task was accomplished by Reddi and Sampath in 1983 when they invented a crude but highly reproducible assay for ectopic bone formation. The assay was based on the activity of alkaline phosphatase and the Calcium content of the newly formed bone. This group also showed that when the protein component was dissociated from the matrix the remaining matrix in itself did not induce new bone formation. When the matrix was reconstituted with the protein, however, it was quite effective as the original matrix in inducing the bone. This conclusively proved that it was not the matrix but actually the protein contained within the matrix that was responsible for ectopic bone formation. The first clinical study was conducted in 1988 by Johnson and associates who studied purified human BMP. Intensive competition followed in gene sequencing for the BMP. Cloning of the bioactive molecules revealed seven structurally related proteins that can be further divided into three groups based on degree of identity of the mature proteins: BMP-2 and -4 (92% identity); BMPs 4-7 (average 75% identity) and BMP-3. Additional family members have been cloned from a wide variety of tissues [2]. In addition to being expressed in mineralizing tissues, transcripts for the BMPs are also found in non-mineralizing tissues. There are both experimentally induced as well as naturally occurring mutations in the BMP family members. Analyses of the discrete effects of inactivating the genes for various BMPs or the BMP related growth and differentiation factors (GDFs) indicate that these proteins have specific functions during development of non-skeletal as well as skeletal elements [27].

While the expression domains for BMP family members often overlap and so, may account for the limited defects seen in some of the gene-deletion studies [2], there are areas with distinct patterns. For example, both BMP-2 and -7 are expressed in the perichondrium in the developing chick digits; however, BMP-7 expression is interrupted at the sites of joint formation, while BMP-2 is expressed in the developing joints [2]. Analyses of the embryonic expression patterns of GDF-5, -6, and -7, a subfamily of BMP-related molecules, shows that while GDF-5 transcripts are present in all limb joints (as well as in the condensing mesenchyme), GDF-6 is found only in the carpal/metacarpal and tarsal/ metatarsal joints, and GDF-7, in the developing shoulder and tendons forming at the tips of the digits [16]. Unlike the ectopic cartilage and bone formation induced by BMPs 2-7, recombinant GDFs 5-7 induce tendon-

like structures when implanted into ectopic sites [16], further demonstrating that members of the BMP family regulate distinct developmental functions. Beyond an understanding of the role of these proteins in regulating embryonic development, there is also an interest in being able to use BMPs in clinical settings to stimulate the repair of bone defects and to promote the more complex regeneration of periodontal tissues [2, 23]. This possibility is based on the potent osteoinductive properties of BMPs 2-7 and the similarity between the differentiations programs they initiate during ectopic bone formation and that of endochondral bone formation during embryogenesis and fracture healing. The diverse roles of BMPs in developmental and regenerative processes have been recognized for some time; however, until recently, it was not clear how this complexity was translated at the molecular level. The recent identification of multiple receptors for the BMPs suggests that the diversity and control of BMP activities may depend on the distribution of specific receptors and perhaps also on their functional state. There is substantial information on the expression profiles of BMPs 2-7 in these systems, thus allowing comparisons to be made with the profiles of the newly identified receptors. In particular, the expression profiles of the BMPs are often coincident with regions involved in the inductive interactions between epithelial and mesenchymal cells that regulate morphogenesis and cell differentiation, as well as in the organizing centers that are critical to establishing anatomical pattern [28]. Since BMPs are secreted proteins and may act at a distance from their site of synthesis, immunohistochemical analyses are ultimately required to determine the range of the proteins' actions. However, an understanding of the function of the individual BMPs requires clear delineation of the nature of the specific receptors and their distribution in the target tissues. Differences in affinity of the receptor complexes for the various ligands, as well as differences in their downstream signaling properties, are all important in evaluation of the ability of a cell to respond to BMPs. Thus, an understanding of how the receptors interact with the BMPs as well as their expression patterns in the target tissues will be important to our understanding of how BMPs regulate development and their involvement in healing and regenerative processes.

BMPs themselves are classified into several subgroups on the basis of sequence similarities and homology. These include BMP2, BMP4, decapentaplegic BMP5, BMP6, BMP7 and BMP8. Although originally named because of their ability to induce ectopic bone formation, cartilage condensation, chondrocyte maturation and interdigital cell death, BMPs are involved in many developmental processes, including cell proliferation and differentiation, apoptosis, and intercellular interactions during morphogenesis. Members of the BMP family function in a gene dosage dependent manner during development and participate in ocular development. Therefore, mutations that alter the level of BMPs or alter the degree of BMP signaling are candidates to contribute to Axenfeld-Rieger syndrome and other conditions involving anterior segment malformation, elevated Intraocular Pressure (IOP), and glaucoma. Interestingly, some BMPs modulate tooth morphogenesis and Axenfeld-Rieger patients present dental abnormallities.

The action of BMP is mediated by heterotetrameric serine/threonine kinase receptors. Specific receptor subunits that bind to BMPs include BMPRI (BMP Receptor Type-I) and BMPRII (BMP Receptor Type-II). The BMPRI phosphorylates specific molecules in

the cell cytoplasm, resulting in increased alkaline phosphatase activity, proteoglycan synthesis and collagen synthesis.

Two specific forms of BMPRI include Type IA (ALK3, BRK1) and Type IB (TSK7L/ ALK2 - Activin Receptor-like Kinase 2, BRKII, RPK1),and are known to dimerize with BMPRII (Act RI - Activin Receptor Type-I, ActRII, ActRIIB, T-ALK) in the presence of BMP2, BMP4 and BMP7. BMP ligands can bind to either Type-I or Type-II receptor subunits independently, but both receptor types are required for high-affinity binding and signaling. Binding of bone morphogenic proteins to the receptor complex, results in the activation of BMPRI, which in turn phosphorylates SMAD1, SMAD5 and SMAD8 molecules.

Upon phosphorylation, these BMP-specific SMADs form a complex with the co-SMAD, SMAD4 and translocate into the nucleus to activate transcription of specific genes. In the nucleus, the SMAD1-SMAD4 complex binds with low affinity to the GCCG or CAGA motif in the promoter regions of many BMP-responsive genes.

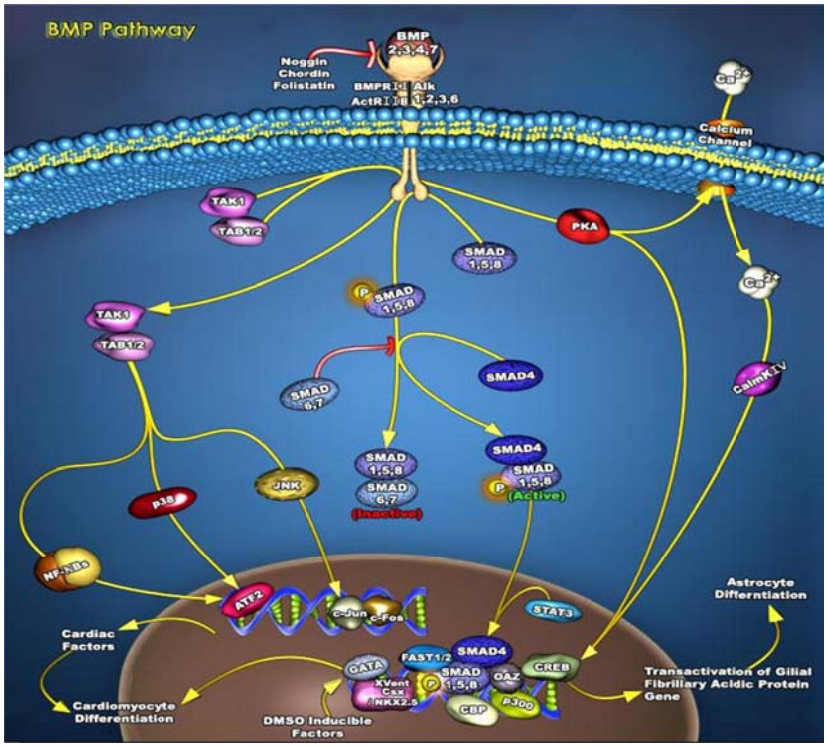

Fig.1 BMP signaling pathway.

In the ectoderm, BMPs activate two biochemical pathways, one mediated by SMADs and a second mediated by the p38/MAPKs (Mitogen-Activated Protein kinase) pathway downstream of TAK1 (TGF-Beta Activated Kinase-1) [10, 32, 1, 5, 9, 20].

BMPs initiate the recruitment of progenitor and stem cells towards the area of bone injury, stimulate both angiogenesis and the proliferation of stem cells from surrounding mesenchymal tissues and promote maturation of stem cells into chondrocytes, osteoblasts and osteocytes. BMPs are also involved in the regulation of other biological processes unrelated to bone formation. They play essential role in early vertebrate embryogenesis such as in mesoderm induction, limb develop-

ment, stimulation of proteoglycan synthesis, alkaline phosphatase activity, collagen synthesis, osteocalcin expression in chondroblasts/osteoblasts and hematopoietic formation.

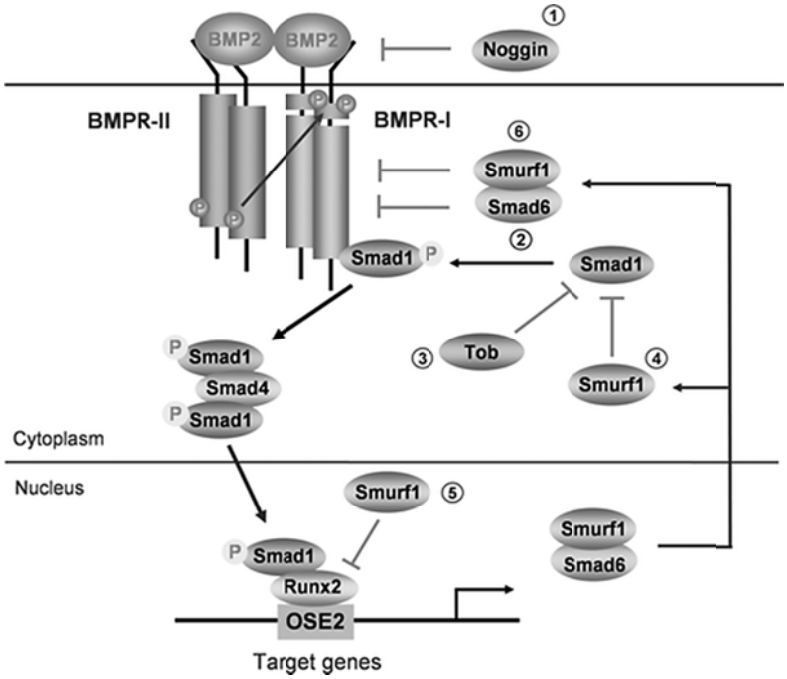

Fig.2 Activation of target genes through BMP receptors type I and II.

Role of BMP during tooth formation

BMP7 has been implicated in such diverse processes as murine hind brain development, tooth development, nephrongenesis, eye development and skeletal patterning. The cloning of BMPs heralds an era where its clinical applications using suitable delivery systems in orthopedic surgery, dentistry, as well as plastic and reconstructtive surgery is truly on the horizon. Although the most effective and optimal delivery system remain to be identified, it is clear that their use to heal or treat severe skeletal defects (axial, craniofacial and periodontal) will have enormous advantages over conventional treatments in clinical contexts.

The mRNA expression patterns of several of the BMPs as well as the results from in vitro studies suggest that these proteins may be involved in regulating both tooth morphogenesis and differentiation of specialized cellular structures in the tooth (28, Gene Expression in Tooth http://honeybee.helsinki.fi/toothexp). At early stages of tooth formation, BMP-2 and BMP-4 are expressed in the dental lamina, while BMP-4 is also expressed in underlying mesenchyme. As tooth development proceeds to the bud stage, BMP-4 expression shifts to the mesenchymal layer, while BMP-2 and now BMP-7 are expressed in the dental epithelium. The region of the forming enamel knot, which is an organizing center believed to be involved in establishing tooth form, expresses elevated levels of both BMP-2 and BMP-7 [21, 6]. At the cap stage, all three BMPs are expressed in the enamel knot. BMP-4 transcripts are also found in the dental mesenchyme, and there is localized expression of BMP-2 in mesenchymal cells deep in the dental papilla. At the bell stage, presumptive

ameloblasts express BMP-4 and odontoblasts express BMP-2, -4, and -7 [6]. Interestingly, no defects in tooth formation were found in the BMP-7 knock-out mouse [6], even though BMP-7 is expressed at all stages. While this suggests that BMP-7 is not im-portant to induction, morphogenesis, or cellular differentiation, it is also possible that other BMPs are compensating for the loss of BMP-7 or that maternal BMP-7 has permitted the initial stages of development to precede [6]. Recently, the combined actions of BMPs-2 and -4 and FGF-8 have been implicated in determining the positioning of the sites of tooth formation [17]. Whether BMPs inhibit FGF signaling or FGF inhibits BMP signaling is not known; however, a possible mechanism for the inhibition of BMP signaling has recently been reported for another tyrosine kinase receptor, the EGF receptor [9]. Whether a similar mechanism occurs for the FGF tyrosine kinase receptor remains to be shown. The BMP receptors involved in mediating these responses have not been formally identified, although mRNAs for BMP types I and II receptors have been detected in dental pulp from adult human molars (ALK-2, ALK-3, ALK-6, BMPRII) [4], adult bovine in-cisors (ALK-2, ALK-3, ALK-6, BMPR II) [29], and adult rat incisors (ALK-2, ALK-3, BMPRII) [30].

Two particularly interesting observations were made in the latter study. First, the smaller of the two ALK-3 transcripts commonly seen in other species is missing in the rat incisor. This is not a species-specific profile, since rat preosteoblastic and osteoblastic cells express two transcripts, although the relative abundance does vary [16]. At this point, the functional significance of the two transcripts is unknown. The second differrence between rat and bovine profiles is the absence of ALK-6 in rat incisor pulp.

The authors speculate that these differences may reflect the embryonic nature of rat incisor pulp. As indicated above, cell cultures prepared from bovine pulp tissue will differentiate in vitro. Temporal changes in the transcripts of a number of TGF-f superfamily members and their receptors occur during the in vitro differentiation of bovine dental pulp cells into preodontoblasts [30]. For example, BMP-4 gradually increases during the differrentiation of the cultures, whereas BMP-7 is not expressed until the cells have differentiated into pre-odontoblasts (day 28), as reflected in extensive nodule formation and the expression of osteocalcin. The receptors also showed distinct patterns of expression. ALK-2 increases from day 7 to day 21, while ALK-3 and BMPRII mRNAs first decrease (day 7 to day 14) and then increase at day 21. Although ALK-6 or ACTRII profiles were not examined, it is evident from the results presented above that differential regulation of several BMP receptors occurs during odontoblast differentiation.

There is limited information on the in situ hybridization profiles for potential BMP receptors during tooth development and no reports as yet on the profile in adult teeth. ALK-3 but not ALK-6 is present in the dental lamina at E12.5 in the embryonic mouse [11], while ALK-2 [33] could not be detected in the tooth primordium of E12.5 mouse embryos. At El 5.5, ALK-3 appears to be present in both mesenchymal and epithelial layers. As indicated previously, early-stage dental lamina also expresses BMP-2 and -4, while mesenchyme expresses BMP-4. In a study of the expression profiles of ALK-3, BMP-2, and BMP-4 in rat molar development [16] showed that, at the cap stage, ALK-3 is strongly expressed in the inner and outer enamel epithelium, enamel organ, and in the dental papilla, while BMP-4 is expressed in the mesen-

chymal cells near the inner and outer enamel epithelium [16].

Thus, there is the possibility of paracrine signaling from the mesenchymal cells to the enamel epithelial cells as well as autocrine signaling between the cells of the dental papilla. At the bell stage, the dental papilla and the enamel epithelium have low levels of ALK-3 transcripts, while the odontoblasts strongly express ALK-3 and BMP -4 with weaker expression of BMP-2. Thus, increasing ALK-3 levels is seen in odontoblasts during both in vivo and in vitro differrentiation.

As indicated above, little is known about the expression profiles for potential BMP type II receptors during tooth development. While ACTRII and ACTRIIB were not detected in developing rat teeth [24], these receptors were not detected in skeletal structures either, in contrast to the results of studies of the skeletal structures in mice [18]. Since ACTRII is expressed in rapidly growing bones of a two-day old rat and in the healing fractures of adult rats either there is a dramatic difference between embryonic rat and newborn or adult rat bone, or a technical problem precluded the detection of the receptors in the embryonic rat skeletal structures.

Interestingly, a small percentage of ACTRII-null mice have variable hypoplasia of the mandible and lacked lower incisors [7], suggesting a role for ACTRII in mandible development, although clearly some factors must be compensating for the lack of ACTRII, since not all mice had the mandible defect. ACTRIIB-null mice has defects in axial patterning and organ asymmetry [18]; however, the dentition was not examined. Whether the defects in activin type II receptors are due to loss of signaling from BMPs or activins is still unknown. However, it is interesting to note that neither of the receptor mutants resulted in a phenotype that matched the activin mutants [15]. Clearly, additional information on the expression profiles of the Type I and Type II receptors are required to map out the potential signaling partners.

The supporting structures of the adult tooth are also responsive to the BMPs. Members of the BMP family are being investigated for their use in repairing or regenerating periodontal tissues [23, 3]. While earlier studies used freeze-dried demineralized bone or partially purified BMP preparations, the availability of recombinant human BMPs (rhBMP) has facilitated the study of the effects of individual BMPs in various model systems. rhBMP-2 and rhBMP-7 have been most extensively studied. In a rat model of periodontal regeneration, both rhBMP-2 [8] and rh BMP-7 [21] stimulated regeneration of the bone and cementum, and maintained the periodontal ligament width (i.e., no ankyloses). In a furcation defect model, implants of either rhBMP-2 [26] or rh BMP- 7 [23] have been reported to stimulate various degrees of periodontal regeneration; however, BMP-2 also induced some ankylosis which may have been due, in part, to the carrier used for delivery [26], whereas BMP-7 did not induce ankylosis. The ankylosis in the BMP-2-treated defects was limited to the cementum-enamal junction.

Whether this represents differences in the responding cell populations at different levels of the defect is unknown. While the receptor profile of the periodontal ligament cells, cementblasts, and the surrounding alveolar bone is unknown, it is intriguing to speculate how differences in the receptor profiles in the responding populations might affect the outcome. For example, periodontal ligament cells can be induced to differentiate into osteoblasts [12]. Perhaps the receptors expressed by periodontal ligament cells were activated by BMP-2 to induce osteoblastic differrentiation but were

either refractory to or did not generate the same set of signals in response to BMP-7 binding.

In this context, it is interesting to note that BMP-7 does not stimulate alkaline-phosphatase expression in cultures of human periodontal ligament cell cultures [19].

The in vitro effects of BMP-2 on periodontal ligament cells are not known. Given the potent osteoinductive properties of BMPs 2-7, misexpression of these BMPs or their receptors could lead to pathological ossification. Interestingly, elevated levels of BMP protein are present in calcifying fibrous epulis originating from the periodontal ligament [19]. The surrounding normal periodontal ligament is only weakly positive, and the gingiva and normal oral mucosa are negative. While neither the cause of neither the elevated expre-ssion of BMP protein nor the nature of the BMP isoform is known, mis-expression of this protein correlates with abnormal mineralization of the periodontal ligament. This multiplicity of receptors, as well as a growing family of downstream signaling molecules, increases the spectrum of physiological responses that can be regulated by these proteins.

SUMMARY

In the developing tooth, complex spatial and temporal expression patterns have been revealed for the receptors as well as the BMPs. Also, distinct differences between the biological responses mediated by two BMP type I receptors (ALK-3 and ALK-6) during development were found with the use of either dominant-negative or constitutively active receptor mutants. Additional differences are likely to be found in the signaling pathways used by the complete receptor complexes comprising both type I and type II receptors. Whether all BMPs signal through all or some of currently identified receptors is still unknown. However, given the large number of BMPs and BMP-related molecules, it is quite likely that additional BMP receptors will be found which will further increase the complexity of this system.

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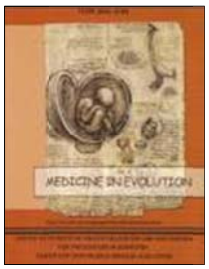
A COMPARATIVE STUDY OF SHADE-MATCH PERCEPTION OF PORCELAIN FUSED TO METAL CROWNS



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ABSTRACT

***Objectives**: Matching the shade of a porcelain-fused-to-metal restoration to that of the natural teeth is an important goal of the restorative dentist. We aimed to compare patients', student's and prosthodontist's satisfaction with the achieved shade of porcelain-fused-to-metal restorations.*

***Methods**: One or more porcelain-fused-to-metal crowns in the anterior zone were completed in 23 patients who needed prosthetic rehabilitation. After treatment's completion, a validated questionnaire to assess satisfaction with the shade match was used in all patients, one student and the prosthodontist who supervised these procedures. The Kendall's tau-b statistic and the Weighted κ Statistic were used to analyze the level of agreement between the ratings given by patients, the prosthodontist and the student.*

***Results**: Patients rated 86.95% of the crowns' shade as good, and 13.04% were rated as satisfactory. The prosthodontist rated 30.55% crowns' shade as good, 52.77% as satisfactory and 16.67 % as poor, and the student rated 69.44% and 30.55% of the crowns' shade as good and satisfactory respectively. The level of agreement between student and prosthodontist, as computed by the weighted κ, was 0.25, and the level of agreement between patients and prosthodontist was 0.09.*

***Conclusions**: With this simple tool, we demonstrated that although the majority of patients were highly satisfied with the shade-match of the restoration, the level of agreement between patients and prosthodontist was low. However the patient satisfaction is the most important goal of the aesthetic restorations and this was achieved in this cohort.*

***Key words**: porcelain-fused-to-metal crown, shade perception, shade guide.*

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INTRODUCTION

One of the most important goals of the dentist-dental technician team is the ability to match the shade of a porcelain-fused-to-metal restoration to that of the natural teeth [1]. Many factors may affect the process of shade matching, including the light source, the patient's clothing and makeup, inconsistencies of the commercial shade guides and insufficient and vague communication with the dental laboratory [2, 3].

Standardized shade guides have been developed to assist in the process of shade selection and to help practitioners communicate effectively with the dental technician. Vita classical and Vitapan 3D Master are the mostly used in dental practice and dental laboratory. However, the successful use of these shade guides depends on the accuracy of the color assessment by the individual choosing the shade, as well as on effective communication with the dental laboratory fabricating the restoration. Some have reported superior intrarater repeatability in shade selection with the use of Vita Lumin Vacuum shade guide [4]. Others consider that the use of Vitapan 3D-Master shade guide notably improved intrarater repeatability among the general practitioners [5].

The purpose of our study was to compare the patients', student's and prosthodontist' s satisfaction with the shade match of the porcelain fused to metal crowns (PFM) and the adjacent natural teeth.

MATERIAL AND METHODS:

For this study we have recruited 23 patients from the Department of Prosthodontics who needed at least one porcelain fused to metal crown in the anterior zone. Twenty-one restorations were performed on central incisors, 11 on lateral incisors, and 4 on cuspids, either by students or by a prosthodontist.

After completing the treatment, patients were asked to express their degree of satisfaction regarding the level of matching of the shade of the restoration with that of the adjacent natural teeth. The following levels of satisfaction were recorded using a simple questionnaire: "*poor*" (obvious color mismatch), "*satisfactory*" (acceptable blend to the adjacent natural dentition, but a color difference could be detected) or "*good*" (good blend into the adjacent natural dentition).

Immediately after obtaining the patients' assessment, a prosthodontist and a student were asked to evaluate the shade match of the fixed PFM restoration to the adjacent teeth using the same rating system and blinded to the patients' assessments. Shade selection is highly affected by viewing conditions, such as light source, wall color, the amount of sunlight, the patient's clothing and makeup, and the viewing angle of the tooth [6, 7]. In our study, both patients and the examiner conducted the test at the same visit under the same controlled conditions. Standard conditions used for evaluating shade match included day light and having the patient seated in an upright position with his or her mouth at the observer's eye level.

The level of agreement between patients, the prosthodontist and the student in this assessment was determined and the results were analyzed for statistical significance. The null hypotheses for this study assumed no difference in the perception of shade match of PFM restorations between

patients and the prosthodontist, or between genders. The Kendall's tau-b statistic and the Weighted κ Statistic were used to analyze the agreement between the ratings given by patients, prosthodontist and student [8] controlling for patient gender and the health professional who was responsible for the restoration.

This study was approved by the Ethical Committee of the University of Medicine and Pharmacy "Victor Babes" Timisoara.

RESULTS

Among the 23 recruited patients, 14 were females and 9 were males, and the mean age of this cohort was 40 years (range 21-58 years). The majority of the patients (86.95%) rated the restoration shade as good, while only 3 patients (13.04%) considered it satisfactory. None of the 23 study participants considered the shade-match poor. Since some of the participants received more than one crown, the patient's ratings considering the number of placed crowns is even more compelling. Out of 36 crowns placed, 33 (91.6%) were considered good, while 3 (8.3%) were considered satisfactory. The distribution of patients' opinions relative to the shade-match of the placed restorations is shown in figure 1 and figure 2.

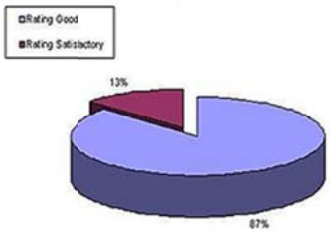

Fig.1 Percentage of patient ratings considering the number of patients.

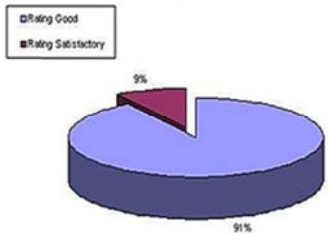

Fig.2 Percentage of patient ratings considering the number of crowns placed.

Among the 20 patients rating the crown shade as good, 13 were women and 7 were men, while among the 3 patients rating the crowns as satisfactory, 1 was a woman and 2 were men. Regarding gender distribution, 2 men and 1 woman gave a "satisfactory" rating. Twenty-eight of the 33 crowns rated as good were placed by a prosthodontist, and 5 by a student.

The 3 crowns considered by patients as satis-factory were all placed by a student. The ratings given by patients for each crown relative to the person who placed the crown are shown in figure 3.

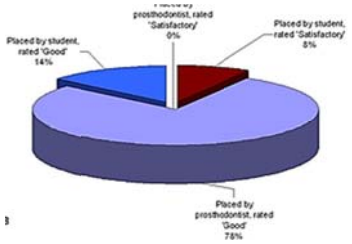

Fig.3 Ratings given by patients for each crown relative to the clinician who placed the crown.

The student rated 25 of 36 crowns (64%) as "Good" (2 placed by a student and 23 placed by the prosthodontist) and 11 crowns (30.5%) as "Satisfactory" (6 placed by a student and 5 placed by the prosthodontist.

The ratings of the prosthodontist were lower: 11 of 36 crowns (30.5%) were rated as "Good", 19 (52.7%) as "Satisfactory" and 6 (16.6%) as "Poor". The crowns rated "Good" by the prosthodontist were all placed by a prosthodontist. Among the crowns rated "Satisfactory" 2 were placed by a student and 17 by a prosthodontist and all 6 crowns rated "Poor" were placed by a student.

In figures 4, 5 and 6 we present clinical examples of the ratings of the tooth shade.

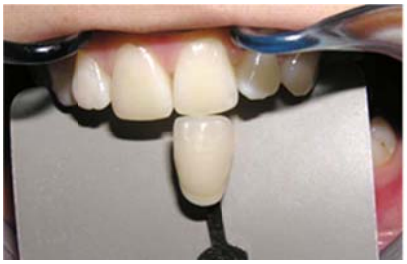

Fig.4 Comparison of a porcelain fused to metal crown on tooth 21 with adjacent natural tooth 11 with Vitapan 3D master shade guide. The dentist's and patient's rating were "good".

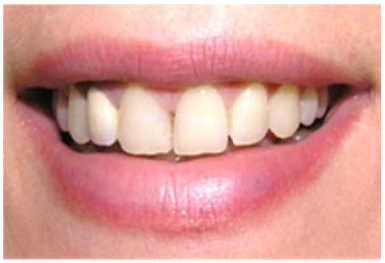

Fig.5 Comparison of porcelain-fused to metal crown on tooth 12 with adjacent teeth. The patient's rating was "satisfactory", but the prosthodontist's rating was "poor".

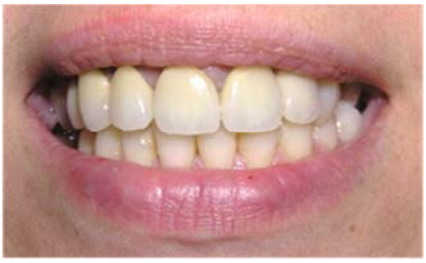

Fig.6 Comparison of porcelain-fused to metal crowns on teeth 11, 12, 13, 21, 22 with adjacent tooth 23, and with the antagonists. The patient's rating was "good" and the prosthodontist's rating was "satisfactory".

The ratings obtained from the prosthodontist and the student is shown in figures 7 and 8 respectively. The level of agreement between patients' satisfaction and the prosthodontist was low (Weighted κ of 0.09). The level of agreement between patients and student as measured by the Weighted κ was 0.34 (95% confidence interval, 0.04 to 0.65). The level of agreement between student and prosthodontist, as computed by the Weighted κ, was 0.25.

Table 1 shows the differences between the satisfaction ratings of the different groups. Similar results regarding the agreement in satisfaction among the tested parties were obtained when analyzed these data using the Kendall tau coefficient (0.226, indicating a rather insignificant level of agreement).

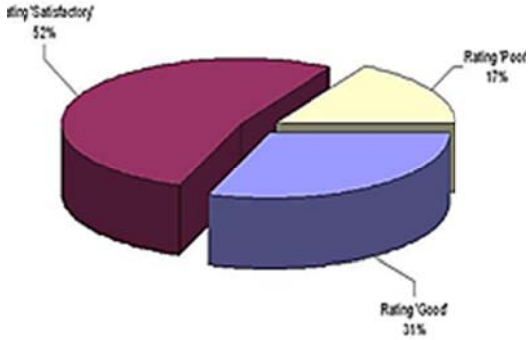

Fig.7 The satisfaction level of the prosthodontist regarding the shade match of porcelain fused to metal crowns.

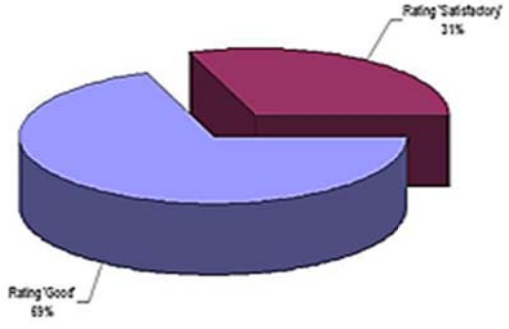

Fig.8 The satisfaction of the students with the shade match between the restorations and natural teeth.

DISCUSSION

This study shows that the majority of the patients analyzed were highly satisfied with the shade-match of the placed restorations, especially if the crowns were placed by the prosthodontist. The highest satisfaction ratings were given by the patients, followed by the student and then by the prosthodontist. None of the crowns rated by patients as "Satisfactory" was rated any higher by a student or prosthodontist. Furthermore, no crown rated "Satisfactory" by a student, was considered "Good" by a prosthodontist.

Men had a tendency to rate lower than women, however due to the very small number of patients rating the crowns as satisfactory; a precise gender-related conclusion cannot be obtained from this analysis.

Several factors are instrumental in achieving an optimal shade-match of the crowns restorations. According to Carsten [9], in addition to external factors such as lighting, specific observer-related conditions play a major role in accurate color perception. Others have reported that an impaired color vision of the dental health-care providers is associated with significantly more errors in the process of shade matching [10]. Culpepper [2] has suggested that dentists consult an assistant for a second opinion during the shade selection process to minimize such errors. However, Ethell & all [11] reported no significant differences between color-defective and non color-defective dental health-care providers in shade matching abilities. Curd & all [12] concluded that dental students' shade matching abilities were better with a light-correcting source than under natural light.

Mollon [13] reported that women are more capable than men in the shade selection and color matching process, although gender and experience were not found to be confounding factors for matching shades in other studies.

We used the weighted κ methods for estimating the level of agreement between the selected categories of subjects who ranked the same items into the certain categories. The κ coefficient is a number between 0 and 1. The closer is the value to 0, the higher the disagreement between various subjects' ratings. In general κ values of 0.8-1 indicate good agreement, whereas κ values of 0.4-0.8 indicate moderate agreement. In this study we found a very low agreement between the prosthodontist and patients' ratings and a rather small level of agreement between patients and student' ratings. The level of agreement is low because the prosthodontist rated visibly lower than the patients and had a tendency to rate lower than students. It is possible that the lower ratings by the prosthodontist may be related to higher expectations with the shade match of an existing restoration by the dental health care provider.

This difference in satisfaction ratings has important clinical signifycance. Patients were more satisfied with the color of porcelain fused to metal restorations placed by a prosthodontist than with the color of restorations placed by a student. This may be a result of the prosthodontist's experience or the patient's perception of quality. By comparing patients' opinions with those of one practitioner and one student, rather than comparing agreement among practitioners, we can also conclude that patients are not always in agreement with the clinician in regard to shade-matching decisions.

Patients' satisfaction with the selected shade of porcelain fused to metal restoration is an important factor to consider, and the opinion of the patient

may differ from that of the practitioner. We believe that the patient's opinion should be considered in the selection of shades for PFM restorations. Thus, collaboration between the patient and dentist should be an integral part of treatment planning. If the patient is satisfied with the shade match of an existing restoration, the dentist should not consider changing the restoration on the basis of his/her assessment alone. These findings support our belief that patients' involvement in shade selection and their satisfaction with the outcome are important to achieve the best esthetic results. To minimize the problem of shade mismatch of PFM restorations, the dentist should not forget that patient satisfaction is an important goal. Furthermore, a systematic method for shade determination, which includes input from both the clinician and the patient, as suggested by Sorensen and Torres [14] always should be followed.

Table 1 *Satisfaction level of groups related to one another*

		Satisfaction level of patient			**Total**
		Good	Satisfactory	Poor	
Satisfaction level of prosthodontist	Good	11	0	0	**11**
	Satisfactory	19	0	0	**19**
	Poor	3	3	0	**6**
Total		**33**	**3**	**0**	**36**

		Satisfaction level of prosthodontist			**Total**
		Good	Satisfactory	Poor	
Satisfaction level of student	Good	11	14	0	**25**
	Satisfactory	0	5	6	**11**
	Poor	0	0	0	**0**
Total		**11**	**19**	**6**	**36**

		Satisfaction level of patient			**Total**
		Good	Satisfactory	Poor	
Satisfaction level of student	Good	25	0	0	**25**
	Satisfactory	8	3	0	**11**
	Poor	0	0	0	**0**
Total		**33**	**3**	**0**	**36**

CONCLUSIONS

In summary, in spite of the limitation associated with the rather small number of cases analysed, we conclude that the majority of the patients were highly satisfied with the shade-match of the restoration. However, the level of agreement between patients and prosthodontist was low.

The patient satisfaction is the most important goal of the aesthetic restorations and this was achieved in this cohort.

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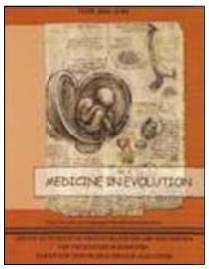
STUDY CONCERNING THE GASTRIC MUCOSA AGGRESSIVITY OF SHORT TERM N.S.A.I.D.S. TREATMENT IN THE PRESENCE OR ABSENCE OF HELICOBACTER PYLORI



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ABSTRACT

Objectives: *The study aims the involvement of Helicobacter pylori in NSAIDs induced gastric disease and the role of this bacterium.*

Material and method: *The study includes 160 patients. The 160 patients with chronic degenerative rheumatic disease were subjected to oral NSAIDs treatment (non-selective COX inhibitors) for 10 days. Endoscopic and histological examinations performed after the anti-inflammatory treatment for the detection of Helicobacter pylori allowed the delimitation of two groups of patients NSAIDs Hp + and NSAIDs Hp -. We compared the two groups based upon previously defined endoscopic and histological parameters.*

Results: *The 160 patients classified into the two groups i.e. NSAIDs Hp+ and NSAIDs Hp- were detected with endoscopic and histological lesions consistent with acute gastritis (abrased epithelium, blood exudation, oedema and capillary congestion more frequent in NSAIDs Hp-) and chronic gastritis changes (hyperaemia, congestion, papulous gastropathy, intestinal metaplasia more frequently encountered in the NSAIDs Hp+ group) [1,4,6].*

Discussions: *Acute and chronic gastritis changes are often multifactorial diseases with a series of risk factors for the occurrence of these endoscopic and histological changes after NSAIDs treatment, Helicobacter pylori being one of these risk factors [6]. Non-selective COX NSAIDs pose a gastric risk by direct action and systemic action by introducing selective COX2 inhibitors, harmful gastrointestinal effects were diminished. NSAIDs effects have been demonstrated by COX2 inhibition and harmful effects occur by inhibition of COX1 [8]. Short term intake of NSAIDs (10 days) may cause acute gastritis lesions by COX1 inhibition [5, 7]. Present literature data on the toxic additive effect of NSAIDs and Helicobacter pylori on the gastric mucosa are still controversial [7].*

Conclusions: *1. in our study, Helicobacter pylori offers protection to the gastric mucosa against drug-induced aggression, reducing both the number of cases who develop erosive gastritis as well as the severity of lesions 6. 2. Both endoscopic and histological acute and chronic changes are most frequently detected in the gastric antrum [4].*

Key words: *non-selective COX NSAIDs, Helicobacter pylori, acute gastritis, chronic gastritis, endoscopic lesions, histological lesions*

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INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are classes of world-wide highly used drugs for their analgesic, anti-inflammatory, anti-pyretic and anti-platelet effects. Anti-inflammatory drugs emerged together with the discovery and commercialization of Aspirin around 1874.

Over 100 years ago, Felix Hofmann from Bayer Industries reported the synthesis of acetyl salicylic acid as the first non-steroidal anti-inflammatory drug which he named Aspirin. After almost 40 years Douthwaite and Lintott endoscopically proved Aspirin caused ulcerations on the gastric mucosa. Since 1970 numerous accessible non-steroidal anti-inflammatory drugs (NSAIDs) were developed encouraging self medication and thus becoming the most frequently used drugs [2, 5, 7].

Around 35 million patients worldwide take NSAIDs daily and around 30% of them develop important gastrointestinal lesions. ARAMIS (Arthritis, Rheumatism and Aging Medical Information System) estimated that one third of NSAIDs treatment costs in rheumatoid arthritis are needed for the treatment of adverse effects (over 2 billion USD per year). Due to differences between types of drugs, dosage and duration of treatment, the estimation of adverse effects is difficult. Generally, 10-20% (with limits between 5-50%) of patients experience dyspepsia during NSAIDs treatment. During a 6 months period, 5-15% of rheumatoid arthritis patients stop the treatment due to adverse effects. The number of hospital admissions caused by complications of NSAIDs treatment decreased during recent years. The mortality rate in patients hospitalized for upper digestive haemorrhage is 5-10%. ARAMIS showed that the mortality rate connected to NSAIDs consumption is 0.22% per year with an annual risk of 4.21 as compared to those who do not take NSAIDs. In the USA, around 107,000 patients are hospitalized yearly for gastrointestinal complications connected to NSAIDs and around 16,500 of rheumatoid arthritis patients die yearly due to the adverse effects of the treatment. The number is comparable to deaths caused by AIDS and is higher than the number of deaths caused by multiple myeloma, asthma or Hodgkin's disease [2, 5, 7].

MATERIAL AND METHODS:

The study includes 160 patients in whom the following exclusion criteria were used

- no history of gastritis, gastric ulcer, gastric cancer;
- no consumption of NSAIDs or other digestive aggressive drugs two months prior to the study.

Diagrams were used to represent classification of patients into lesion groups to suggest acute or chronic gastritis and the presence or absence of *Helicobacter pylori*, highlighting the results as absolute and percent values.

The 160 patients with chronic degenerative rheumatism followed oral NSAIDs treatment (non-selective COX inhibitors) for 10 days with various formulae, mainly aspirin, diclofenac and ketoprofen. At the end of the anti-inflammatory treatment, endoscopic and histological examination for the detection of *Helicobacter pylori* allowed the delimitation of two groups of patients i.e. NSAIDs Hp+ and NSAIDs Hp-. We compared the two groups based upon previously defined endoscopic and histological parameters.

RESULTS

The 160 patients divided into the two groups i.e. NSAIDs Hp+ and NSAIDs Hp- were detected with acute gastritis endoscopic and histological lesions (abrased epithelium, blood exudation, capillary oedema and congestion more frequently observed in NSAIDs Hp-) and chronic gastritis changes (hyperaemia, congestion, papulous gastropathy, intestinal metaplasia more frequently encountered in the NSAIDs Hp+ group).

The NSAIDs Hp+ group includes 95 patients, 56 women and 39 men.

The NSAIDs Hp- group includes 65 patients, 43 women and 22 men.

The endoscopic lesions detected in the two groups were the following:

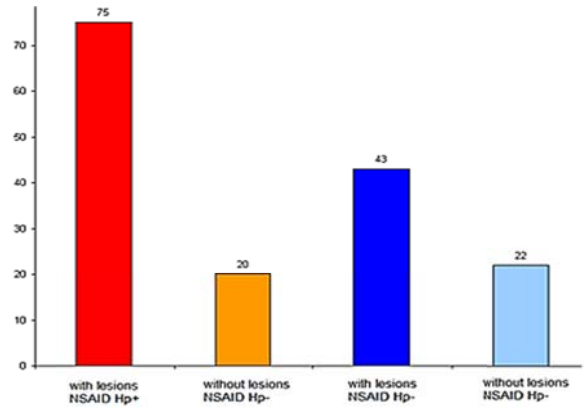

Fig.1 Proportion of cases with and without lesions, correlated to the presence of Hp.

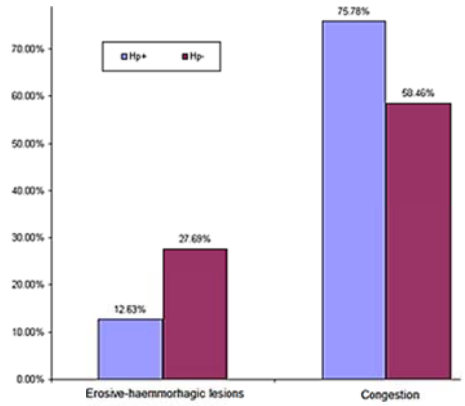

Fig.2 Endoscopically detected lesions

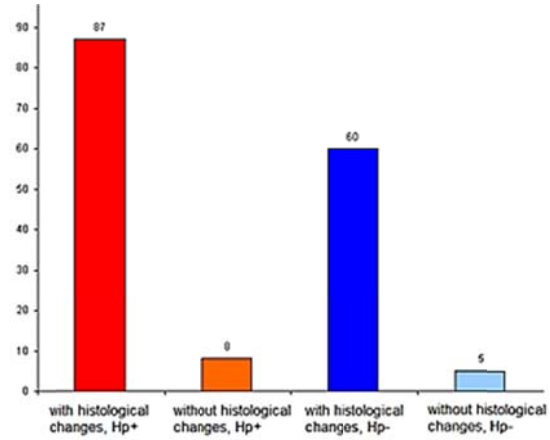

Fig.3 Histological changes.

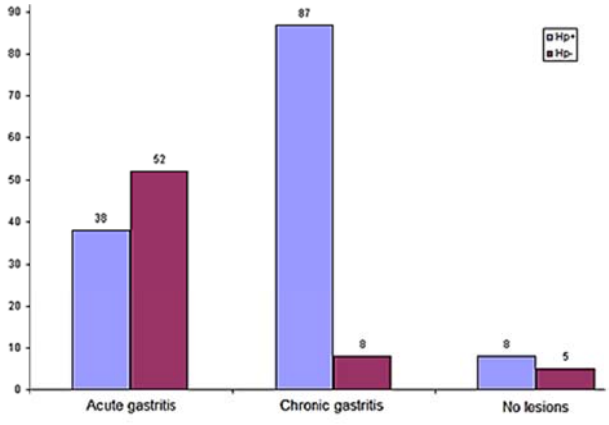

Fig.4 Complete picture of histological changes.

Out of the total number of 95 NSAIDs Hp+ patients, 75 had endoscopic lesions and 20 were lesion free, and in the group of 65 NSAIDs Hp- patients, 43 had endoscopic lesions and 22 had no lesions. The detected lesions are as follows: (fig. 2)

- Erosive-haemorrhagic lesions predominantly in the NSAIDs Hp- group detected in 18 patients (27.69%) and in only 12 patients (12.63%) of the Hp+ group
- Congestion was more frequently found in the NSAIDs Hp+ group in

72 patients (75.78%), and in the NSAIDs Hp- group congestion was found in only 38 patients (58.46%).

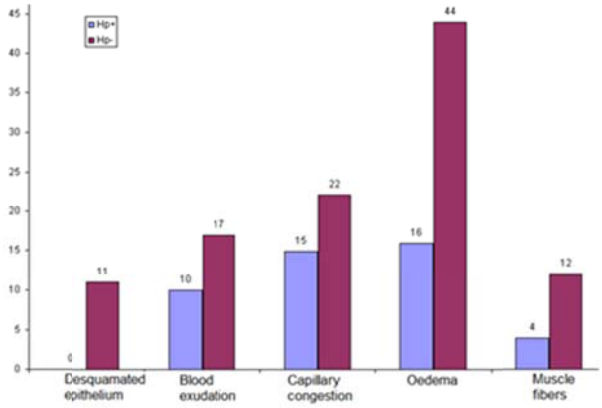

Fig.5 Acute gastritis.

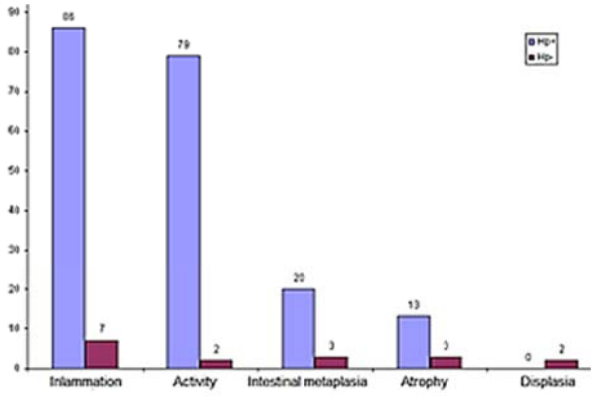

Fig.6 Chronic gastritis.

Histological changes were found in 87 patients in the NSAIDs Hp+ group and in 60 patients of the NSAIDs Hp- group.

Of the 95 NSAIDs Hp+ cases, 38 (40%) had additional acute gastritis changes on pre-existent histological chronic bacterial gastritis (type B) in 87 cases (95%). Eight cases (8.42%) had no histological changes.

Of the 65 NSAIDs Hp- cases, 52 (65%) had histological changes characteristic for acute gastritis and 8 patients (12.30%) had chronic gastritis changes and other 5 patients had no histological changes.

Acute gastritis characteristic histological changes were: (fig. 5) polymorphonuclear infiltrate, erosive lesions (discontinuity of the superficial epithelium) vascular congestion and haemorrhages and oedema in the corion [1, 4, 7]. Thus, in our NSAIDs Hp- cases, the following patients had acute gastritis histological lesions: 11 with desquamated epithelium, 17 with blood exudation, 22 with capillary congestion, 44 with oedema, and 14 with muscle fibers, and in the NSAIDs Hp+ group, a lower number of patients i.e. 10 with blood exudation, 15 with capillary congestion, 16 with oedema, 4 with muscle fibers.

Chronic gastritis changes (fig. 6) were hyperaemia, petechial congestion, papulous gastropathy with lympho-plasmocitary infiltrate (exponent of inflammation), intestinal metaplasia changes and even atrophic and dysplastic changes. In chronic gastritis we may define an active form when polymorphonuclear cells are predominant in the infiltrate and inactive when mononuclear cells are predominant [1, 4, 7]. The classification as acute gastritis for the entire study was made also taking into account the history of acute dyspepsia and endoscopic examination, while the chronic gastritis diagnosis was supported by endoscopic and histological elements.

Based upon figures 5 and 6, acute gastritis histological changes are represented by oedema and capillary congestion, abrased epithelium, blood exudation more frequently found in the NSAIDs Hp- group, while chronic inflammatory changes – polymorphonuclear or lympho-plasmocite infiltrates, papulous changes, intestinal metaplasia were observed especially in the NSAIDs Hp+ group.

CONCLUSIONS

1. After 10 days of treatment the NSAIDs aggressivity on the gastric mucosa is suggested by dyspeptic symptoms and endoscopic changes in 73.75% of cases (75 patients in the NSAIDs Hp+ and 43 patients in the NSAIDs Hp- groups, respectively – total number of patients with endoscopic lesions: 118 of the 160 investigated patients) and also by histological lesions even in those cases where macroscopic endoscopic lesions were not evident (87 patients in the NSAIDs Hp+ and 60 patients in the NSAIDs Hp- groups, respectively; a total of 147 of the 160 investigated patients, representing 91.86%)
2. The symptomatic *Helicobacter pylori* infection of the gastric mucosa was histologically proven in 57.5% of the analysed cases.
3. Considering that most patients with acute (endoscopic) gastritis changes had dyspepsia, the paper somewhat highlights the predictive character of this dyspepsia for the presence and severity of NSAIDs induced gastropathy. [1, 4, 7].
4. Under the reserve of the individual aggressivity of oral anti-inflammatory drugs, our study suggests that *Helicobacter pylori* offers gastric protection against drug aggressivity, idea supported by the lower number of cases with dyspepsia, haemorrhagic erosion, finally with erosive acute gastritis endoscopic and histological changes in the NSAIDs Hp+ group [6].
5. The most exposed gastric area was the antral mucosa where most endoscopic and histological changes were demonstrated [4, 7].
6. Another conclusion revealed by the study is that the NSAIDs Hp- is more disturbing than the NSAIDs Hp+ gastropathy due to the immediate complications (dyspepsia and even erosion induced haemorrhages). After treatment cessasion, though, these manifestations regress.
7. Although, initially, NSAIDs Hp+ gastropathy is less dramatic, sometimes even asymptomatic, it still remains severe by the complexity of endoscopic and histological lesions and by the immediate or delayed evolutive potential [6].

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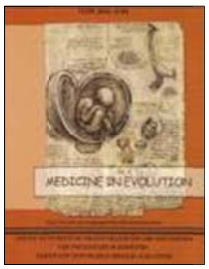
ARE 2D-STRAIN PARAMETERS AFFECTED BY ELECTRICAL DYSSYNCHRONY IN PATIENTS WITH NON-ISCHEMIC HEART FAILURE SCHEDULED FOR CARDIAC RESYNCHRONIZATION THERAPY?



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ABSTRACT

Left ventricular (LV) torsion and strain are essential components of ventricular performance. Cardiac dyssynchrony can reduce LV ejection fraction (LVEF) and decrease cardiac output. We evaluate the influence of electrical dyssynchrony on 2D-strain parameters in patients with non-ischemic heart failure (HF).

***Methods**: Echocardiography was performed into three groups: without evidence of heart disease (group 1, 30 patients), with HF with reduced LVEF without electrical dyssynchrony (group 2, 37 patients) and with electrical dyssynchrony (group 3, 29 patients).*

***Results**: LVEF, LV strain, torsion and twist were significantly better in group 1 compared to group 2 and 3. LV torsion, twist, strain and LVEF were significantly lower in group 3 compared to group 2 (0.8±0.4 versus 1.2±0.2°/cm, p<0.001; 5.2±2.6 vs 8.2±1.4°, p<0.001; -4.9±2.4 vs -6.8±1.6%, p=0.003; and 30 ±4 vs 35±9%, p=0.015, respectively).*

***Conclusions**: In patients with non-ischemic HF the presence of electrical dyssynchrony induces a reduction of longitudinal and rotational deformation.*

***Key words**: Cardiac dyssynchrony, Heart Failure, Speckle Tracking Echocardiography.*

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INTRODUCTION

Heart failure (HF) remains one of the major public health problems in developed countries [1]. Left ventricular (LV) function results from the contraction and relaxation of helically oriented myofibres. The human heart has a specific helical arrangement of the myofibers wit a right-hand orientation from the base toward the apex in the endocardial layers and a left-hand orientation in the epicardial layers [1, 2]. This spiral architecture of the myofibers leads to a LV systolic wringing motion as a result of an opposite rotation of LV apex and base. This characteristic of the LV contributes significantly to LV systolic function, in addition to myocardial shortening and thickening.

LV longitudinal strain, rotation, twist, and torsion are important aspects of the cardiac mechanics. With technical improvements in the temporal and spatial resolutions of two-dimensional (2D) echocardiography, the myocardial deformation and rotation can now be measured using the 2D-strain with the speckle tracking method (STE) [3].

Current clinical guidelines support cardiac resynchronization therapy for symptomatic HF patients wit a depressed LV ejection fraction (LVEF) and electrocardiographic widened QRS duration >120 ms [1]. Although imaging measures of mechanical dyssynchrony have shown promise to improve patient response rate to resynchronization therapy, they have not yet replaced widened QRS duration as a surrogate for mechanical dyssynchrony in current practice guidelines.

AIM AND OBJECTIVES

The purpose of our study was to evaluate the influence of electrical dyssynchrony on 2D-strain parameters in patients with non-ischemic HF scheduled for cardiac resynchronization therapy.

MATERIALS AND METHODS

We analyzed 88 consecutive patients in sinusal rhythm with a history of symptomatic HF and reduced LVEF [1]. Etiology of HF was considered ischemic in the presence of significant coronary artery disease (>50% stenosis in ≥1 major epicardial coronary artery) on coronary angiography and/or a history of myocardial infarction or revascularization. Twenty two patients were excluded for inadequate echocardiogramphic images, ischemic HF, primary valvular heart disease, valvular prosthesis, congenital heart disease, paced rhythm. The 66 remaining patients were included in the analysis and represented the study population. In addition, 30 subjects without evidence of structural heart disease, frequency matched for age, sex, and body surface area, were included as a normal control group, selected from an echocardiographic database.

All study participants underwent clinical examination, 12-lead electrocardiogram, and transthoracic echocardiogram. The QRS duration was determined by automated computerized measurements and confirmed manually. According to current guidelines, patients with electrical dyssynchrony scheduled for cardiac resynchronization therapy presented New York Heart Association (NYHA) functional

class III to IV, sinus rhythm, LVEF ≤35%, and QRS duration ≥120 ms [1].

Patients were divided into three groups: group 1 including the 30 patients without evidence of heart disease, group 2 including 37 patients with HF with reduced LVEF (<50%) without electrical dyssynchrony, and group 3 including 29 patients with HF with reduced LVEF with electrical dyssynchrony. The study was approved by local institutional review boards. Informed written consent was obtained from all patients.

Echocardiography

Conventional echocardiography was performed with an ultrasonographic system (Vivid 7 General Electric, Milwaukee, WI). All images were digitally stored and analyzed off-line with EchoPac PC Dimension software (GE Medical). LV ejection fraction was calculated from apical two- and four-chamber views using LV volumes by the modified biplane Simpson rule in accordance with guidelines [1]. Cardiac rotation was computed using STE. Grayscale digital cine loops triggered to QRS complexes were acquired from two LV short-axis planes, at LV basal level and at apical level, and stored on hard disk for subsequent off-line analysis [3, 4]. The LV twist curve was generated by calculating the difference between apical and basal rotations at each corresponding time point. The peak difference between rotation angles at the apex and base was used in our study. Peak LV torsion was derived from LV twist divided by LV diastolic longitudinal length as previously described previously [3]. STE was also used for myocardial deformation measurements; longitudinal peak systolic strain was determined from apical planes (four-, three- and two-chamber view) in a 17 LV segments model by averaging all 17 LV segments [3, 4].

Statistical analysis

Numeric variables are presented as mean value ± standard variation (SD) and compared using Student's t-tests or analysis of variance, as appropriate. Categorical variables as absolute values and frequency percentages and compared with χ^2 tests.

Receiver operating characteristic (ROC) curves were constructed to determine optimal sensitivity and specificity. All statistical analyses used the software package SPSS version 11.5 (SPSS Inc, Chicago, IL). A p value of < 0.05 was accepted as statistically significant.

RESULTS

The study included 66 consecutive patients with non-ischemic HF with reduced LVEF, in sinus rhythm and 30 patients whithout evidence of heart disease. The characteristics of the study population are presented in Table 1.

In our control group (group 1) the LVEF (61 ± 10 %), LV strain (-15.2 ± 2.3 %), LV torsion (2.3 ± 0.48 °/cm) and LV twist (15.3 ± 3.1 °) were significantly better (each p < 0.001) compared to group 2 and 3 (figure 1). In patients with HF with reduced LVEF and electrical dyssynchrony (group 3), LV torsion, LV twist, LV strain and LVEF were significantly lower compared to group 2 (LV torsion: 0.8 ± 0.4 versus 1.2 ± 0.2 °/cm, p < 0.001; LV twist: 5.2 ± 2.6 vs 8.2 ± 1.4 °, p < 0.001; LV strain: - 4.9 ± 2.4 vs - 6.8 ± 1.6 %, p = 0.003; and LVEF: 30 ± 4 vs 35 ± 9 %, p = 0.015, respectively).

Table 1 *Baseline characteristics of the study population.*

Characteristics	Group 1 (n = 30)	Group 2 (n = 37)	Group 3 (n = 29)
Age, years	49 ± 13	53 ± 15	55 ± 11
Men/women, n	19 / 11	22 / 15	17 / 12
Body surface area, m2	1.7 ± 0.5	1.9 ± 0.4	1.8 ± 0.6
Heart rate, beats/min	71 ± 16	81 ± 13	84 ± 12
New York Heart Association class	-	2.5 ± 0.7	3.43 ± 0.4
QRS duration, ms	83 ± 9	97 ± 20	147 ± 26
LV diastolic volume, ml	78 ± 15	165 ± 28	194 ± 31
LV systolic volume, ml	30 ± 13	107 ± 25	135 ± 28
LV ejection fraction (%)	61 ± 10	35 ± 9	30 ± 4
Severe functional MR, n (%)	-	3 (8%)	10 (34%)
Medication			
Beta-blockers, n (%)	-	31 (83%)	23 (79%)
ACEI/ARB, n (%)	-	33 (89%)	24 (83%)
Diuretics, n (%)	-	36 (97%)	27 (100%)
Digitalis, n (%)	-	16 (43%)	23 (79%)

Legend: data are presented as mean ± SD or absolute values (%);
ACEI/ARB = angiotensin conversion enzime inhibitor / angiotensin receptor blocker;
MR = mitral regurgitation

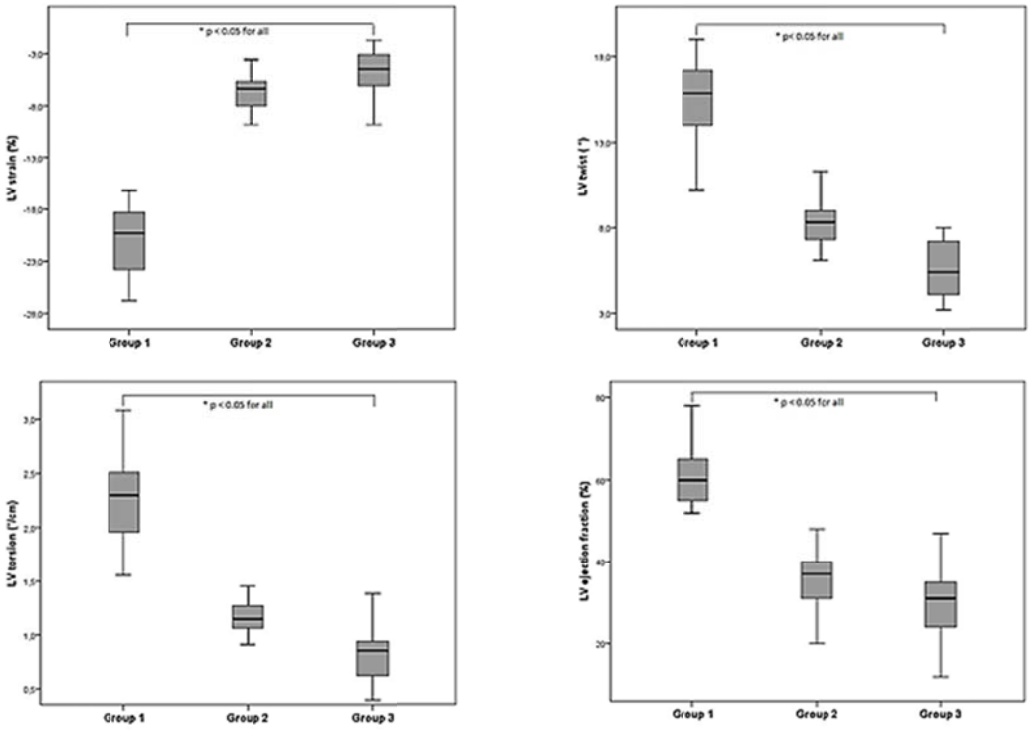

Fig.1 Comparison of left ventricular (LV) global longitudinal strain (a), twist (b), torsion (c) and ejection fraction (d) among groups. See text for a definition of groups. * $p < 0.05$.

The apical rotation of the LV represents the most important component of the LV twist[12]. Comparing our 3 groups, the apical rotation was maximal in group 1 (9.7 ± 3.5 °), followed by group 2 apical rotation (2.5 ± 1.2 °) and by group 3 apical rotation (1.9 ± 1.1 °), each $p < 0.05$.

If we analysed the ability of STE parameters to detect the patients wit indication of cardiac resinchronization therapy LV twist and torsion presented the highest area under the receiver operating characteristic curves (AUC = 0.85 and AUC = 0.82 respectivelly); for the LV global longitudinal strain the AUC was only 0.75. The apical rotation of LV presented a value of 0.76 for the AUC to identify patients wit indication of resincronization therapy acording to the current guidelines (figure 2). A statistical comparison of the ROC curves for LV twist and LV torsion didn't identified a significant difference regarding the accuracy to detect this kind of patients ($p > 0.50$).

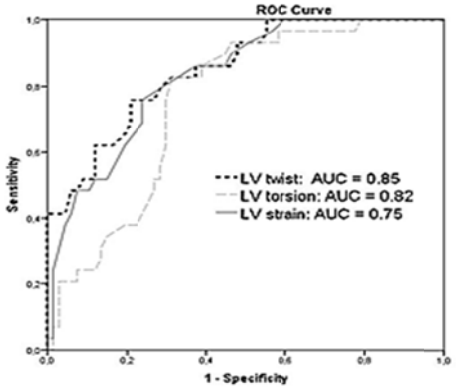

Fig.2 Receiver operating characteristic (ROC) curves for left ventricular (LV) global longitudinal strain, twist and torsion in prediction of electrical dyssinchrony in patients with non-ischemic heart failure. AUC = area under the ROC curve.

DISCUSSION

The most important finding of this study was that the presence of electrical dyssynchrony defined according to the current guidelines in patients' with non-ischemic HF scheduled for cardiac resynchronization therapy induces a significant reduction of LV apical rotation, torsion, twist and global longitudinal strain.

Several techniques have been applied for the assessment and quantification of LV deformation. Tagged cardiac magnetic resonance imaging and sonomicrometry are considered the gold standard, but the most recent STE allows accurate measurement of the magnitude, timing, and dynamics of LV torsion and shows excellent correlation with magnetic resonance imaging data [3]. However, because the STE is not dependent on Doppler angle, it is able to determine the mechanical alterations that cannot be determined by tissue Doppler. Speckle tracking strain analysis is a novel method based on grayscale 2D-images, which permits the assessment of myocardial deformation in 2 dimensions [3, 4]. LV longitudenal strain reflects LV long axis function, controlled predominantly by subendocardial fibres [2]. Altered LV ventricular geometry resulting from cardiac remodeling (fibrosis, dilatation, wall thining, and reduction in fiber angles) is associated with reduction of the systolic torsion and longitudinal strain.

Taber et al explored the impact of this changing transmural myofiber orientation on LV rotational mechanics [5]. The contraction of the epicardial fibers rotate the apical end of the model in the counterclockwise direction and the base in the clockwise direction. Conversely, shortening of the subendocardial fibers rotate the apex and base in clockwise and counterclockwise directions, respectively. When both layers are coupled to contract simultaneously, a larger radius of rotation for the outer epicardial layer resulted in the epicardial fibers having a mechanical advantage in dominating the overall direction of rotation. The endocardial layer does provide some opposition to epicardial motion. Elimination of twist decreases epicardial shortening at the expense of an increase in endocardial shortening and reduces the efficiency of LV systolic performance.

The geometric apical remodeling accompanied by the changes of contractile performance can result to the loss of shearing and less twisting for ejection and suction[21]. Recently, Delgado et al demonstrated that RV apical pacing induced a dyssynchronous mechanical activation of the LV, as measured by radial strain and a subsequent significant decrease in LV global longitudinal shortening and LV twist [6]. Electric conduction defects in HF are associated with a decrease in contractile performance, development or prolongation of MR, and wasted cardiac work as a result of development of mechanical asynchrony. The fibrosis impairs both the conductive tissue and the myocardium [2]. The present study demonstrates that evaluation of LV function in patients wit HF and indication for cardiac resincronization therapy using speckle tracking strain analysis is feasible and the both, longitudinal and transversal deformations types are afected. The ROC analysis showed in our study that LV twist and torsion were more acurate than longitudinal strain to detect patients with indication for cardiac resinchronization therapy.

This result is similar with a previous report by Bertini et al where the rotational mechanics may have additive value in the overall assessment of LV dyssynchrony to achieve higher sensitivities [4].

The ability of LV torsion assessment by integrating apical mechanics likely increases the sensitivity of this technique. Electrical asynchronism contributes to a vicious circle of LV wall stress, asymmetric hypertrophy, and dilatation that progressively deteriorates LV function as previously demonstred [2, 4, 6].

CONCLUSIONS

This preliminary study indicates that in patients with non-ischemic HF scheduled for cardiac resynchronization therapy, the presence of electrical dyssynchrony induces a significant reduction of LV global longitudinal strain, LV torsion, twist and apical rotation. LV torsion and LV twist are more accurate than longitudinal strain to detect patients scheduled for cardiac resynchronization. Alterations in the rotational mechanics can therefore bring new aspects to our understanding of LV dyssynchrony.

Acknoledgement

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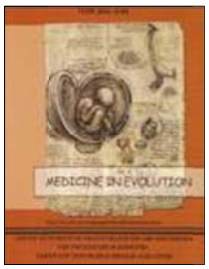
PRIMARY AND METASTATIC MALIGNANT TUMORS ON THE LIVER - REVIEW -



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ABSTRACT

The liver represents the most common localization of primary and metastatic hepatic tumors. This paper aims to review hepatic premalignant lesions and the histopathological classification of malignant tumors of the liver, describing the main growth patterns, citoarchitectural features and immunohistochemical profile of epithelial and mesenchymal hepatic tumors. Clinical aspects, differential diagnosis criteria and developmental-prognostic behavior are discussed.

***Key words**: hepatic tumors, hepatocellular carcinoma, immunohistochemisty, Hep Par-1, a-fetoprotein.*

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PRIMARY EPITHELIAL TUMORS

1. HEPATOCELLULAR CARCINOMA

Hepatocellular carcinoma (HCC), a common neoplasm worldwide with an incidence of one million new cases per year, is more frequently found in Asia and Central Africa, with a decreased frequency in North America and Europe. The most important risk factors are viral infections with B and C viruses, hemochromatosis, cirrhosis, alcoholic liver disease, as well as a variety of drugs and toxins (Anthony P.P., 2002); HCC is rarely associated with metabolic disorders or other liver diseases. The viral DNA of hepatitis B integrated into the host genome is considered to be decisive in initiating liver carcinogenesis. Repeated and persistent infection followed by inflammation and regeneration triggers a cascade of events in the process of carcinogenesis (De Souza A., 1997). HCV infection is also associated with a high incidence of HCC. HCV is an RNA virus with a different replication than that of HBV; it is not integrated into the host genome, the mechanism of hepatocarcinogenesis being probably different.

Clinical features

Clinical manifestations of HCC are varied and nonspecific, including abdominal pain, weight loss, and with progression of the disease, hepatomegaly, jaundice and signs of obstruction of the bile ducts. Abnormal but nonspecific liver tests often reflect a liver disease. Although not entirely specific, high serum values of α-fetoprotein (AFP) indicate the appearance of HCC. Even if imaging techniques can show early lesions, HCCs ≤ 1.5 cm often remain undetected. Large tumors have a poor prognosis, while the fibrolamellar variant of HCC may have a somewhat better prognosis (Stuart K.E., 1996).

HCC can be solitary or multinodular, in the latter case the multicentric origin of the tumor versus intrahepatic metastases being discussed. About 90% of HCCs are developed in a liver with underlying hepatic cirrhosis (especially in areas with a higher incidence), these neoplasms being also found in noncirrhotic liver.

HCC has a predilection for intravascular dissemination, most frequently for the portal vein system. Hepatic veins can also be involved, with obstruction of venous out-flow (Budd-Chiari syndrome). The tumor can extend towards the inferior vena cava, the right atrium, associating even esophageal and gastric varices (Anthony P.P., 2002). Invasion of bile ducts can determin hemobilia or symptoms related with obstruction of large bile ducts. Invasion of adjacent organs, such as stomach and duodenum, can also be possible. Rarely, HCC can go through a spontaneous regression.

Histopathological classification

Although numerous criteria of classification have been proposed, the revised WHO classification (Table 1) is widely accepted, emphasizing the importance of architectural and cytologic features (International Working Party, 1995).

The histopathological classification shows several architectural patterns:

The trabecular or sinusoidal pattern (Fig. 1) is the most common pattern, made up of thickened liver trabeculae, sometimes as thick as 15-20 cells separated by sinusoids that maintain the endothelial lining cells (Ishak K.G., 1994). The endothelial cells cand be identified using IHC markers such as factor VIII, CD31 and CD34 (Fig. 11). Solid areas, necrosis foci and Kupffer cells in reduced numbers can be present.

Acinar or pseudoglandular pattern – with glandlike structures formed by hepatocytes that contain fibrin, bile, and even histiocytes. The bile that can be seen in the cytoplasm of tumor cells is pathognomonic, being observed more frequently in the acinar variant. Many HCCs can have a mixed pattern, with both trabecular and acinar areas (Fig. 2).

The solid, compact, or pelioid pattern (Fig. 3, 4) – made up of hepatocyte trabeculae of several cells thick, often compressed, giving the impression of a solid neoplasm. Sometimes large pseudovascular spaces full with blood, similar to those of peliosis hepatis can be observed. The rupture of these tumors can cause hemoperitoneum.

Histologic variants of HCC (Table 2).

Many well-differentiated HCCs can be formed by hepatocytes with a quite normal aspect and minimal cytologic a-typia: polygonal shaped cells with eo-sinophilic finely granular cytoplasm, with round but atypical, enlarged and hyperchromatic nuclei, irregular nuc-lear contour chromatin. Nuclei of dys-plastic cells often show cytologic atypia (prominent eosinophilic nucleoli) more expressed than in the nuclei of well-differentiated HCC.

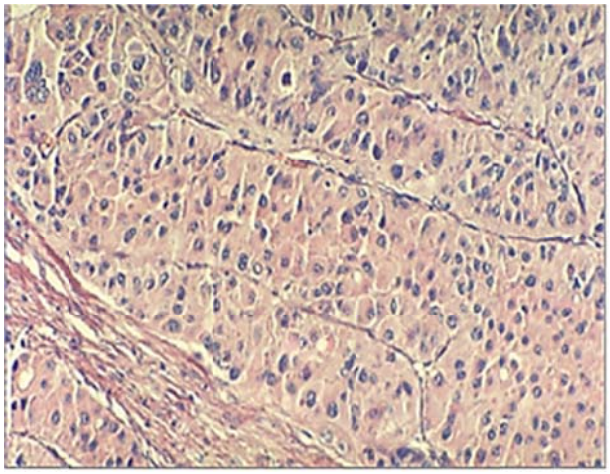

Fig.1 HCC with trabecular pattern. HE

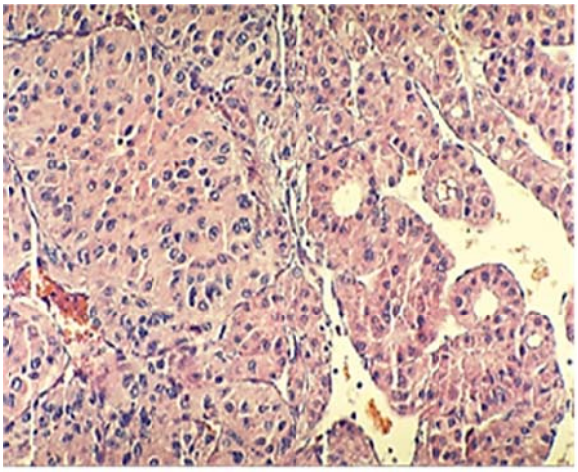

Fig.2 HCC with mixed pattern (trabecular and acinar).HE

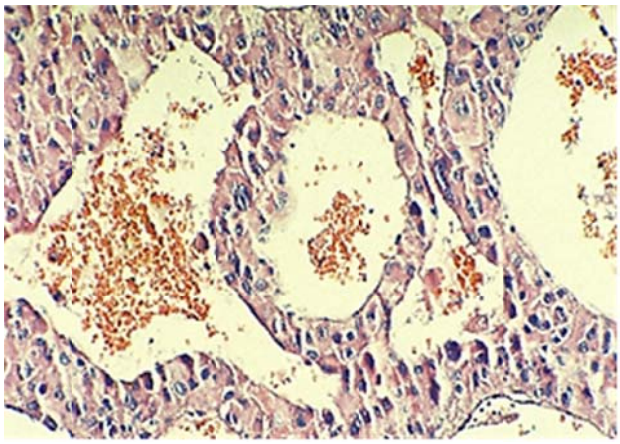

Fig.3 HCC with peliosis-type pseudovascular spaces. HE

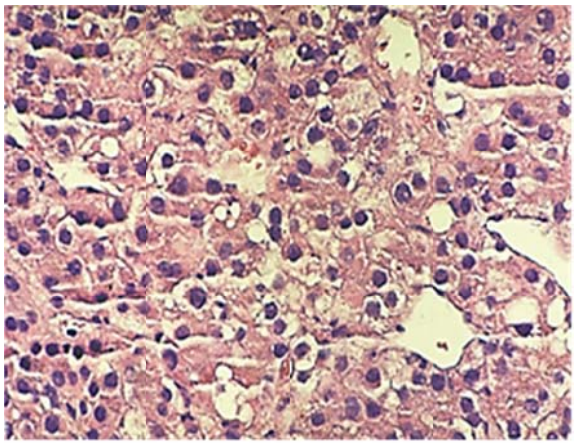

Fig.4 HCC with solid, compact pattern. HE

Tabel 1 *Hepatic primary epithelial tumors (revised WHO classification)*

1 Hepatocellular carcinoma
2 Hepatoblastoma
3 Cholangiocarcinoma
4 Mixed hepatocholangiocarcinoma
5 Hepatobiliary cystadenocarcinoma

Tabel 2 *Histologic variants of hepatocellular carcinoma*

HCC the classic type
Sclerosing HCC
Clear cell HCC
Spindle cell (sarcomatoid) HCC
Pleomorphic HCC (anaplastic, giant cell)
Fibrolamellar HCC

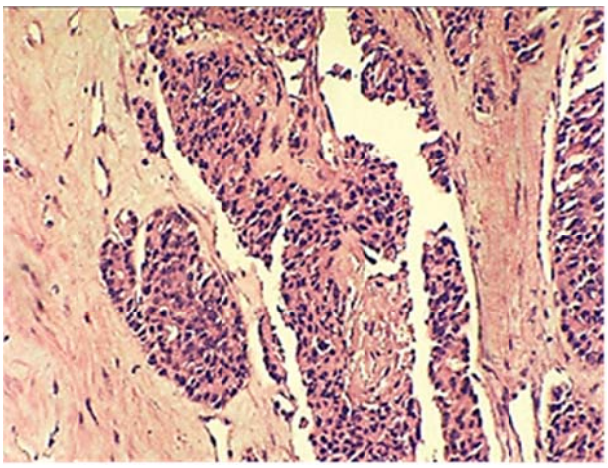

Fig.5 HCC sclerosing type. HE

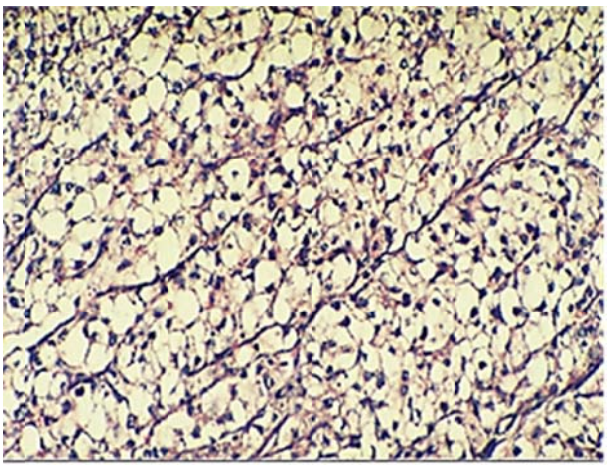

Fig.6 HCC clear cell type. HE

a) Hepatocellular carcinoma the classic type

Can be made up of cells similar to nonneoplastic hepatocytes, arranged in trabeculae of several cells thick, with a slightly basophilic cytoplasm and variable degrees of nuclear pleomorphism. Tumor cells can contain a variety of intracytoplasmic inclusions: Mallory-like material, fibrinogen, albumin, pale bodies, megamitochondria, intranuclear eosinophilic pseudoinclusions (representing intranuclear focal invaginations of the cytoplasm). The presence of mucin (unusual in HCC) should suggest a cholangiocarcinoma or a metastatic adenocarcinoma. In spite of the association between HCC and HBV infection, viral antigens are rarely demonstrated in tumor cells (Subramony C., 1993). The differential diagnosis of classic HCC is made with macroregenerative nodules (MRNs), hepatocellular adenoma (HA), cholangiocarcinoma

(CC) and hepatoblastoma. Distinguishing between HCC and MRNs with liver cell dysplasia can be difficult. In well-differentiated HCC, liver cells can have an aspect similar to benign hepatocytes, while dysnplastic hepatocytes often show marked cytologic atypia. Both lesions can have a similar architectural pattern, but hepatocyte trabeculae is usually thicker in HCC. On hepatic biopsy the differential diagnosis can be extremely difficult, sections colored with silver impregnation that emphasize attenuation or even disappearance of the reticulin network in the majority of HCC being needed.

Similar diagnostic difficulties can also be found in differentiation between HCC and HA. While the majority of HCC develop on underlying cirrhosis, HA appears in the absence of cirrhosis, like a tumor with solid architecture (sometimes with focal acinar patter) and keeping the reticulin network, but without significant cytologic atypia. Differentiation between acinar HCC and CC can also be difficult, especially on hepatic biopsies (Lau S., 2002). The presence of intracytoplasmic mucin pleads for a CC, while intracytoplasmic bile suggests a HCC. Desmoplasia is more evident in CC, but is not always helpful, being also found in sclerosing HCC.

b) Sclerosing hepatocellular carcinoma

The sclerosing variant of HCC shows a trabecular, acinar, or mixed (trabecular-acinar) architectural pattern, with malignant hepatocytes embedded in fibrous, abundant, relatively dense, hypocellular stroma (Fig. 5). The tumor, seen more frequently in elderly patients, is often associated with hypercalcemia (Anthony P.P., 2002).

Differentiation between the sclerosing variant of fibrolamellar HCC, cholangiocarcinoma and metastatic adenocarcinoma can be difficult. Large, polygonal hepatocytes, with eosinophillic cytoplasm and the presence of a large number of pale intracitoplasmic bodies, disposed in a fibrous stroma of lamellar aspect, as well as a higher incidence in younger patients plead for fibrolamellar HCC. Metastatic adenocarcinomas (arising in the pancreas and biliary tree) can also have a significant desmoplastic stroma, but not as abundant as in sclerosing HCC. Immunohistochemical reactions can be useful for a correct diagnosis.

c) Clear cell hepatocellular carcinoma (Fig. 6)

Is made up of tumor cells with clear cytoplasm, abundant in glycogen, alternating with tumor cells resembling nonneoplastic hepatocytes. Often, the tumor can be associated with hypoglycemia and can have a somewhat better prognosis. It is difficult to differentiate this tumor from a hepatic metastasis of clear cell renal carcinoma, but the association of cirrhosis as well as the negative immunoreaction for vimentin (characteristic of renal carcinomas) and the presence of bile in tumor cells favor a primary hepatic tumor (Craig J.R., 1989).

d) Spindle cell (sarcomatoid) hepatocellular carcinoma

The sarcomatoid variant (spindle cell) of HCC is a rare tumor consisting of spindle cells arranged in fascicles or in an organoid pattern, often presenting multinucleated giant cells (Haratake J., 1991). Differentiation from true sarcomas (leiomyosarcomas and fibrosarcomas) can be difficult, positive expression for vimentin suggesting a mesenchymal tumor.

e) Pleomorphic (anaplastic, giant cell) hepatocellular carcinoma (Fig. 7)

Is the rarest variant of HCC, composed of tumor cells with bizarre nuclear features and aspects of multi-

nucleation, arranged in solid sheets (Hood D.L., 1990).

f) Fibrolamellar hepatocellular carcinoma

A tumor with incompletly known pathogenesis and a generally better prognosis, is more frequently found in younger adults (affecting both sexes equally), being developped in the absence of cirrhosis (LeBrun D.P., 1991). It is composed of tumor cells with abundant eosinophilic granular cytoplasm (oncocytic type) arranged in sheets separated by a pale, paucicellular, fibrous stroma of lamellar aspect, offering a characteristic microscopic appearance to the tumor. Tumor cells can contain bile, pale bodies (immunoreactive for fibrinogen), eosinophilic intracytoplasmic globules (composed of C reactive protein, fibrinogen and α1-antitrypsin), copper and copper-binding protein (present in most cases).

Histologic grading of hepatocellular carcinoma has not proven useful in appreciating the prognosis. Edmondson and Steiner proposed four grades of HCC. Most HCCs are grade II and III; well-differentiated HCC (grade I) may be difficult to distinguish from hepatocellular adenomas or macroregenerative nodules with dysplasia and grade IV tumors are hardly distinguished from undifferentiated adenocarcinomas with other primary localization.

Immunohistochemistry – Specific and reliable immunohistochemical markers for HCC are not known. Hepatic α-fetoprotein (AFP) can be demonstrable in 10%-50% of HCCs (Fig. 10). While hepatocytes (benign and malignant) show strong immunoreactivity for low molecular weight keratins (CAM 5.2, CK8) but not for high molecular weight keratins (CK7, CK19), cholangiocarcinoma can be positive for both types of markers (Ma C.K., 1993). α1-antitrypsin (α1-AT), α1-antichemotripsin (α1-ACT), polyclonal carcinoembrionic antigen (that reacts with biliary canaliculae) and Hep Par-1 (Fig. 9) can be useful in establishing the diagnosis of HCC (Ljubimova J.Y., 1997).

HCCs with neuroendocrine differentiation are immunoreactive for neuroendocrine markers such as chromogranin and synaptophysin (Subramony C., 1993); tumor cells can also be positive for estrogen and progesterone receptors, as well as for human chorionic gonadotropin. Proliferation markers, such as proliferating cell nuclear antigen (PCNA) and Ki-67 (MIB-1) (Fig. 12), may be useful in assessing the proliferation rate of tumor cells, but are not useful in establishing the diagnosis (Ojanguren I., 1993). Flow cytometry and morphometric studies led to contradictory results. Image analysis can be useful in differentiating between dysplasia, well-differentiated and poorly differentiated HCCs, electron microscopy being rarely used in routine practice (An C., 1997). Molecular biology studies showed significant differrences in the expression of various oncogenes in nonneoplastic liver and HCC, including hepatocyte growth factor (HGF) and its receptors, c-met and c-myc. HGF RNA is not expressed in normal or cirrhotic liver tissue, but it is present in HA and HCC. C-met was also elevated in HA and HCC, as compared to normal liver, and the expression of HGF and c-met was associated with higher expression of c-myc protooncogene. The role of a stem cell in regeneration and carcinogenesis is still obscure. Computer-assisted imaging system can be useful in differentiating hepatocellular large cell dysplasia from HCC (Ljubimova J.Y., 1997).

2. PREMALIGNANT LESIONS

Liver cell dysplasia was described for the first time 20 years ago in cirrhotic nodules under the form of atypical hepatocytes, being considered a

preneoplstic lesion and defined as large cell and small cell dysplasia. Because the significance and premalignant potential of these lesions remain controversial, it was recommended to replace the term of dysplasia with large cell and small cell change (Wanless I., 1995).

Large cell change is characterrized by large hepatocytes with enlarged hyperchromatic nuclei and prominent nucleoli, with aspects of multinucleation, but with normal nucleus to cytoplasm ratio and often with intranuclear inclusions. Although large cell change was predominantly associated with HCC, it is frequently found in cirrhotic liver (after a chronic infection with HBV or HCV), being considered a premalignant process. As compared to HCC, large cell change is characterized by a normal nucleus to cytoplasm ratio, the absence of mitoses, low proliferation rate and the absence of p53 mutations. A recent study states that the predictive value of large cell change for HCC is <20%; but it can represent a regenerative or degenerative phenomenon, or it reflects a response to prolonged liver cholestasis (Natarajan S., 1997).

Small cell change (more rarely found) is characterized by hepatocytes smaller than the normal ones, with increased nucleus to cytoplasm ratio and nuclear hyperchromasia, high proliferative activity and overexpression of p53 protein. When it appears in small foci it can be associated with HCC, more frequently than large cell change. Diffuse or poorly defined areas of small cell change, without nodular configuration, can represent a regenerative process or they can appear in chronic biliary lesions, probably not being neoplastic (Su Q., 1997). The term of dysplasia is used to describe a population of cells with abnormal histologic features caused by supposed genetic alterations, but without any sure criteria of malignancy (Wanless I., 1995). Because the genetic alterations are not established, the diagnosis and classification of dysplasia is based upon the topography and morphologic features of abnormal cell nests: the nests with dysplastic hepatocytes of <1mm in diameter are named dysplastic foci, and the ones over 1 mm- dysplastic nodules, that are classified on the basis of cytological features in low and high grade dysplastic nodules.

Displastic foci can be seen in chronic hepatitis with HBV and HCV, in α1-antitripsin deficit and tyrosinemia (Ishak K.G., 2001). These foci have distinct but irregular margins and are made up of usually uniform cells that are different from the surrounding hepatocytes by the spectrum of nuclear atypia (variable from minimum to severe), cytoplasmic stain and the content of fats or glycogen. It is considered that ***low grade displastic nodules*** (DN) from cirrhotic liver represent a clone proliferation of hepatocytes, with clinical and pathological features similar to macroregenerative nodules (MRN). Low grade DNs are made up of a more uniform population of hepatocytes, without specific morphologic features. In the absence of clone studies, the terms of MRN and low grade DN are used to describe the nodules without cytological or architectural features of high grade dysplasia.

High grade displastic nodules (DN), also known as borderline nodules, type II MRN, atypical MRNs and atypical adenomatous hyperplasia can appear almost always in a cirrhotic liver. Serum AFP is normal or in the limits found in chronic hepatic disease or cirrhosis. In the case of these lesions considered premalignant processes, surgical excision is recommended (Ferrell L., 1994). Macroscopically, high grade DNs has an aspect similar to MRNs and low grade DNs, but with an irregular, poorly circumscribed outline.

Microscopically, dysplastic changes can be present uniformly in the nodule or as foci. Usually the nodule is recognized through the areas with small cell change with increased nucleus to cytoplasm ratio and nuclear density (estimated number of hepatocyte nuclei/microscopic field), as compared to normal liver (Ferrell L., 1993). Large cell change is rarely a feature of high grade DN, but when it is present, the focus consists of an area with atypical cells and not under the form of dispersed nuclei in the nodule. Other features commonly found in high grade DN include: focal hepatocyte trabeculae 3 cells thick, zonal reduction of the reticulin network, slight dilatation of sinusoids and sometimes areas with acinar (pseudoglandular) architecture, Mallory bodies, steatosis, clear cell changes, basophilic cytoplasm, bile, the presence of portal spaces and the absence of iron deposits (these being common in low grade DN or in MRN). Differentiation between high grade DN and HCC is based on the presence of trabeculae ≥3 cells thick, moderate mitotic activity, nuclear density twice higher than normal, the collapse of reticulin network, numerous arteries and the absence of portal spaces – features that confirm the diagnosis of HCC.

3. HEPATOBLASTOMA

Hepatoblastoma is a primary hepatic tumor that affects especially male children under the age of 3 years old and appears rarely in adults. The tumor was associated with congenital anomalies such as Down syndrome, Beckwith-Wiedemann syndrome, nephronblastoma, the absence of right adrenal gland, fetal hydrops, Meckel diverticulum and umbilical hernia, familial cases being rarely described. Clinic, it presents as an abdominal tumor mass with rapid growth, invading surrounding tissues and organs, being associated with high serum AFP values (Koneru B., 1991). Histopathologically, the tumor comprises irregular tumor nodules (with epithelial and mesenchym al components) separated by thin fibrous strands, resembling a cirrhotic liver.

Epithelial hepatoblastoma is made up of elongated or spindle embryonal cells, arranged in cords or forming rosette-like structures and fetal cells that resemble fetal hepatocytes arranged in two or three-cell-thick cords; the fetal cells contain variable amounts of glycogen and neutral fat; extramedullary hematopoiesis foci can be observed. Some hepatoblastomas (with macrotrabecular growth pattern) are similar to HCC and have a poorer prognosis (Wakely P.E., 1990).

Mixed epithelial - mesenchymal hepatoblastoma has, in addition to the epithelial component, mesenchymal tissue composed of fibroblasts, collagen fibers and osteoid formation, sometimes these tumors being described as teratoid hepatoblastomas.

Anaplastic hepatoblastoma is composed of small, undifferentiated cells with reduced cytoplasm and hyperchromatic nuclei, having a worse prognosis than that of other types (Goldstein R.M., 1993). Combined hepatoblastomas and Yolk sac tumor have also been described (Cross S.S., 1992).

Immunohistochemically, hepatoblastoma shows positive expression for high and low molecular weight keratins, CAM 5.2 and AE 1/3, for AFP, S-100 protein and vimentin (especially hepatoblastomas with embryonal spindle cells). Hepatoblastomas with neuroendocrine differentiation that are positive for chromogranin and neuron-specific enolase (NSE), as well as tumors that secrete human chorionic gonadotropin (demonstrable on tissue sections), or hepatoblastomas that contain melanin have been described (Ruck P., 1993). The prognosis is not favorable, sometimes patients present with dis-

tant metatatses (in lungs, brain, or bone marrow) when diagnosed. After liver transplantation, survival is generally better in hepatoblastoma than in HCC (Koneru B., 1991). Hepatoblastomas with fetal pattern can respond to therapy.

4. CHOLANGIOCARCINOMA

Cholangiocarcinoma (CC) is an adenocarcinoma with origin in bile ducts with intrahepatic (peripheral), hilar (Klatskin tumor), or extrahepatic localization. Usually, it is a solitary tumor that develops in the absence of cirrhosis, in patients with ages between 50-70 years, affecting both sexes equally. Factors associated with CC include primary sclerosing cholangitis, parasite infestation (oriental), congenital biliary cysts (including Caroli disease and choledochal cyst) and exposure to thorium dioxide (Thorotrast) (Ruiz J., 1992).

Peripheral CC – the most common form, it affects small intrahepatic bile ducts; clinic symptoms manifest late, when the tumor has unresectable significant dimensions. The hilar variant of CC associates incipient clinic manifestations, patients presenting jaundice and symptoms of bile duct obstruction.

Extrahepatic CC can have its origin between the hepatic duct and ampulla of Vater. The hilar and extrahepatic variants affect predominantly men in the sixth and seventh decades of life. Histopathologically, CC is a tumor with glandular architecture (and sometimes papillary or solid), with marked desmoplastic stromal reaction. The tumor is composed of columnar or cuboidal cells with mildly basophilic cytoplasm (with or without intracytoplsmic mucin), round or ovoid nuclei and indistinct nucleoli. CCs are tumors with variable degrees of differentiation: the poorly differentiated and usually the papillary variant presents a signet ring component; tumors with focal squamous differentiation were also described (with features of mucoepidermoid or adenosquamous carcinoma). The tumor infiltrates portal spaces and invades periportal sinusoids; it leads to metastases in regional lymph nodes (already present at the time of diagnosis) and distant metastases in lungs and peritoneal surface. CC may be impossible to distinguish from adenocarcinoma originnating from pancreas or the extrahepatic biliary tree, and sometimes from HCC with acinar architecture. Positive IHC reactions for epithelial membrane antigen (EMA), high molecular weight keratins, CEA, blood group antigens, tissue polypeptide antigens and carbohydrate antigen CA 19-9 support the diagnosis of cholangiocarcinoma (Lau S., 2002).

5. MIXED CHOLANGIO-HEPATOCELLULAR CARCINOMA

Mixed cholangiohepatocellular carcinoma is a rare tumor that associates histological features of HCC and CC and elevated serum AFP values. The diagnosis needs immunohistochemical and electronic microscopy investigations.

6. HEPATOID TUMOR

Hepatoid tumors represent a separate group of malignant HCC-like neoplasias that produce AFP. This kind of tumors was described in the ovary, stomach, gallbladder, pancreas, lung, kidney and endometrium (Gardiner G.W., 1992).

7. HEPATOBILIARY MUCINOUS CYSTADENOCARCINOMA

Hepatobiliary mucinous cystadenocarcinoma with mesenchymal stroma (similar to ovarian tumor) is a rare malignant tumor that occurs almost exclusively in women. It develops in the setting of congenital hepatic fibrosis, choledochal cyst or of a pre-existing hepatobiliary cystadenoma. It can also

arise from ectopic rests of duct epithelium or gallbladder. Histopathologically, it is a multilocular cystic tumor with cystic cavities lined with columnar or simple cuboidal epithelium, with or without mucin secretion, separated by a distinctive mesenchymal stroma composed of spindle cells (resembling ovarian stroma or fetal mesenchyme). The tumor expresses hormonal receptors for estrogen and progesterone. It is a less aggressive tumor and the hepatic resection in incipient stages can be curative. The differential diagnosis is made with CC and CC with hepatic cysts (Weihing R.R., 1997).

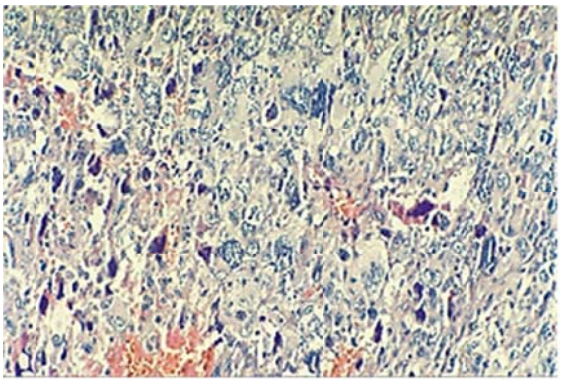

Fig.7 Anaplastic HCC. HE

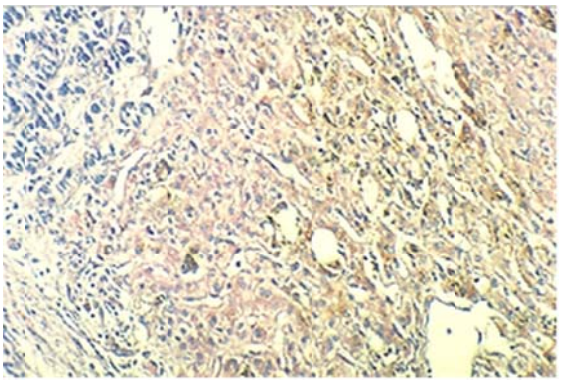

Fig.8 Hepatic metastasis of a lung carcinoma with small cells. HE

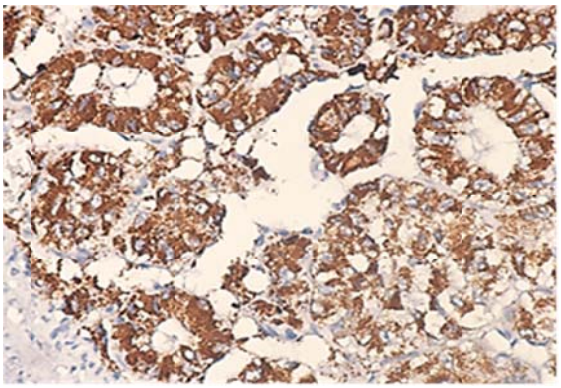

Fig.9 Hep Par-1 in acinar HCC. EnVision

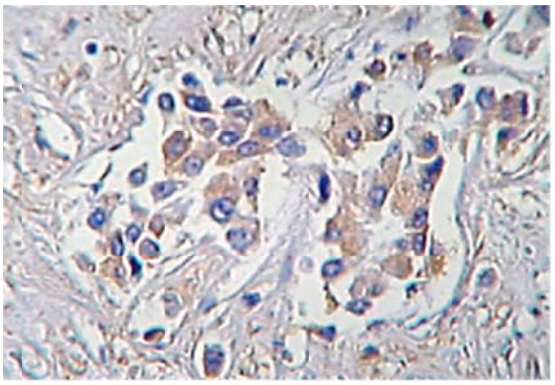

Fig.10 HCC positive for α-fetoprotein. Peroxidase LSAB

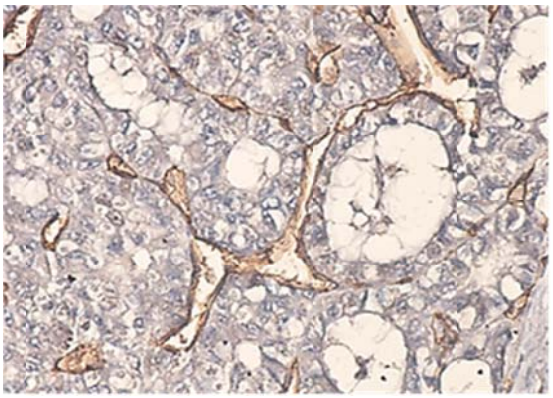

Fig.11 CD34 expression in acinar HCC. Peroxidase LSAB

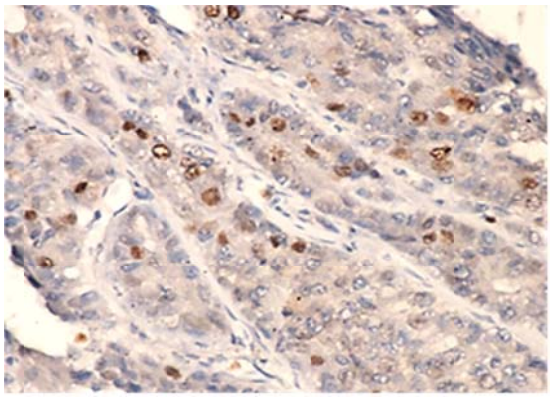

Fig.12 Ki-67 in trabecular HCC. Peroxidase LSAB

NON-EPITHELIAL PRIMARY MALIGNANT TUMORS

1. ANGIOSARCOMA

Angiosarcoma is the most common primary malignant mesenchymal neoplasm of the liver that appears more frequently in males, in parallel with increasing age. Long-term exposure to vinyl chloride, thorium dioxide, arsenic and steroids are known risk factors; associations with alcoholic cirrhosis and hemochromatosis were also described (Rojter S.E., 1995). On hepatic biopsy, the diagnosis can be difficult and differentiation from epithelioid hemangioendothelioma almost impossible. While angiosarcoma has an unfavorable prognosis, hemangioendothelioma can be treated by transplantation. Four distinct growth patterns were described: diffuse micronodular, diffuse multinodular, massive and mixed, as well as several histopathological patterns often combined:

The sinusoidal or cavernous pattern is made up of sinusoid-like or dilated vascular spaces lined by enlarged malignant spindle epithelial cells, with iregular hyperchromatic nuclei and often with bizarre nuclear features. Tumor cells are positive for endothelial markers: factor VIII, Ulex Europeaus, CD 31, and CD34.

Tumors with solid pattern of growth are composed of spindle cells without obvious vascular spaces, the differential diagnosis being problematic; less differentiated tumors show weak immunoreactivity for specific endothelial markers.

The differential diagnosis is made with other sarcomas, especially on biopsy material.

Tabel 3 *Non-epithelial primary malignant tumors of the liver*

Angiosarcoma
Epithelioid hemangioendothelioma
Kaposi sarcoma
Spindle cell (sarcomatoid) HCC
Lymphomas
Histiocytosis X
Other

2. EPITHELIOID HEMANGIOENDOTHELIOMA

Epithelioid hemangioendothelioma (EHE) is a relatively rare malignant vascular tumor, generally with intermediate or low malignancy grade, females being affected twice as often as males, anytime between the second and the seventh decade of life. Usually it is a slow growing tumor, patient's accusing abdominal pain, malaise, weight loss, jaundice and sometimes Budd-Chiari syndrome. The tumor is multinodular, often with confluent nodules, giving the impression of one massive nodule. Histopathologically, two patterns are recognized and described:

A dendritic pattern with stellate and spindle cells included in a dense and myxoid fibrous stroma, with vacuolation of tumor cells (representing lumens of primitive vessels), sometimes presenting a glandular appearance. IHC, tumor cells are positive for factor VIII,

Ulex, CD31, and CD34, ultrastructurelly presenting endothelial differentiation, tight junctions, pinocytotic vesicles and Weibel-Palade bodies in approximately 30% of cases.

The epithelioid pattern is composed of large atypical cells with abundant cytoplasm, arranged in solid areas and surrounded by inflammatory infiltrate with lymphocytes, polymorphonuclear and eosinophilic leukocytes. EHE has a propensity for vascular dissemination, often mimicking venoocclusive disease.

The differential diagnosis includes other vascular tumors (angiosarcoma, Kaposi sarcoma, bacillary angiomatosis), as well as non-neoplastic lesions (venoocclusive disease).

3. KAPOSI SARCOMA

Kaposi sarcoma is a neoplastic proliferation of endothelial cells, most likely of lymphatic origin, appearing as hemorrhagic, well defined hepatic. Patients with hepatic Kaposi sarcoma are almost always seropositive for HIV, being in an advanced stage of AIDS, with multiple lesions in several regions of the body. Histopathologically, it`s made up of spindle tumor cells, often with intracytoplasmic inclusions (probably representing phagocytosed erythrocytes), with a relatively normal aspect of the nuclei, without significant cytologic atypia or an expressed mitotic activity. In addition, vascular spaces without endothelial lining, containing extravasated red blood cells, hemosiderin-laden macrophages and lymphocytes can be observed. IHC stains for endothelial markers (factor VIII, CD31 and CD34) are rarely positive.

The differential diagnosis is made with angiosarcoma.

Bacillary angiomatosis occurs similar to Kaposi sarcoma, in patients with AIDS and is caused by Bartonella quintana/henselae, which can be demonstrated ultrastucturally with Warthin-Starry stain or by polymerase chain reaction (Leboit P.E., 1989).

LYMPHOPROLIFERATIVE LESIONS OF THE LIVER

The liver can be secondarily involved in leukemias and lymphomas (Hodgkin and non-Hodgkin) (Marcelin A.G., 2004). Involvement of the liver in leukemia shows a diffuse pattern with infiltration of the sinusoids with leukemic cells, excepting lymphocytic chronic and lymfoblastic acute leukemia that involve often portal spaces, similar to the infiltration pattern from lymphomas. Leukemia with hairy cells can be associated with formation of lesions such as hepatic peliosis, with dilated sinusoids surrounded by tumor cells. Liver involvement in Hodgkin lymphoma appears like nodular masses in portal spaces. Although the certitude of diagnosis is given by Steinberg-Reed cells, the presence of cell infiltrate composed of lymphocytes, plasma cells, eosinophilic cells and numerous atypic cells is enough to establish the diagnosis of Hodgkin lymphoma, if this was confirmed in another primary localization. Occasionally, epithelioid granulomas can be observed in the parenchyma or portal spaces, but their presence without other features previously described is insufficient for the diagnosis. Rarely, intrahepatic cholestasis can be observed, occasionally associated with biliary duct loss.

Non-Hodgkin lymphoma involves the liver in the context of disseminated disease, with formation of nodular masses in portal spaces. In some lymphomas sinusoid infiltration with tumor cells can be observed, aspects similar with those of leukemia. The liver is involved especially in periferic

lymphomas with T cells (in about 50%) of patients. Also, epithelioid granulomas and intrahepatic cholestasis can be noted, similar to Hodgkin lymphoma (Scheimberg I., 1995).

Primary hepatic lymphomas are rare (0, 4% of extranodal lymphomas), presenting as single or multiple masses, rarely with diffuse infiltration of the liver. The majority are diffuse lymphomas with type B big cells, sometimes can be Burkitt lymphomas and low grade lymphomas with B cells, MALT type. Associations with AIDS, hepatitis B and C, autoimmune disease, primary biliary cirrhosis and antisupressive therapy were described (Page R.D., 2001).

Hepatosplenic lymphoma with T cells with origin in gamma delta citotoxic T lymphocytes involves the liver with sinusoid and portal space infiltration, the spleen and bone marrow being affected at the same time. These tumors are very aggressive, with a mean survival of 1 year (Santos E.S., 2003).

THE LIVER IN HISTIOCYTOSIS X

Histiocytosis X is a term used to define the spectrum of three diseases: Hand-Schüller-Christian, Letterer-Siwe and eosinophilic granuloma of the bone. Rarely, patients show clinical manifestations similar to sclerosing cholangitis, with jaundice and portal hypertension. Hepatic biopsy shows: (1) cellular infiltrate composed of many eosinophilic leukocytes and S-100-positive Langerhans-type cells (containing Birbeck granules), present in portal spaces, with a tendency to intralobular extension and (2) interlobular bile ducts with epithelial changes similar to those of primary sclerosing cholangitis and small bile duct proliferation (Pirovino M., 1988).

OTHER RARE MALIGNANT TUMORS

Other rare malignant hepatic tumors includes:

(1) primary malignant neuroendocrine tumors (gastrinoma and hepatic apudoma) described rarely in the liver and in the biliary tree; (2) ectopic hepatic cell carcinoma from the abdominal cavity; (3) primary hepatic malignant melanoma, also undifferentiated sarcoma (embryonal, mesenchy-mal), choriocarcinoma, endodermal si-nus tumor, adrenal or pancreatic rest tumor, rhabdomyosarcoma, leiomyosarcoma, fibrosarcoma, osteosarcoma, liposarcoma, malignant schwannoma, hemangiopericytoma, malignant fibrous histiocytoma and squamous cell carcinoma (Alonso J.F., 1992).

METASTATIC TUMORS

The liver represents a common, special site for numerous metastatic neoplastic processes and almost any tumor can give metastases in the liver (Fig. 8).

Hepatic biopsies are often performed to determine if a hepatic tumor mass is primary or secondary. Fine-needle aspiration of the liver is generally not useful in the diagnosis of liver diseases, but being necessary in tumor diagnosis. Immunohistochemical studies are also useful (Kondo Y., 1991).

CHANGES ASSOCIATED WITH MASS LESIONS

In hepatic parenchyma adjacent to neoplastic or non-neoplastic lesion (mass) typical changes can be highlighted, such as: bile duct proliferation with periductal inflammatory infiltrate with polimorphonuclear leukocytes, dilated sinusoids and cholestasis.

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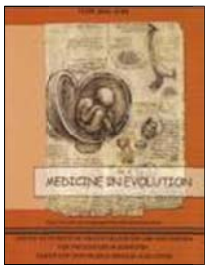
THE ROLE OF SALIVA IN THE UROLITHIASIS - ODONTOLITHIASIS RELATION AS FAVORING FACTOR OF PERIODONTAL PATHOLOGY



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ABSTRACT

The study was performed in a group of 50 urolithiasis cases in the County Clinical Emergency Hospital Timisoara, during the period 2009-2010. A randomly selected group A, including 30 CRF cases in pre-dialysis stage, and a control group B, of 20 cases without CRF, were studied. Patients were subjected to a standard general clinical and biological examination of the urinary apparatus and a dental examination for diagnostic purposes. The role of saliva in the formation of periodontal lesions and the presence of salivary and serum tartar as local favoring factor were comparatively monitored in the group with urolithiasis induced CRF (in pre-dialysis and dialysis stages) and in the group without CRF. Patients with CRF caused by urinary lithiasis were found with significant changes in the ionic composition of saliva with decreased Ca and increased phosphate concentrations and had a more alkaline saliva as compared to patients without CRF and this phenomenon is not entirely due to the urease synthesized by oral bacteria but it is also based upon other causes (e.g. the phosphate buffer system). In conclusion, by extrapolating these results, even though the observed changes do not represent the exclusive cause of oral and periodontal pathology, significantly more frequently in patients with urolithiasis induced CRF, they may contribute as a general favoring factor together with tartar as local favoring factor in the occurrence of this pathology.

***Key words**: urinary lithiasis, urinary lithiasis induced CRF, dental tartar, periodontal lesions.*

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INTRODUCTION

Despite the fact it is mostly composed of saliva, the oral fluid has a complex composition, including the transudate of oral mucosa and ginvival grooves, exsudate from the periodontal pockets, nasopharingeal mucus, transitory liquids, and sometimes even regurgitated gastric secretion. The fact that the exsudate in the periodontal pockets directly increases with the intensity of inflammation must be kept in mind.

Saliva plays an important role in maintaining the normal trophicity of oral tissues. The lubrication of soft structures, keeping the ecologic balance of oral flora, bacterial aggregation, the antibacterial function, keeping the local acid-basic balance are only some of the essential functions of saliva. Because of these multiple functions, as well as due to the local interactive role, saliva may represent a "mirror" of general pathology and a "diagnostic fluid" in detecting viral, immunologic and metabolic diseases (including the chronic renal failure caused by urinary lithiasis). In CRF caused by urinary lithiasis the metabolic acidosis, the changes in the phosphocalcic balance and hyperparathyroidism are constantly present.

The concentration of calcium, phosphate and magnesium in the saliva, the local pH play an important role in the formation of periodontal lesions. Also, the rhythm of dental tartar formation depends on the individual variation of the salivary flow, and mineralization follows the local increase of the calcium and phosphate ion saturation and is achieved by the increase of the salivary pH determining the decrease of the precipitation point of calcium and phosphate and precipitation of proteins – colloidal proteins in the saliva chelate calcium and phosphate ions generating an oversaturated solution which becomes unstable due to the stagnation of saliva, colloids become insoluble and they sediment and mineral salts precipitate. Depending also on the main origin of its components i.e. predominantly salivary and sanguine gingival exudates, respectively, dental tartar may be supragingival or salivary tartar or subgingival or serum tartar. The salivary proteins present in the supragingival tartar are not found in the subgingival tartar.

Many oral germs are able to synthesize urease. Urease decomposes urea into ammonium which may increase the salivary pH, may influence the rhythm of tartar formation and may also generate inflammatory lesions or may exacerbate irritative lesions caused by the presence of tartar.

In cases of urea excess (CRF) this phenomenon is amplified and may be at the origin of erythematous and ulcerative gingival lesions in uremic patients.

MATERIALS AND METHODS

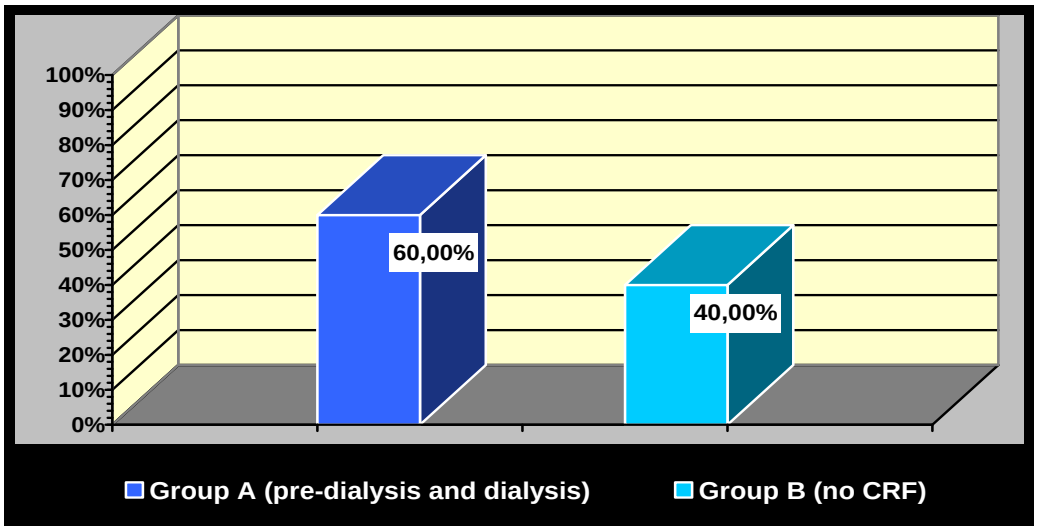
The study was performed in a group of 50 patients with urinary lithiasis selected from the cases of the County Clinical Emergency Hospital Timisoara during the period 2009-2010.

The study included a randomly selected group A of 30 cases with predialysis and dialysis CRF, and a control group B without CRF. Patients were subjected to a standard clinical and biological examination for the urinary apparatus and a dental examination for diagnostic purposes. The role of saliva in the formation of periodontal lesions and the presence of salivary and serum tartar as local favoring factor were

comparatively monitored in the group with urolithiasis-induced CRF (in pre-dialysis and dialysis stages) and in the group without CRF.

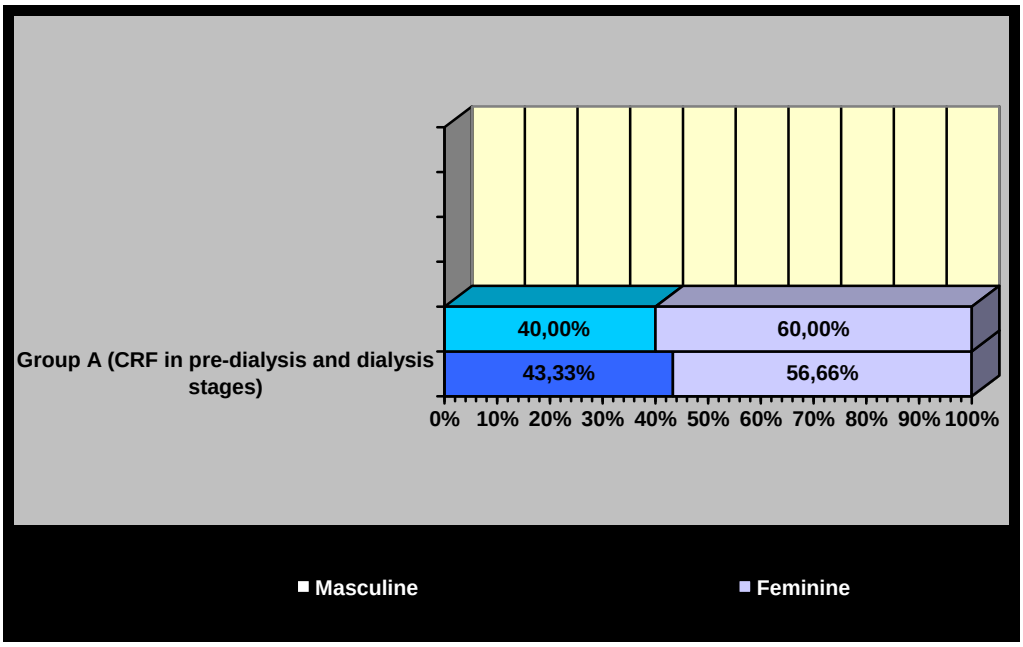
RESULTS

The total group of 50 cases (100%) composed of two study groups:
Group A (CRF in dialysis and pre-dialysis stages): 30 cases (60.00%)
Group B (without CRF): 20 cases (40.00%)



Graph 1: Group allocation of cases

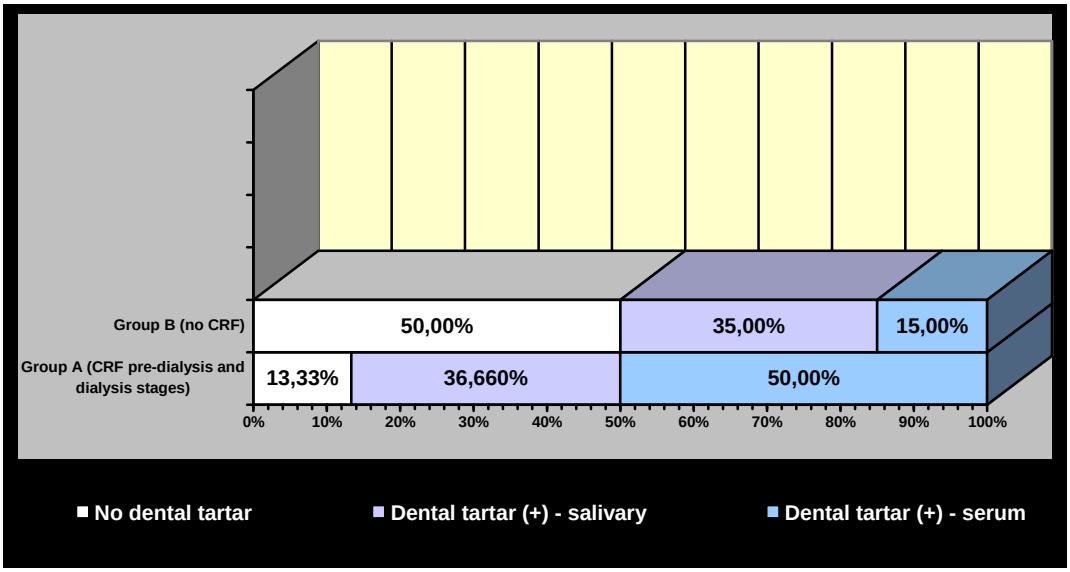
Composition of studied groups:



***Graph 2*:** M/F gender distribution of cases

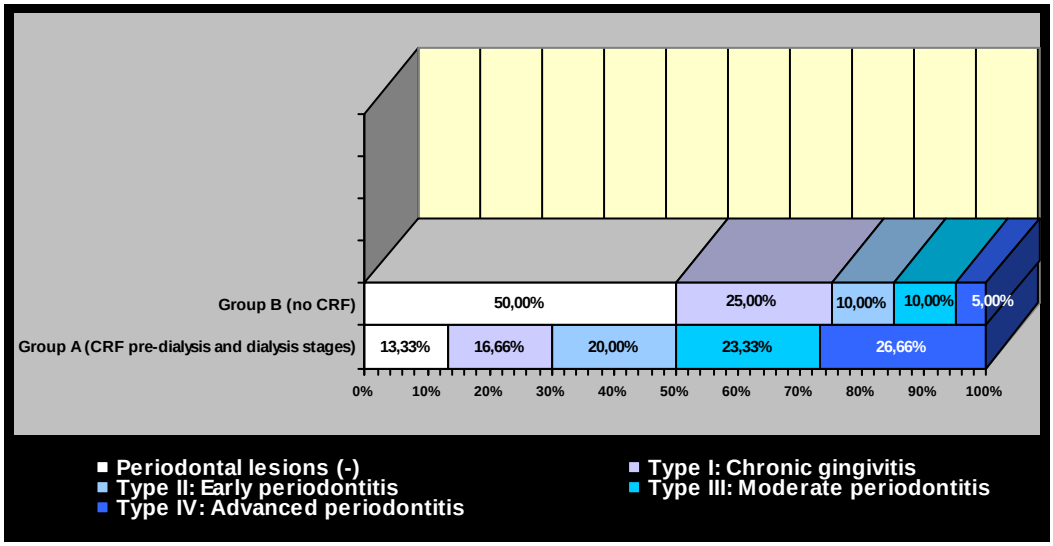
Dental diagnosis:

Depending on the presence or absence of dentally diagnosed tartar: absence of dental tartar (-), total dental tartar present (+) (salivary, serum), the following were observed:



Graph 3: Case distribution according to the dental diagnosis

Depending on the presence or absence and type of dentally diagnosed periodontal lesions, the following were found:



Graph 4: Case distribution according to dental diagnosis

The ionic saliva composition is similar to that of the plasma but relative concentrations significantly differ proving that saliva is not a simple plasma ultrafiltrate. While Na, Ca, Mg, chloride and bicarbonate concentrations are lower than plasma levels, usually phosphate and K levels are higher. At the normal oral pH level, saliva is an oversaturated Ca and phosphate solution where no spontaneous christalization may occur unless some precipitation inductors are present (collagen, glycosaminoglycans, lipids, sialoprote-

ins). The lack of spontaneous precipitation capacity of an oversaturated solution is due to specific, generally organic, inhibitors such as staterine and some proline rich proteins present in the saliva. The significant change of the ionic composition of the saliva may alter this balance thus favoring the precipitation of calcium phosphate which may participate in the formation of periodontal lesions.[3,4,8,9]

In the studied groups we found a statistically significant difference in the total saliva composition with respect to Ca and PO4 in CRF cases induced by urinary lithiasis as compared to those withouit CRF. The significance degree differs and has the highest importance where the phosphate concentration is concerned and even though there are also changes in the Mg composition, the difference is not statistically significant. Lower saliva concentrations of Ca and higher levels of phosphate and magnesium may have multiple causes in CRF patients. In these patients, the basic disturbance in the phosphocalcic balance is due to the decrease of renal phosphate elimination, leading to hyperphosphataemia and hypocalcaemia. Hypocalcaemia induces the increase of parathormone synthesis, with the tendency to correct the calcium through renal calcium and phosphate elimination, through the mobilization of calcium from the bones, and through the increase of intestinal calcium reabsorbtion. In further phases of CRF this will lead to secondary hyperparathyroidism (the augmentation of body intoxication → excess of parathormone → uremic toxin and secondary bone changes. Usually, interventions on this modified balance address the decrease of parathyroid stimulation and normalizing the phosphocalcic balance by reducing the oral intake of proteins to 0.6 g/kg body weight / 24hrs and implicitly of phosphates to 600-700 mg / 24hrs) and the increase of the oral calcium intake. These diet changes may significantly influence the ionic composition of saliva but also the protein saliva content. The consequences of these changes may be multiple: the change of the buffer capacity of saliva phosphates (leading to changes of the saliva pH), changes in the balance between precipitation and precipitation inhibitors (calcium phosphate precipitation), changes of the dental enamel solubility (favoring the occurence of dental plaque, tartar and caries), alterations in the mechanisms of local antibacterial defense. As it may be observed, all these consequences may favor the occurence of periodontal lesions.[10,12,14]

The value of the saliva pH depends on many factors: age and gender (acid pH values are predominant in women and in elderly people), the month when the measurement was performed (saliva pH decreases in April and December), period of the day (saliva pH decreases before meals), sugar or alcohol consumption, pregnancy (pH decreases), deficient oral health and hygiene (pH becomes acid). In the investigated groups, the saliva pH was significantly higher in cases with CRF as compared to the control group where CRF was absent. Saliva pH changes in patients with CRF depend on the value of nitrogen retention and on the presence of urea synthethizing flora in the oral cavity. Urea is the most difusable and non-toxic substance resulting from the proteic catabolism. Increased urea serum levels are accompanied by increased salivary levels of urea. In this context, based upon excessive levels of substrate, bacteria synthethizing urease (many species encountered in the oral cavity decompose urea into ammonium and may generate a significant increase of the saliva pH, and ammonium may induce gingival aseptic irritation lesions favoring the occurence of gingival lesions.[15,16,17,18]

The main saliva buffer systems are represented by bicarbonates, phosphates and some saliva proteins. Normally, the saliva bicarbonate buffer system largely depends on plasma bicarbonate levels. Between meals, the concentration of saliva bicarbonate is similar to plasma levels, low quantities of metabolic CO2 are produced and a large part of the bicarbonate is reabsorbed in saliva channels; the buffer capacity of the saliva is low and according to the Henderson – Hasselbach formula (pH = pK + log[HCO3-/H2CO3]) the pH value is very close to the pK value of approximately 6.1. Upon stimulation of the saliva secretion, the saliva flow increases, the bicarbonate reabsorbtion decreases and the saliva buffer capacity is increased based upon the bicarbonate buffer system. The CRF patient usually has metabolic acidosis in various degrees (depending on the severity of the CRF) and a general reduction of the saliva flow. For these reasons, the buffer capacity of saliva bicarbonates is seriously diminished and the increase of the saliva pH level, as observed in the investigated groups, must be dependent on other factors. A possible factor might be the effect of the buffer system represented by saliva proteins, but this system becomes active only at pH levels under 5 which are practically outside in vivo physiological ranges. Thus, the idea that probably the important role in keeping the saliva pH level is played by a normally secondary system represented by saliva phosphates comes into attention.[10,11,12,14]

The phosphate buffer system is represented by H2PO4(-)/HPO4 (--). This buffer system has a pK level of 6.8 and it is used by the body at total phosphate levels. In this system, the relatively constant concentration of HPO4 (--) is very important as it may influence the ionic Ca (++) x HPO4 (--) product which in its turn determins a critical pH level. This critical pH is defined as the pH value at which the fluid on the tooth surface becomes unsaturated in hydroxiapatite and allows the mobilization of the enamel calcium phosphate. The intense involvement of the phosphate buffer system in maintaining the pH level of the saliva, may induce instability in the phosphocalcic ionic product and this way, together with the effects of the bacterial plaque, it may favor the occurence of tartar and caries in CRF patients.[2,4,5,19,20,21]

Another aspect which should be considered is the content of organic phosphates (phitates) in the ionic composition of the saliva of CRF patients. These phitates appear to react with phosphocalcic salts in the enamel and reduce the solubility of the enamel surface. In CRF, there is an objective need to reduce the oral intake of phosphates and phitates with the purpose of reducing the phosphocalcic changes which are present in these patients. This reduction of the alimentary intake of phosphates is already advisable at low values of nitrogen retention and it may be associated to the necessity of phosphate-chelating agents (aluminium oxydes and hydroxydes, carbonated or acetic calcium salts, etc.). Practically all CRF patients in this study were on various degrees of phosphate restrictive diets at the moment of examination. In this context, it is obvious that a significantly more alkaline saliva has a lower Ca content and a significantly higher phosphate composition, suggesting the intervention of more factors in the achievement of the new balance for the ionic saliva composition. The consequences of these changes are difficult to analyze in a singular manner, but they certainly contribute, in associatioon to other factors, to a significantly wider oral pathology in CRF patients as compared to those without CRF.[10,12,14]

CONCLUSIONS

In conclusion, we may state that patients with CRF caused by urinary lithiasis show significant changes in the ionic composition of saliva with a decrease of Ca and an increase in phosphate levels. CRF patients have a more alkaline total saliva as compared to those without CRF and this phenomenon is not entirely due to urease synthesis by the oral bacteria but it is also based on other causes (e.g. the phosphate buffer system).

Extrapolating these results, it may be stated that, even though the encountered changes do not represent the exclusive cause of the significantly more frequent oral and, implicitly, periodontal pathology in the urinary lithiasis induced CRF patients, they may contribute as a general favoring factor together with tartar as local favoring factor in an important proportion to the occurence of this pathology.

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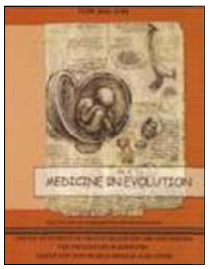
THE ROLE AND IMPORTANCE OF THE SOCIAL AND MEDICAL INQUIRY THROUGH THE QUESTIONNAIRE IN THE SCIENTIFIC APPROACH OF THE EDUCATION FOR HEALTH



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ABSTRACT

The dental health education is very important for the good of our society, and of course very important is to evaluate cares' knowledge of oral health; to provide a high quality, consistent, oral health training programme for careers in residential homes; to evaluate the quality of this programme by examining both cares' changes in knowledge and any changes in cares' behavior as reported by residents and to assess any changes in the oral health of the elderly residents after one year[1]. These are usually done by questionnaire where the people of the community are answer. In one study made in England in 2009 they discovered that Carers' baseline knowledge about oral health was poor; the oral health training programme was enjoyed and their knowledge gain after one week was high. However, the elderly residents perceived no change in the oral care given by carers either after one week or after one year and there are no measurable improvement in the oral health of residents after carer training, except for an increase in filled coronal surfaces. Few of the carers originally trained were still working in the same residential homes after one year[2].

***Key words**: dental health, health education, questionnaire, social, surveyes.*

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INTRODUCTION

The health education is not randomely done. Before starting an action, a campaign for health, a program must be elaborated. The program could be on short term, on an average term or on long term.

The objectives of the program define its intentions, in broad lines. Then, the next step consists in the transformation of the objectives of the program in the objectives of the campaign for promoting health. To achieve the intended objecttives, the people have to take into account to which group of population they are addressing, the level of sani-tary knowledge of that group, by esta-blishing the best strategies.

The mechanic use of some educational - sanitary forms (lectures, lessons, conversations etc.) without taking into account **where? When? To who**?

They are addressed to, represents an unreal and an inefficient way of approaching the sanitary education of the population[1].

MATERIALS AND METHODS

The scientific approach of the education for health cannot be conceived without research. The purpose of the research is to find out the level of sanitary knowledge of the population, before the campaign begins and after it too, being also a modality of controlling the efficiency of the action.

The methods of research can be:

A) *quantitative*: the analysis of the statistic existent data, *surveys by questionnnaires,* fast methods.

B) *Qualitative*: The observation (direct or participatory); the interview (semistructurated, about the background, in group); the conversation (individual, « focus group »). The surveys are useful in the selection of the channels of communication and in the activities of the institutional networks which are preferred by the target groups and with a high popularity.

Also, important is the use of the surveys in applying the educational materials, in a group of individuals, selected from the target groups, before their larger use. It is a cheap means of identifying the possible mistakes from the process of communication, but it cannot guarantee the complete success. Also, based on the results of the inquiry it does, it will coordinate the educational - sanitary activity, elaborating programs, starting campaigns of education for health, evaluating proprietary objectives and showing the corresponding forms of approaching them. The questionnaire is a set of questions addressed to the students or to the adults, verbally or in writing, for them to answer in writing or orally. In one of the educational projects we have used written questionnaires for a homogenous group of adults. Also, people can use written questionnaires for the children, parents of the evaluated children and teachers who collaborated concerning the consolidation of the information about health and the motivation of the pupils to adopt it.

To facilitate the answers and to increase their relevance, reducing the possibility of the apparition of some inadequate answers, we have used the tests based in multiple choices, especially for the little pupils.

These tests consisted in questions to which more variants of possible answers are given, from which the pupil has to choose the one that suits him or her or the one which is the closest to the situation he was. As an

example of questionnaire, we have the one intended for a pilot test for a research in the field of education for oral health.

The questionnaire

The questionnaire needs to have an ID, a date for the interview and a signature. The questionnaire has the following parts:

A. ***Social demography***: the birth date (day, month, year), the gender (male/female), the place where you live (urban or rural), the current address (town/ village, street and number), if you are an employee (yes/ no), the last school you have graduated (primary school, secondary school, highschool, vocational school, college, university, still a student), members of the family (number), family income (less than 1000 lei, between 1000 and 2000, between 2000- 5000 lei, more than 5000 lei, I don't know).

*B. **Nutrition.*** Nutrition has the following subparts [3]:

B1. How many meals or drinks a day do you have?

B2. How often do you eat or drink, even in small quantities the following: fresh fruits, biscuits, cookies, sweets such as chocolate, ice cream, candies, coke, juice, lemonade, jam, honey, sugar chewing gum, dairy products such as milk, yoghurt, cheese, cream, supplements of calcium and Vitamin D.

B3. How often do you have: warm food, food or cold drinks from the fridge or ice, fizzy drinks (besides water). You can answer with: daily, a few times/ week, a few times/ month, never, I don't know.

B4. Is the water you drink: spring water, tap water, flat water, mineral water?

*C. **Lifestyle.*** Lifestyle has the following subparts:

C1: Do you drink coffee or black tea? You can answer with yes. No. If yes then how often? Please answer with: once a day, 2-3 times a day, more than 3 times a day, 2-3 times a week, rarely.

C2: Do you smoke? Yes/ no. if yes, then how much? Please answer with: 5 cigarettes /day, 5-10 cigarettes /day, 10-20 cigarettes /day, more than cigarettes /day, occasionally.

C3: How often do you drink alcohol? Please answer relying on the experience of the latest year: beer, wine, alcohol. Please answer with: 1 glass/day, 5-6 glasses/week, 2-4 glasses/week, 1 glass/week, 1-3 glasses/ month, 1 glass/month, never.

D. The oral dental hygiene. The oral dental hygiene. Have the following parts:

D1: When do you brush your teeth?

Please answer with: never, when somebody tells me to brush, in the morning and in the evening, after each meal, before bedtime.

D2: Do you ever use toothpicks?

Please answer with: after each meal, when I feel I have leftovers in my teeth, never.

D3: Do you use dental floss?

Please answer with: each time I brush my teeth, in the evening when I brush my teeth before bedtime, sometimes, never, I don't know what it is or how it is used.

D4: Do you use mouth water?

Please answer with: each time I brush my teeth, in the evening when I brush my teeth before bedtime, sometimes, never

D5: Do you have extracted teeth?

Please answer with: none, less than 3 teeth, 3-5 teeth, 5-10 teeth, 10-20 teeth, all, I don't know.

D6: Do you have dentures?

If the answer is yes, then whe did you purchased the last one? Please an-

swer with: 1-2 years ago, 3-4 years ago, 5-9 years ago, more than 10 years ago, I don't know.

D7: How often did you meet the following in the last months**?**

1. *Difficulties you came across at the consumption of food due to some dental problems or problems at the mouth level.*
2. *Difficulties you came across at chewing, biting, due to some dental problems or problems at the mouth level.*
3. *Aches at the gums, teeth, or spots on the throat.*
4. *a feeling of tension in the teeth, gums, or because of the braces.*
5. *have you been embarassed due to the look of your teeth, mouth?*
6. *Do you ever avoid a conversation due to your dental look, the smell of your mouth or the look of the braces?*
7. *have you ever not attended a social event because of some problems with the mouth or the teeth?*

E. The Accessibility to dental medical services. The Accessibility to dental medical services has the following subparts:

E1: When did you last visit a dentist for dental problems, dentures?

Please answer with: less than a year, between 1 and 2 years, between 2 and 5 years, more than 5 years, never, I don't know.

E2: How often did you see a dentist in the last 12 months?

E3: What was the main reason for your last visit to the dentist?

Please answer with: consultation, evaluation, scaling, brushing, a normal treatment, an emergency treatment, a spontaneous treatment, I don't know.

E4: What was the main reason for which you did not see a dentist in the last two years?

Please answer with**:** it is too expensive**,** you did not want to spend money on dental care, you are afraid or you do not like to go to the dental office, you do not have dental problems which should require treatment, the dental office is too far away, you do not have teeth anymore or dentures, you have other medical problems which impedit you to go, I do not know.

E5: Would it be possible for you to go to a dentist whenever neccessary, at a distance of less than 30 minutes from your home or workplace?

Please answer with: Yes, no, I don't know

E6: If you need dental care, do you easily have access to a dental office or to a dental clinic?

Please answer with: Yes, no, I don't know

E7: Generally, whenever you need dental care where do you go to?

Please answer with: at a dental office or at a private clinic, at a dental office which has a contract with CJAS, at a clinic or at a university, at the nearest dental office, others, I don't know.

CONCLUSIONS

The various directions of orientation of the education for health emphasize the complexity of these activities and altogether attract the attention to some possible difficulties concerning the obtaining of some efficient results [4].

Although the carer training programme is very well received, no changes in oral health practice resulted. Barriers to practice of oral care by carers remained and training, even when including practical skills, evaluation by peers and a high knowledge gain, failed to reduce these barriers [2].

The approach of any direction implies the respecting of certain beha-

vior and without that, the reaching of the basic objectives of education for health is hard to be accomplished. We must not forget that after all, any action of the education for health, irrespective of the direction of the orientation, follows the reaching of several objetives.

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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

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