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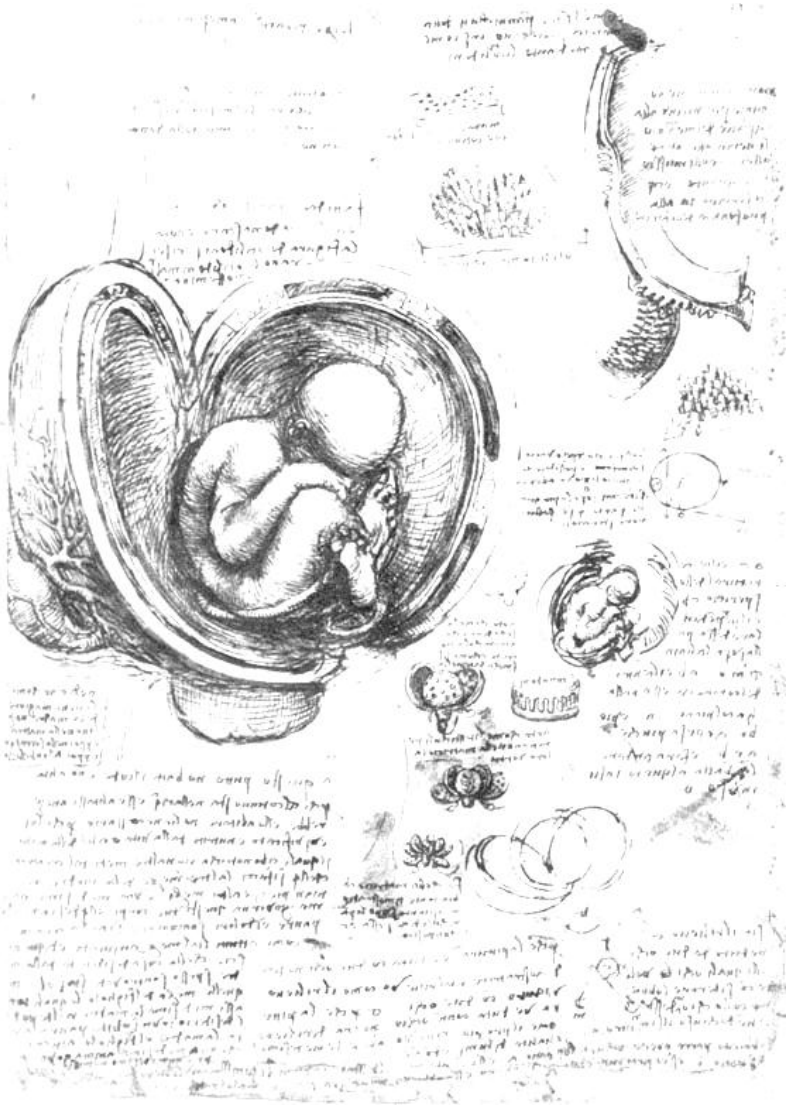

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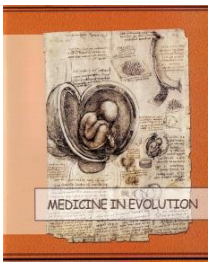
SEXUAL DYSFUNCTION CAUSED BY CYCLING- A REVIEW OF THE MEDICAL LITERATURE IN THE FIELD



CLARA MATEI[1], MIRCEA TAMPA[1], ISABELA SARBU[2], ALEXANDRA LIMBAU[1], ADRIAN DUMITRU[1], TEODOR POTECA[1], SIMONA-ROXANA GEORGESCU[1]

[1]"Carol Davila" University of Medicine and Pharmacy, Bucharest
[2]"Victor Babes" Hospital for Infectious and Tropical Diseases, Bucharest

ABSTRACT

Cycling is a sport whose regular practice presents numerous benefits for the body, especially related to the decrease in the risk for cardiovascular afflictions; at the same time however, cycling is a risk factor for various urogenital disorders, the perineal compression during cycling activities probably being responsible for the occurrence of erectile dysfunction. This can be due to the compression of the nerves but also of the arteries, therefore determining a pronounced decrease in the penile arterial blood flow. Numerous factors influence the risk of sexual dysfunction occurring after cycling: the form of the bicycle saddle, the position of the handlebars comparative to the height of the saddle, the correct posture when cycling, especially for long distances. The present article aims to look over the medical literature in this field.

Key words: *erectile dysfunction, cycling, sexology*

Correspondence to:

Clara Matei
MD, PhD
Address: 37 Dionisie LupuStr, Bucharest
E-mail address: matei_clara@yahoo.com

INTRODUCTION

Cycling is a sport whose regular practice presents numerous benefits for the organism, including weight loss, the decrease in the risk for cardiovascular afflictions etc; moreover, large scale cycling presents benefic effects on the ecosystem through the decrease in pollution and the need for fossil fuel (1). In the same time however, cycling is a risk factor for numerous urogenital disorders, including urethritis, prostatitis, hematuria, testicular torsion, perineal and scrotal paresthesia (1,2). Perianal compression during cycling activities also seems to be responsible, at least temporary, for the occurrence of erectile dysfunction. This can be due to both nerve compression (pudendal and cavernous nerves) and arterial compression, leading to the reduction in the penile arterial blood flow. The present article aims to look over the medical literature in the field.

Among the first reported cases one is the case presented by Desai and Gingell in 1989, of a young 27 year old male with no other health problems, who after a long bicycle ride with no prior practice, accused total loss of erections for three weeks; the sexual dysfunction was resolved over a three month period. (3)

In 1997, Andersen and Bovim applied a questionnaire to the participants at an annual tour of Norway over a distance of 540 km. 22% of the respondents accused perineal innervation (pudendal and cavernous nerves) related manifestations, including paresthesias which, in some cases, exceeded 30 days, as well as erectile dysfunction in 13% of the cases, lasting more than a week in 6% of the respondents (4).

A study published in 2001 followed the change in partial oxygen pressure (pO2) in the glans before, during and after a bicycle ride. A marked reduction (from 60.5 mmHg to 17.9 mmHg) was observed during cycling- temporary reduction with a return to the previous values after effort cessation and 10 minutes of orthostatic position; cycling in a leaning position did not produce any disturbances in the oxygen pressure, thus proving the importance of the influence of posture during cycling on the perineal compression (5).

Since perineal disorder is caused by the compression of vasculo-nervous packs, various types of saddles projected to reduce this discomfort appeared on the market. Taylor et. al analyses the effect of such saddles on the perineal symptomatology on a group of cyclists, observing a decrease from 79% to 14% (6).

Other afflictions can also be caused by cycling. For example, De Rose et. al report two cases of arterial priapism due to arterial-lacunar fistula following cycling (2). Also, cases of testicular torsion secondary to forces exercised between the moving thighs and the saddle have been reported (1). Furthermore, it seems like there are associations between cycling (like in riding) and testicular cancer, partially explained by the exposure of testicles to repeated minor trauma and the contact with the leather of the saddle and textiles impregnated with various chemical agents (1).

Schwarzeret. al analyze the changes in the penile pO2 in athletes after cycling using more types of saddles and notice that the form of the saddle counts more than its firmness; thereby, it seems like a wider saddle is more efficient in reducing the effect of pelvic compression than a soft saddle (7).

Southorn shows that the pudendal nerve, originating in the sacral plexus, passes, along with the homonym artery and vein, through Alcock's Canal, close to the insertion of the ischiotuberal ligaments on the ischial bone, towards the emergence of the canal under the pubic symphysis.

The mechanic compression exercised during cycling is an important factor determining the alteration, at least temporary, of the arterial and nervous functionality. (1).

Jeonget. al showed in a study published in 2002, that the decrease in the penile blood flow is more important when using a narrow saddle as compared to using a wide saddle (8).

Other studies also involve the effect exercised by the form of the saddle on the normal sexual functionality in males; therefore, Schrader et al showed in 2008 a reduction of 66% of pelvic compression on a group of 90 policemen on bicycles who spent at least 24 hours on the bicycle during work, by using modified saddles which assure a more reduced pressure on the pudendalvasculo-nervous pack ("no-nose saddle") (9).

Dettori et al showed in 2004 that numerous factors can be involved in the reduction of the risk of erectile dysfunction in cyclists; among those, the most important seem to be the use of a racing bike as compared to a mountain-bike, the use of wide saddles, without cut-outs and maintaining the handlebars in a lower position as compared to the level of the saddle; the study was performed on a group of 463 cyclists without previous erectile dysfunction, after the end of a competition track of 320 km; 4.2% of those presented erectile dysfunction after ending the race (10).

CONCLUSIONS

Cycling presents numerous benefits on health; in the same time however it can present the risk of secondary erectile dysfunction; this is due to the decrease in the penile blood flow and the compression of the pudendalvasculo-nervous pack. Various factors influence the degree of this compression; of those, it seems like the design of the saddle is more important than the firmness/elasticity of the material it is produced from. There exist, at present, numerous types of saddles whose design is realized such that they try to reduce perineal compression. Medical studies attesting the role of perineal compression resulted after cycling have had and have an important role in the production of these special saddles, intended to reduce the negative impact of cycling on male sexual function.

Acknowledgement

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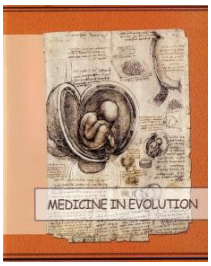

STUDY DESIGN: DETERMINATION OF HEAVY METALS CONCENTRATION IN VEGETABLES GROWN IN HISTORICALLY POLLUTED SOILS AND HEALTH RISK ASSESSMENT OF EXPOSED POPULATION

MIRELA NEDELESCU[1], DAN BĂLĂLĂU[2], DANIELA BACONI[2], CRISTIAN BĂLĂLĂU[3]

[1]Department of Hygiene and Environmental Health,"Carol Davila"University of Medicine and Pharmacy, Bucharest, Romania
[2]Department of Toxicology, Faculty of Pharmacy,"Carol Davila"University of Medicine and Pharmacy, Bucharest, Romania
[3]Department of General Surgery,"Carol Davila"University of Medicine and Pharmacy, Bucharest, Romania;"Sf. Pantelimon"Emergency University Hospital, Bucharest, Romania

ABSTRACT

Heavy metal pollution represents a major public health concern and despite the global efforts to limit the effects of exposure, the problem persists due to high remanence in the environment.

This paper design the steps within a research of estimation the quantity of heavy metals intake by population from vegetables grown in industrial and mining areas which soils are highly polluted with heavy metals. Environmental measurements can be used to predict heavy metal levels of the resident population in order to determine the appropriate actions to be laid out. This approach refines the necessary tools for assessing the public health implications from exposure to heavy metals and reviews the uptake parameters of exposure and estimation of blood levels of heavy metals in exposed populations.

Key words: *dietary intake, heavy metals, risk assessment, soil pollution*

Correspondence to:

Prof. Daniela Baconi
Address:"Carol Davila"University of Medicine and Pharmacy Faculty of Farmacy, Department of Toxicology, Traian Vuia Street, 2nd district, Bucharest
E-mail address: daniela_baconi@yahoo.com

INTRODUCTION

Heavy metals are chemical substances with a specific gravity at least 5 times the specific gravity of water which is 1 at 4°C (Lide, 1992) and the definition includes those metals and semi-metals which are potentially toxic for the human body or the environment. Heavy metals such arsenic, cadmium, lead or mercury are listed in the top 10 chemicals or groups of chemicals of major public health concern (WHO, 2010).

Heavy metals are spread ubiquitously in the environment, both naturally in the nature (in rocks and mineral deposits), but most of the heavy metals releases are the result of human activities such extraction and/or processing of non-ferrous metals, battery manufacturing, refineries, materials from the demolition of houses, old paintings, PVC plastics, X-ray shielding, production of glass and pesticides.

Routes of exposure to heavy metals include all the environmental factors: air, water, soil, vegetation, food, and other consumer products. These compounds get into the body through inhalation and ingestion for the general population, and secondary also by dermal route for the occupational exposed population. For lead exposure through inhalation of airborne lead particles the absorption rates in adults are between 30%–50% depending on factors such as particle size and ventilation rate. In the lower respiratory tract lead is almost completely absorbed. For young children (1–6 years of age) oral intake of heavy metals is a more important route of exposure and can occur from ingestion of contaminated food, soil or water.

In the past years, the levels of heavy metals in the atmosphere of industrial areas have decreased, due to the legislative efforts to limit the health risks associated with exposure of these chemicals. However, should not be overlooked health implications of bioaccumulation of these substances in soil, vegetation and other environmental factors, but also in the human body. Otherwise, the most frequent contaminants in soil in Europe are heavy metals, as reported European Union in 2013 [15].

Soil contamination and the increased metal uptake by food crops, vegetables and fruits grown on contaminated soils are still important health risk issues (Damian et al., 2010). Concentration of heavy metals in soil and vegetables is an indicator of environmental pollution, and most important an indicator of the human exposure to these substances.

Chronic exposure to heavy metals leads to bioaccumulatation of these toxics in the soft tissues of the body and even in bones when the exposure is a long time period. Heavy metals are compounds with a systemic toxicity that affects organs and systems in the body with multiple effects: neurological (encephalopathy, behavioral disorders, peripheral neuropathy), hematological (anemia due to the action of the lead on enzymes involved in hem biosynthesis), renal disorders (chronic renal failure), cardiovascular and digestive diseases, degenerative diseases including Alzheimer's and Parkinson's disease.

For some heavy metals, the toxicity level can be just above the concentrations found in nature. In industrial areas, which are historically polluted, the concentration of these metals in the environment might be in amounts of up to a hundred times more than the maximum permissible level.

STUDY DESIGN OF HUMAN RISK ASSESSMENT OF HEAVY METAL EXPOSURE

There are four steps in human risk assessment: hazard identification, hazard characterization, exposure assessment and risk characterization. These steps are completed by risk management with risk evaluation, monitoring and review, and finally the risk communication through an interactive exchange of information and opinions concerning risks (Kemm et al., 2004).

The standard study design require the following steps: specifying the objectives of the study; mapping the area and establish the points and time from where soil samples and vegetables are collected; set the parameters to be measured; establish the methods of sampling and processing of samples; establish the methods for determining the proposed parameters; perform analysis using element-specific method; statistical processing and interpretation of results; establish / assess the measures for remediation of negative effects; sizing the results / information and transmission to all interested factors.

The starting point of any study of risk assessment are the maximum permitted levels for the risk factors, in this case the concentration of heavy metals in environment, in conjuction with the values found following the analyses performed, at the end analysing the effects of these values.

Table no. 1 includes a set of regulations and documents, mostly developed by regulatory agencies or other bodies, that provide information concerning the assessment of heavy metals in environment and their effects on human health.

Table 1. Regulations and documents concerning heavy metals assessment in environment

International authority	**Regulations / Documents**
European Commission (EU)	Council Directive 86/278/EEC on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture Commission Regulation (EU) No. 836/2011 amending Commission Regulation (EC) No. 333/2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs Commission Regulation (EU) No. 420/2011 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs
US Environmental Protection Agency (US EPA)	Standards, Toxic Substances Control Act, Clean Air Act, Safe Drinking Water Act
European Food Safety Authority (EFSA)	Scientific Opinion of EFSA Panel on Contaminants in the Food Chain (CONTAM)
US Center for Disease Control (CDC)	Research studies, publications
US Agency for Toxic Studies and Disease Registry (ATSDR)	Case studies in environmental medicine
US National Institute for Occupational Health and Safety (NIOSH)	Standard methods for assessing occupational exposure
US Food and Drug Administration (FDA)	Regulations and supervision of food safety

COLLECTION AND PREPARATION OF VEGETABLES AND SOIL SAMPLES

Sampling and analysis for the official control of the levels of heavy metals are established by the international regulations. It is very important that the samples collection, treatment and analysis are carried out systematically and unitary during the entire study. Samples should be representative for the entire area of the study (considered historically polluted with heavy metals), and the density of sampling points should reflect the degree of pollution already determined in previous research, if there are any.

Usually, soil samples will be taken from 2 layers: the surface layer (0–20 cm) and the layer below (20-40 cm) using a bamboo shovel putting them in paper or polyethylene bags. Methods for processing solid or semi-solid samples may be necessary and include mechanical grinding, mixing, rolling, agitating, stirring, chopping, crushing, macerating, mincing, pressing, pulverizing, or any other reasonable means of comminuting the sample. The preparation of soil samples involve manually removal of impurities and air-drying at room temperature. The soil samples should be reduced by grounding to pass through 80 meshes (0.2 mm) (Chang, 2014, Mumba, 2008). For vegetable samples, after removing surface dirt using tap water, the edible parts of vegetables are rinsed with deionised water and dried in an oven at 80 °C to a constant weight (Singh, 2010). The dry vegetable samples will be ground in a mortar with a wooden hammer and passed through a 0,2 mm sieve (80 meshes).

The soil and vegetable samples will be digested in acid medium according to the method chosen for the determination of heavy metal concentration. Furthermore, the samples will be analyzed for the assessment of the proposed parameters.

METHODS FOR DETERMINING HEAVY METALS IN SOIL AND VEGETABLES

The choice of the methodology for assessing the amount of heavy metals in soil and vegetables will depend on the number of elements that we want to monitor, the available apparatus and materials, and the expected levels to be measured in samples. For the determination of some elements, atomic absorption may be acceptable, both flame atomic absorption spectrometry (F-AAS) or graphite furnace atomic absorption spectrometry (GF-AAS). For most elements, it is anticipated that optical emission spectrometry with inductively coupled plasma (ICP-OES) or inductively coupled plasma-atomic emission spectrometry (ICP-AES) will be the method of choice. For some, particularly in difficult matrices and very low levels, inductively coupled plasma mass spectrometry (ICP-MS) may be necessary [19].

HEALTH RISK ASSESSMENT ASSOCIATED WITH HEAVY METALS INTAKE

Oral intake of heavy metals is an important route of exposure and can occur from ingestion of contaminated food, soil or water. Especially for young children (1–6 years of age), soil and dust are important pathways for exposure, ingestion of soil and dust can occur through normal hand-to-mouth activity.

For the characterization of risks due to the heavy metal exposure via vegetables consumption some parameters might be calculated in order to quantify the possible risks to

humans exposed for a large period of time. A number of methods and models have been used to estimate potential risks from exposure to heavy metals.

Tolerable Weekly Intake (TWI) and Tolerable Daily Intake (TDI) are the most common factors used in risk assessment of human exposure and represent the weekly, respectively the daily amount of a chemical that has been assessed safe for humans.

Minimal risk levels (MRL) were developed by ATSDR to estimate the daily human exposure to a dose of a chemical that is likely to be without an appreciable risk of adverse, non-cancerous effects over a specified duration of exposure and are analogous to the reference doses and the reference concentrations developed by US EPA [1]. MRLs are derived from No-observed-adverse-effect-levels (NOAEL) or Lowest-observed-adverse-effects-levels (LOAEL) and are intended to assist in determining the safety of communities near hazardous waste sites. For example, an exposure level below the MRL suggests that there is little likelihood of adverse, non-cancer human health effects occurring, whereas an exposure level exceeding the MRL alerts the health authorities that a more detailed evaluation using site-specific and chemical-specific information is required.

A method for the risk characterization of heavy metal exposure is to calculate the estimated daily intake of heavy metals. The estimated daily intake (EDI) is calculated by the following equation:

$$EDI = \frac{C_{\text{metal}} \cdot D_{\text{food intake}}}{B_{\text{averageweight}}} \quad (1)$$

In *Equation no. 1* C_{metal} is Heavy metal concentration in vegetables, $D_{\text{food intake}}$ is daily intake of vegetable (kg/person) and $B_{\text{average weight}}$ is average body weight.

The average adult body weight is considered to be 70 kg [8], while average daily vegetable intakes for adults in Europe is 0,22 kg/person/day [9].

Another method for exposure assessment is the use of prevalence data for estimating lead blood levels. In this case, the lead blood measurements can be made at one site and extrapolated to other sites with similar environmental and demographic data (ATSDR, 2007).

For the estimation of lead blood level (PbB), the formula below is used:

$$PbB=\delta_S TPb_S +\delta_D TPb_D + \delta_W TPb_W + \delta_{AO} TPb_{AO} + \delta_{AI} TPb_{AI} + \delta_F TPb_F \quad (2)$$

where, Pb_S=soil lead concentration

Pb_D=dust lead concentration

Pb_W=water lead concentration

Pb_{AO}=outside air lead concentration

Pb_{AI}=inside air concentration

Pb_F=food lead concentration

T=relative time spent

δ=the respective slope factor for specific environmental factor

The slope factors are estimated values designated to describe the association between environmental measures of lead in environment and the blood lead levels. For this estimation, the environmental levels for outdoor air, indoor air, food, water, soil, and dust are needed. If these data are missing, default values can be used or an unknown value can be estimated from a known value. For example, EPA has suggested that indoor air can be considered 0.03×the level of outdoor air [6].

Limitations of this method include bioavailability issues and the fact that lead blood level (PbB) is only an indicator of recent exposure (<90 days) and estimation of past exposures can be problematic because of redistribution of lead out of the blood compartment.

The Integrated Exposure Uptake Biokinetic Model (IEUBK) developed by EPA is one of the most extensive

efforts for making population-based predictions of blood lead concentration based on environmental data from the historically polluted region [19]. The model assess lead exposure from air, water, soil, dust, diet, paint and other sources and may be adapted also for other heavy metals. IEUBK incorporates both exposure/uptake parameters and a biokinetic component to estimate the distribution of lead burden in population at risk.

CONCLUSIONS

Heavy metals show long-term contamination and a significant accumulation in soils which led to contamination of food crops. The amount of metals in soil and vegetables is an indicator of environmental pollution and of the human exposure to these compounds. The increased heavy metal intake may lead various disorders and diseases, which reclaim the necessity of monitoring these chemical compounds in the human diet.

The methodology of sampling and determination of heavy metals amount in soil and vegetables is an important key in risk assessment studies and the expected levels to be measured in samples are useful to be known. In interpreting data it is important to look for biologically related patterns and toxicological data available from other research. Outcomes from other studies can help in targeting the potential modes of action. Interpretation of data found will always reported to the reference values established by the public health authorities and other regulatory bodies.

Conflict of interests: None to declare.

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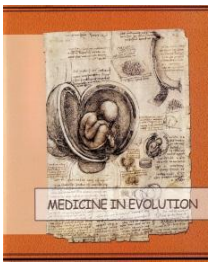
VALUE OF THE PLACENTAL CA AND MG IN PREGNANCIES WITH GESTATIONAL HYPERTENSIONS AND PREECLAMPSIA



MICULITA MARIUS[1], TOTH GHEORGHE ANDREI[2], MARIANA ANGHEL[3], CORINA SAMOILA[3], ANASTASIU DORU[2], ANASTASIU DIANA MARIA[2]

[1]Municipal Emergency Hospital Timisoara - University Clinic of Obstetrics and Gynecology Timisoara, University of Medicine and Pharmaceutics"Victor Babes"Timisoara
[2]Emergency County Hospital Timisoara - University Clinic of Obstetrics and Gynecology"Bega", University of Medicine and Pharmaceutics"Victor Babes"Timisoara
[3]Department IV Biochemistry and Pharmacology – Biochemistry, University of Medicine and Pharmaceutics"Victor Babes"Timisoara

ABSTRACT

Objective

There were followed 29 pregnancies that evolved with gestational hypertension and preeclampsia in witch serum levels of Ca and Mg were in normal range. Placental Ca and Mg was determined from dry placental tissue.

Methods and Material

After births 5g of placental tissue was collected witch underwent a drying process at 105 degree C. The obtained samples were calculated in the muffle furnace at 600 degree C for 6h. The ash obtained was weighted with an analytical balance and it was solubilised in 10mL of 1M HNO3 solution being evaporated to dry. Ca and Mg concentration was determined in the filtrate by flame atomic absorption spectrophotometry.

Results were compared to the normal range values of Ca and Mg concentration from placental tissue of normal pregnancies.

Conclusions

Placental Ca and Mg concentration from pregnancies with gestational hypertension and preeclampsia were lower in 82,75% respectively 86,28 % of the cases compared to the values from the pregnancies with evolved with no complications. There was an insignificant increase of 17,2 % Ca concentration and 13,79% Mg concentration in the cases that evolved with severe preeclampsia

Key words: *magnesemia, calcemia, placenta*

Correspondence to:

Prof. Dr. Anastasiu Doru Mihai
Address: Str. Eugeniu de Savoya nr. 9, ap 4Timisoara
Phone: +40748331318/+40744471327
E-mail address: doru_anastasiu@yahoo.com

INTRODUCTION

The placenta plays an important role in the evolution of any pregnancy, being"a valuable journal of prenatal development of the human being". [10]

Any change of its localization as well as the structural changes may lead to pathological developments of pregnancy with repercussions on the evolution of the newborn.

On the other hand, the presence of chorionic villi in a woman's body is the cause that determines the induction of pregnancy induced arterial hypertension, the eclamptic syndrome that it can generate, being the third leading cause of maternal mortality after hemorrhagic and infectious syndrome.

Starting from the observation of the favorable effect of the treatment with calcium channel inhibitors and the treatment with magnesium sulfate in pregnancy-induced hypertension, we studied the involvement of placentalcalcium and magnesium in homeostasis of elements during pregnancies that have evolved with pregnancy-induced arterial hypertension and preeclampsia.

Pregnancy is considered a carential state in calcium and magnesium, which is why during monitoring of pregnancy, during pregnancy, it is proceeded to a supplementation of calcium at 28 and 34 weeks of gestation in order to achieve an effective prophylaxis of rachitis, as well as a supplementation of magnesium in the first trimester, some authors believe that in this way it is achieved a prophylaxis ofHTAis.

MATERIAL AND METHODS

We studied a group of 29 pregnant women at which the pregnancies have evolved with HTAis or preeclampsia.

Criteria for inclusion in the group were those from the practical guide for diagnosis and treatment in HTAis, systolic blood pressure being greater than 140 mmHg and diastolic blood pressure over 90 mmHg, this occurring in a normotensive pregnant woman until 24 weeks of gestation or outside pregnancy.

The second criterion was that the serum calcium and magnesium values to be established on registration of the pregnant woman and balance examinations at 28 weeks of gestation in order to make their correlation with placental calcium and magnesium.

From every pregnant woman in the group was harvested a placental fragment of at least 5 g which was processed in the laboratory to determine the placental calcium and magnesium. The 5 g of placental tissue samples taken from the women participating in the study were weighed with analytical scale and dried in an oven at 105 ° C for 2 hours. The samples obtained were calcinated in quartz calcination capsules at a temperature of 600 ° C for 6h in a calcination furnace (Snol 8.2, AB Umega, Lithuania). The mass of dry matter obtained from the calcination was determined using an analytical scale, the ash obtained was then dissolved in 10 ml of HNO3 1M solution. The solution was evaporated to dryness. The ash obtained after evaporation was eluted into 100 mL of HNO3 0.5 M solution and filtered through quantitative filter paper and then transferred to a 100 mL volumetric flask made of glass. To determine the concentration of Ca and Mg ions in the filtrate obtained we used the method of atomic absorption spectrophotometry in flame and the apparatus Model ContrAAAnalytik Jena, Germany. To prepare standards

and other reagents we used bidistilled water (spectroscopically pure). Glassware used was treated with 20% (v/v) Pierce solution, washed with tap water, treated with 20% (v/v) HNO3 solution and then again with bidistilled water.The samples were analyzed in triplicate, the average being calculated, and the results were expressed in mg / kg of dry matter. For construction of the standard calibration curves we used standard solutions of calcium and magnesium in concentrations ranging from 2-30 mg / L (Merck). The parameters of the standard curve for the analysis of Ca^{2+}: λ=422, R^2=0.9965, y=0.045+0.0056x, RSD%=0.9-4%, and for the analysis of Mg^{2+}: λ=285, R^2=0.9108, y=1,085+0.058x, RSD%=0.2-2.2%.

The obtained data were statistically processed

Presentation of material:

We have studied 29 pregnant women with HTAis or preeclampsia who gave birth in Clinic of Obstetrics and Gynecology within the Municipal Clinical Hospital Timisoara and County Emergency Clinical Hospital Timisoara during 01.01.2011-31.08.2014.

After the type ofHTAis, 2 (6,89%) were mild forms, 5 (17,24%) were moderate forms, 12 (41,37%) were severe formsand 10 (34,48%) evolved with preeclampsia.

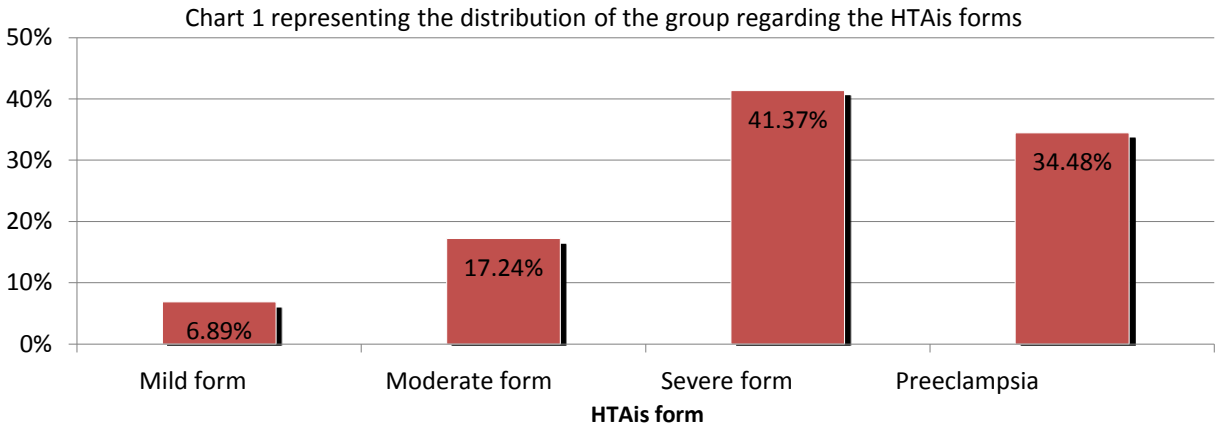


Chart 1. The distribution of the group regarding the HTAis forms

After the onset of the condition, 8 (27.59%) were early forms which started at a gestational age below 28 weeks of gestation, 17 (58.62%) were late forms debuting after 28 weeks of gestation, and 4 (13.79%) were pre-existing hypertension in pregnancy in which the preeclampsia was over-added after pregnancy.

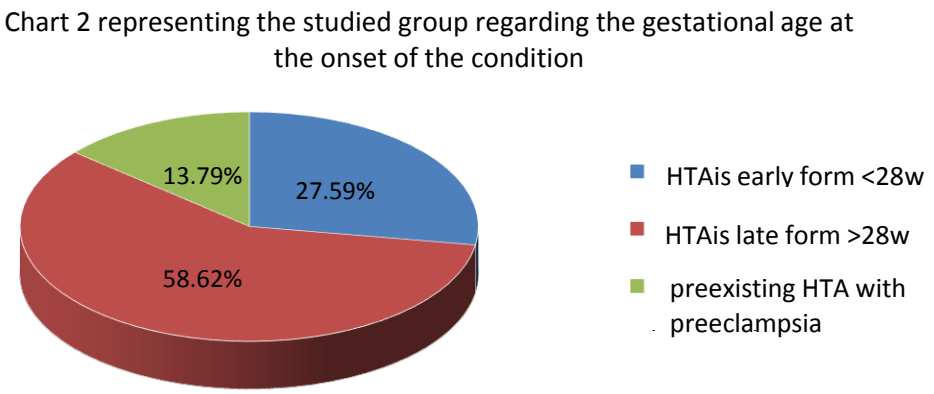


Chart 2. The studied group regarding the gestational age at the onset of the condition

By age, 21 (72.4%) cases were aged between 21-25 years old, favorable fertility age, the entire group having a mean age of 29 years 3 months and 6 days.

By gestation, 13 (44.82%) were at first pregnancy this also corresponding to data found in literature, fitting the profile of women prone to HTAis, 8 (27.58%) were at the second pregnancy, 2 cases each representing 6.89% tetra and multi-pregnancies and 4 were at third pregnancy representing 13.78%.

By parity, the predominantly were primiparous and secundiparous representing the 26 cases, which corresponds to 89.64%, the rest being third and multiparous.

By area of origin 16 (55.17%) came from urban areas, the remaining 13 (44.83%) from rural areas, the difference not being significant.

Chart 3 representing the studied goup by area of origin

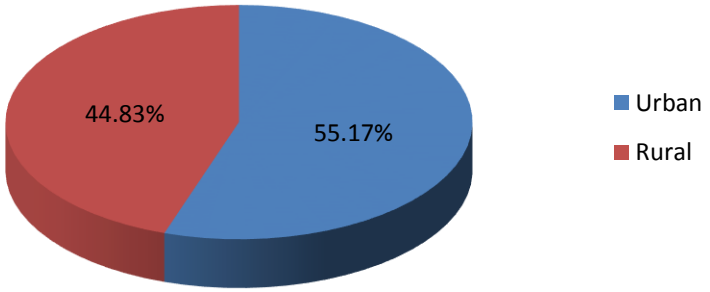


Chart 3. The studied goup by area of origin

Presentation of results:

In the performed analysis we monitored calcium and magnesium values in HNO3 5M solution, the amount of Ca in mg/g wet tissue and the amounts thereof in mg/g of dry calcinated placental tissue.

In our considerations we took into account more and more the latter criterion.

Table I. The studied group by the quantity of mg Ca/g dry placental tissue

<10mg		10.1-15 mg		15.1-20 mg		20.1-30 mg		30.1-40 mg		40.1-50 mg		50.1-70 mg		70.1-100mg		>100mg		Total	
nr	%	nr	%	nr	%	nr	%	nr	%	nr	%	nr	%	nr	%	nr	%	nr	%
1	3.44	3	10.34	3	10.34	3	10.34	4	13.79	5	17.24	5	17.24	2	6.89	3	10.34	29	99.96

The average values of calcium in mg/g dry calcinated placental tissue is 39,182 mg Ca/g dry calcinated tissue placental.

In cases of HTAis, in 11 (57.89%) cases the value of placental calcium is below the mean value of the study group and in 8 (42.10%) of the cases the value is much higher than the average, of about 25-50 % higher.

In cases of preeclampsia, in 80% of cases the level of calcium in dry calcinated placental tissue was higher than the average value of the group, of which in 62.5% of cases the values being above 100 mg calcium/g dry calcinated placental tissue.

Regarding the amount of mg magnesium/gram dry placental tissue, its average value in the study group was 7.26 mg Mg/g dry calcinated placental tissue.

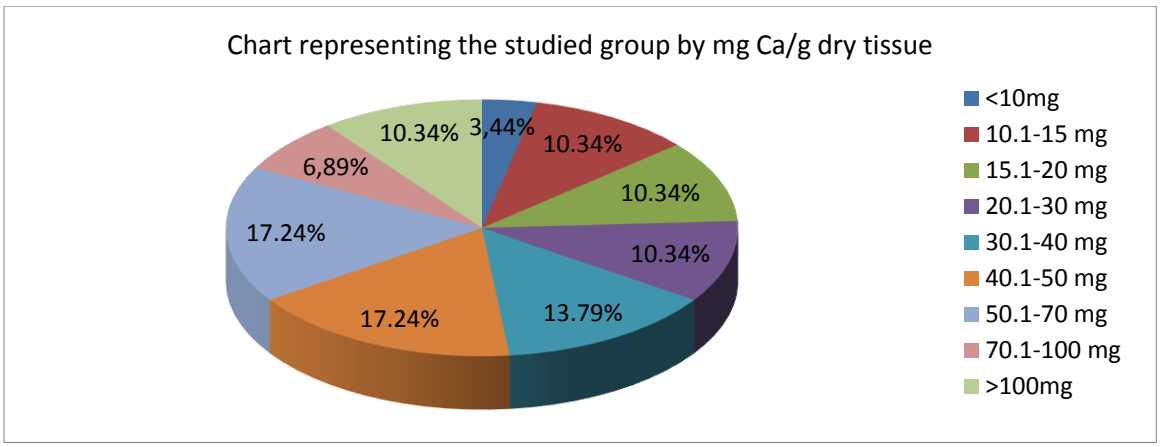


Chart 4. The studied group by mg Ca/g dry tissue

Table II. The studied group by the quantity of mg Mg/gram dry placental tissue

Quantity	No	%
0,5 – 1	1	3,44
1,1 – 4	8	27,58
4,1 – 7,3	9	31,03
7,4 – 14	7	24,13
14,1 – 20	3	10,34
> 20	1	3,44
Total	29	99,96

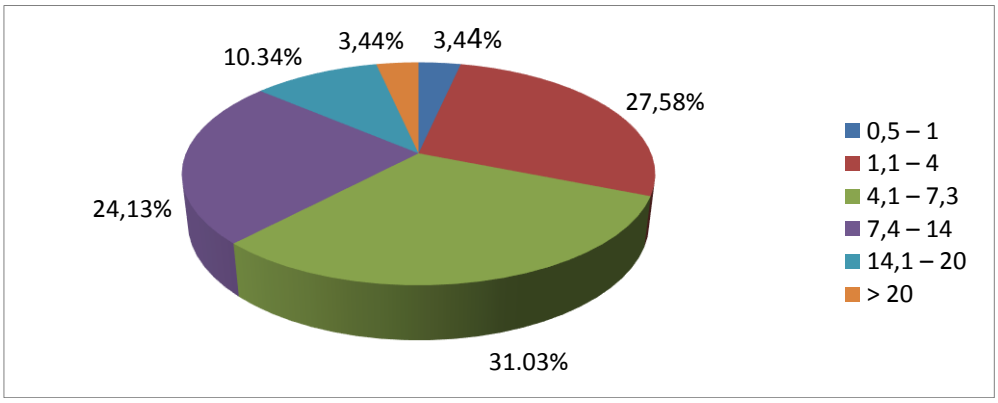


Chart 5.

We mention that in only 27.58%, representing 8 cases, magnesium values were higher than the average study group, in other cases representing 72.41% (21 cases) were below the mean values of the studied group.

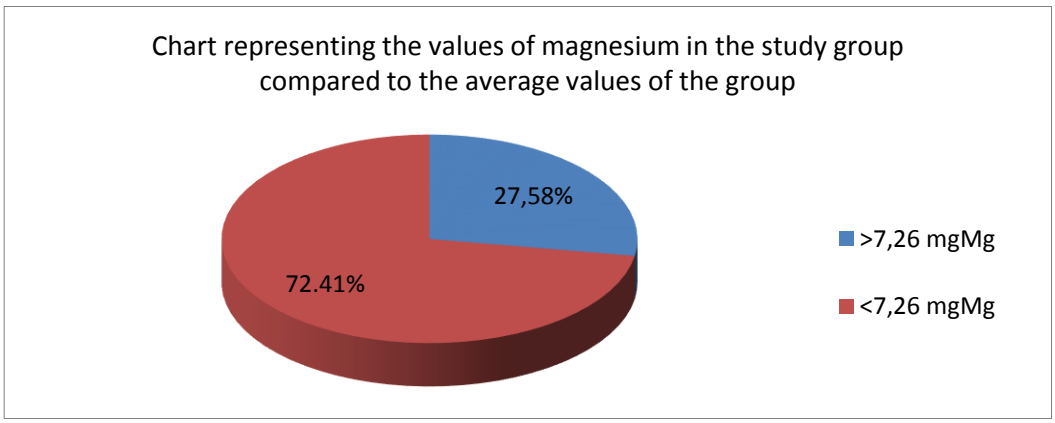


Chart 6. The values of magnesium in the study group compared to the average values of the group

Separating the cases of pregnancy-induced arterial hypertension from those of preeclampsia, we found that out of the 19 cases of HTAis, 17 cases (89.47%) had values lower than the average of the study group and 2 (10.52 %) had higher values.

In cases of preeclampsia, 8 (80%) had higher values than the average group and 2 (20%) had lower values.

DISCUSSIONS

We can appreciate that placental calcium and magnesium values can influence the occurrence of HTAis syndrome. The difference between the values of placental calcium in cases of pregnancy-induced hypertension compared to the value averaged over the study group of 39.18 mg Ca/gram calcinated tissue over 15% could be significant.

In cases of preeclampsia, the difference from the mean is obvious, in 80% of cases of preeclampsia, the placental calcium value being greatly increased, in 62.5% of cases the values being above 100 mg calcium/g dry calcinated placental tissue.

Regarding the placental magnesium, we found that its values were lower in 72.41% of cases. In cases HTAis, in 89.47% we had values lower than the average of the studied group, unlike cases of preeclampsia, in which were found higher values.

Analyzing the results obtained in the study group and given that in these pregnant women serum calcemia and serum magnesemia were normal, we conclude that in cases of HTAis, placental calcium and magnesium values are lower, unlike the cases of preeclampsia, in which their values are above the average of placental calcium and magnesium levels, where it can be concluded that supplementation with calcium and magnesium may contribute to prevention of HTAis.

Comparing the results with mean values of a group of 53 pregnant women who developed physiologically, in which the mean value of placental calcium and magnesium was 68.65 mg Ca/g dry placental tissue, respectively 14.57 mg Mg/g placental tissue dry, we found that in cases of pregnant women who evolved with HTAis and preeclampsia, 24 (82.75%) had placental calcium levels lower than the mean values the from physiological pregnant women, and in 5 cases (17.2%) all with severe preeclampsia, its values were higher.

Regarding the placental magnesium, in 86.20% of cases their values are below the mean value of 14.57 mg Mg / g dry placental tissue, and only 4 (13.79%) had values higher than the average value of placental magnesium in pregnant women that evolved physiologically.

We can conclude that in 80% of cases placental calcium and magnesium values per gram of dry placental tissue are lower than the mean values found in a group of pregnant women that have evolved physiologically.

CONCLUSIONS

1. Placental calcium and magnesium in pregnancies that evolved with pregnancy induced hypertension are involved in homeostasis of elements in the metabolism of pregnant women.
2. There is some variation of calcium and magnesium in placental weight in HTAis and preeclampsia. In HTAis, placental magnesium values being, in 89.47% of the cases, lower than the mean values, and

placental calcium values in 57.89% of cases are also lower.
3. In cases of HTAis severe forms and preeclampsia, placental calcium and magnesium values are increased in proportion of 13-18% of cases.
4. Reported to the mean values observed in a group of pregnant women who have evolved physiologically, there are values lower in 82.75% of cases for placental calcium and in 86.20% for placental magnesium.
5. We can appreciate that the supplementation with calcium and magnesium in pregnant women may be a method of prevention of pregnancy induced arterial hypertension.

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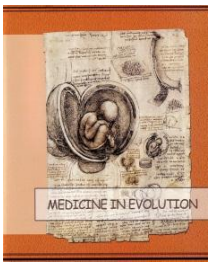

HYPERTENSION, MICROALBUMINURIA AND SUBCLINICAL VASCULAR DAMAGE IN CONTROLLED AND UNCONTROLLED HYPERTENSION

ARDELEANU ELENA[1], LIGHEZAN DANIEL[2], LIGHEZAN RODICA[3], PURCĂRIȚĂ DACIAN[1], DOROBANȚU MARIA[4], DARABONT ROXANA[4], GURGUS DANIELA[1], DELEANU ALEXANDRA[5], NICOLA PATRICIA[1], VIRGIL MIHAI LUCA[1], SHAMUSA BAAJ[6]

[1]Family Medicine Discipline, University of Medicine and Pharmacy „Victor Babeş"Timişoara, România
[2]Medical Semiology Discipline, University of Medicine and Pharmacy „Victor Babeş"Timişoara, România
[3]Parasitology Discipline, University of Medicine and Pharmacy „Victor Babeş"Timişoara, România
[4]Cardiology Department, University of Medicine and Pharmacy „Carol Davila", Bucharest, Romania
[5]Gastroenterology Discipline, University of Medicine and Pharmacy „Victor Babeş"Timişoara, România
[6]Student, University of Medicine and Pharmacy „Victor Babeş"Timişoara, România

ABSTRACT

Objectives. To analyse the association between hypertension, MAU and subclinical carotid vascular damage in controlled and uncontrolled hypertension and to evaluate those correlation with risk factors.

Material and Methods. During 2010-2014 we evaluated clinically and by laboratory a total number of 910 hypertensive patients from 19 family medicine offices of Timiş County. The patients with MAU underwent carotid ultrasound for intima media thickness (IMT) and plaques.

Results.Microalbuminuria (MAU) was present 5.09% of controlled primary hypertension patients and in 9.11% of uncontrolled hypertension (P>0.05). The mean age of the MAU patients was 56±13.1 years and the duration of hypertension was under 5 years in 4 (2.44%) patients, between 5-10 years in 35 (57.3%) and over 10 years in 22 (36%). Six patients with MAU (9.83%) had mild hypertension, 25 (40.9%) moderate and 30 (49.1%) severe hypertension. 68,8% of hypertensive patients with MAUpresentedcarotid vascular damage consisting in mean cIMT of 0.93±0.45 and atherosclerotic plaques, present in28%, versuspatients without MAU (mean IMT 0.82±0.39 mm and plaques in 15 %).

Conclusions. MAU in hypertension was statistical significant associated with high systolic and diastolic blood pressure, mean 24 h blood pressure, obesity and carotid injury.

Key words: *hypertension-microalbuminuria-vascular damage*

Correspondence to:

Prof. Univ. Dr. Elena Ardeleanu
Address: Bul UMF"Victor Babeş"Timişoara, Disciplina Medicina Familiei, Spl. Tudor Vladimirescu nr. 14, Timişoara
Phone: +40729959616
E-mail address: ardeleanu.elena@clicknet.ro

INTRODUCTION

The European Society of Cardiology Hypertension Guidelines 2013 outline that it becomes more and more important to determine the target organ damage secondary to hypertension(1). Studies have demonstrated that the presence of MAU in patients with essential hypertension is related to cardiovascular morbidity and mortality, independent of other well known risk factors. Correlations between cardiovascular and renal pathologies are less clearly defined in the early stages of cardiovascular diseases (1, 2, 3).

THE OBJECTIVES OF THE STUDY were:

- To analyse the association between hypertension, microalbuminuria and subclinical carotid vascular damagein controlled and uncontrolled hypertension.

To evaluate the risk factors associated in hypertension with MAU and subclinical vascular damage

MATERIAL AND METHODS

During 2010-2014we evaluated a total number of 910 patients, of which 360 with uncontrolled hypertension and 550 with controlled hypertension for MAU and carotid vascular damage, in collaboration with 19 family medicine offices of Timiş County.

The general practitioners took a standardized history, performed a physical examination, measured height, weight, casual blood pressure and heart rate for all the study patients. Body mass index (BMI) was calculated as BMI = weight/height^2. A spot urine sample was tested for microalbuminuria (by urinary albumin creatinine ratio) at the family doctor office with Arkray test strips (Japan), which can provide a reading of"negative", a ≥10 mg/L, ≥30 mg/L, ≥80 mg/L and a ≥150 mg/L urinary albumin. Creatinine readings were for concentrations of 10 mg/dl, 50 mg/dl, 100 mg/dl, 200 mg/dl and 300 mg/dl. For the purposes of the present study, microalbuminuria was defined as any reading except"negative". Carotid arteries were imaged with an ultrasound system Sonoscape SSI 8000 with high-resolution B-mode system and linear ultrasound transducer of 7.5 MHz, respecting the Mannheim Consensus. We examined a minimum of 10 mm length of both common carotid arteries (CCA), 5 mm below the carotid bulb. The system was equipped with software that automatically identified the borders of the CCA and calculated carotid intima media thickness (cIMT). Plaques were defined as >50% focal wall thickening from the surrounding vessel wall, distinct from the adjacent boundary (4).

Only patients in whom all measurements were complete for height, weight, plasma creatinine, plasma glucose, 24 h blood pressure monitoring and carotid ultrasound were included in the study.

STATISTICAL ANALYSIS

Data were presented as frequencies and percentages for qualitative variables and as mean ± SD for quantitative variables. The independent variables with a $p < 0.05$, were considered as having statistical significance. All the statistical analyses were performed using the software Stata 9.2. Differences between groups of variables were assessed with the Pearson x^2 for qualitative variables and the Student t test for quantitative data.

RESULTS

During the period of 2010-2014 we evaluated a total number of 910 patients from 19 family offices of Timiş County, of which 550 with controlled hypertension and 360 with uncontrolled hypertension. Microalbuminuria was present in 106 cases (11.02%), of which 29 cases (8.05%) with controlled and 77 (14%) with uncontrolled hypertension. After exclusion of patients with a history of renal disease and with diabetes mellitus, microalbuminuria remained present in 61 cases (7.1%), of which 22 (4.3%) with controlled and 39 (9.11%) with uncontrolled hypertension (figure 1).

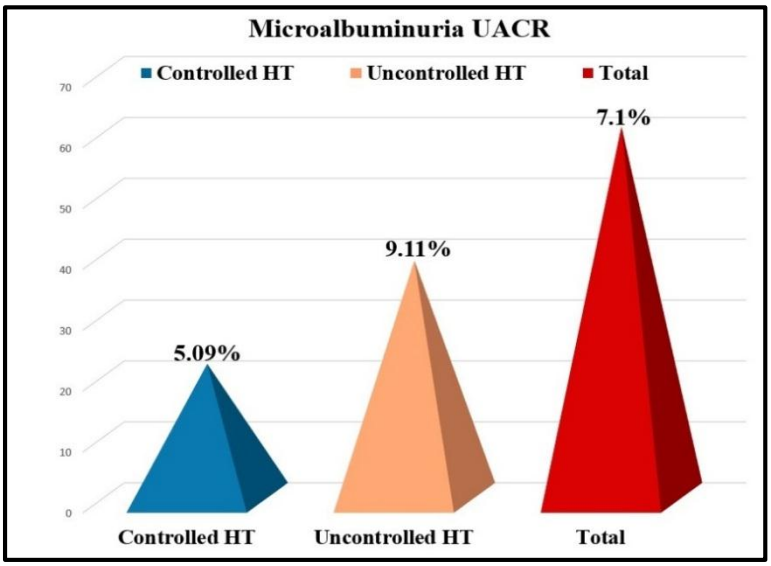

Figure 1. Microalbuminuria determined by UACR in controlled and uncontrolled hypertension after exclusion of diabetes and renal diseases

The mean age of the patients was 56 ± 13.1 years, ranging from 29 to 79 years, the majority having between 50 and 60 years. There were 3 cases (4.9%) with MAU under 40 years, 6 cases (9.8%) between 40-50 years, 31 cases (50.8%) between 50-60 years and 21 cases (34.4%) over the age of 60 years (figure 2).

The evolution in time of hypertension showed that the duration of hypertension was under 5 years in 4 (2.44%) patients, between 5-10 years in 35 patients (57.3%) and more than 10 years in 22 (36%), (figure 3).

The severity degree of hypertension (figure 4) and risk factors were evaluated after the recommendations of the 2013 European Society of Cardiology Hypertension Guidelines (1, 5).

Mean office systolic and diastolic blood pressure, mean 24 hours systolic and diastolic blood pressure, mean daytime and night-time systolic and diastolic blood pressure values of hypertensive patients with MAU are presented in figure 5.

Concerning the cardiovascular risk of the patients that associated microalbuminuria, 47 cases (77.8%) had a high and very high risk, 12 cases (19.6%) a moderate risk and 2 cases (3.2%) a low CV risk (figure 6).

The main cardiovascular risk factors (7, 8) among hypertensive subjects with MAU (figure 7)were: physical inactivity in 37 cases (60.6%), smoking in 13 cases (21.3%), family history of premature cardiovascular disease in 15 cases (24.5%), obesity in 25 cases (40.98%), (19.6%), lipid disorders in 31 cases (50.8%) and metabolic syndrome in 32 (52.4%).

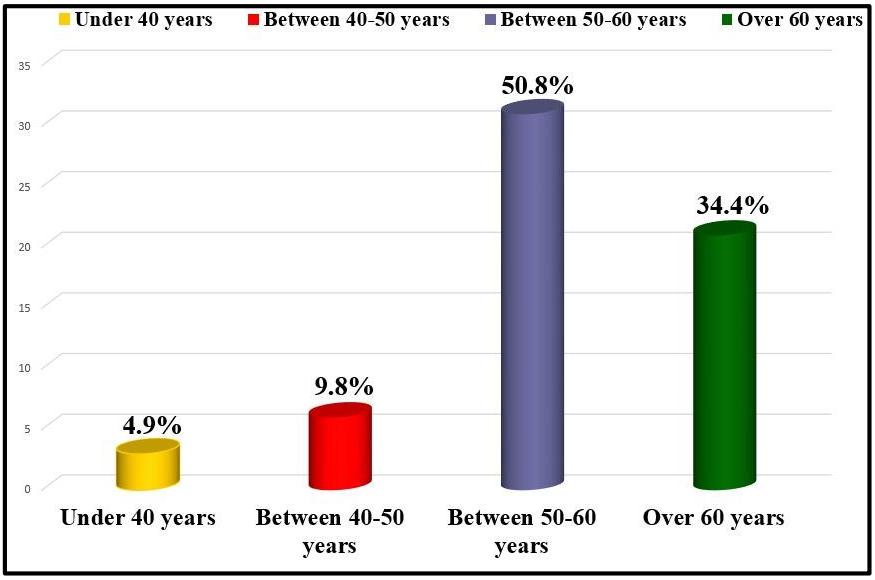

Figure 2. Age groups of patients with microalbuminuria

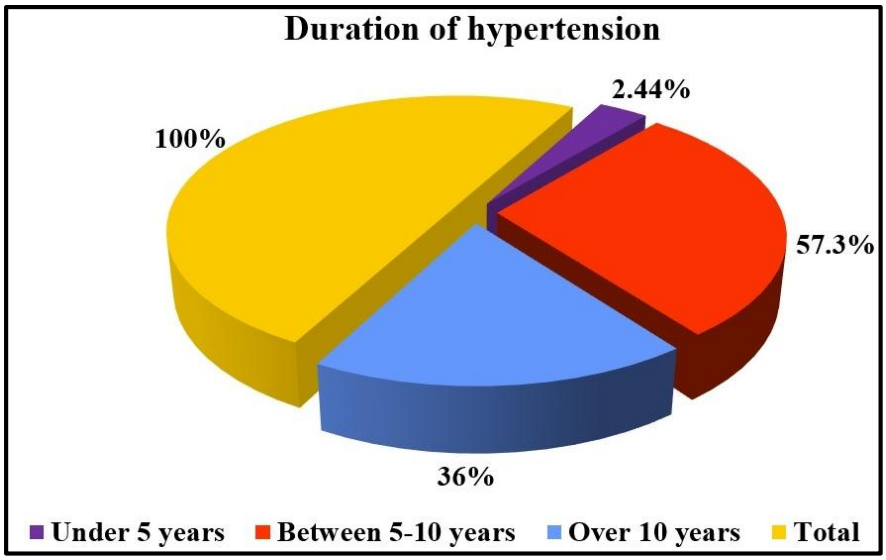

Figure 3. Duration of hypertension

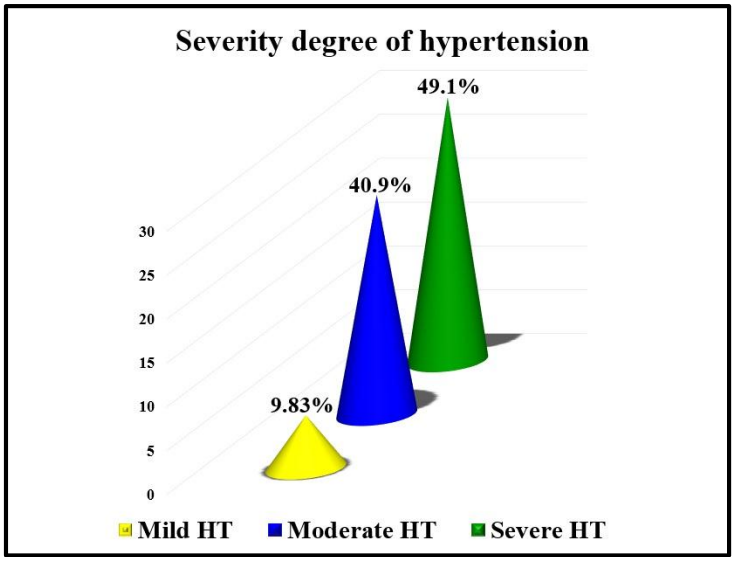

Figure 4. Severity degree of hypertension

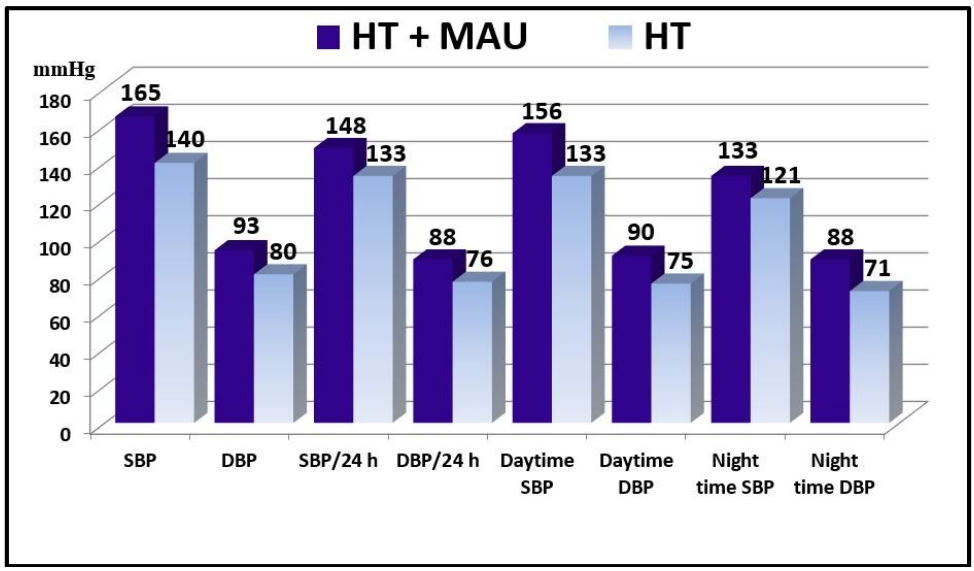

Figure 5. Blood pressure values of the MAU study group

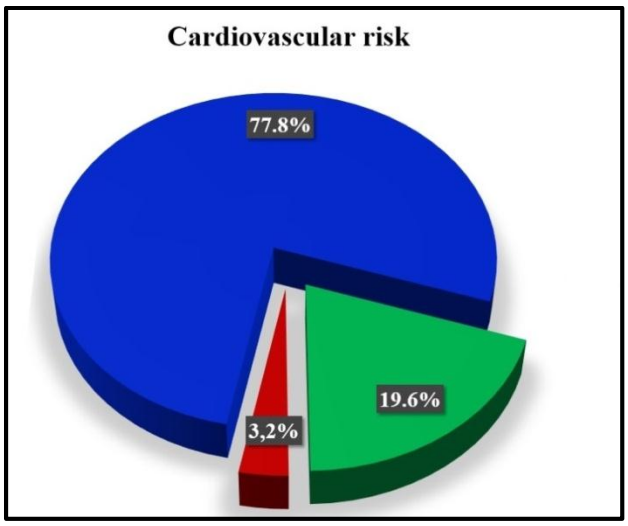

Figure 6. Cardiovascular risk of hypertension associating microalbuminuria

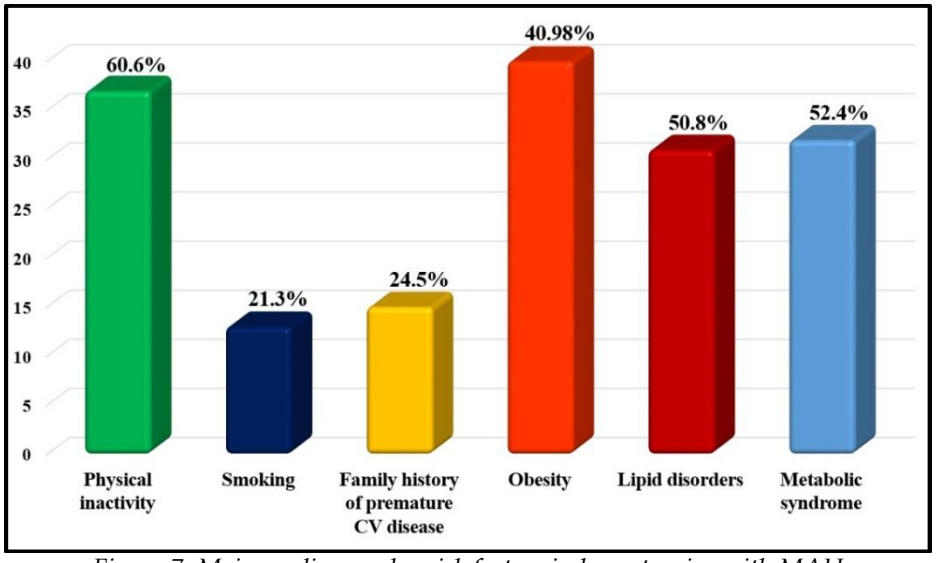

Figure 7. Main cardiovascular risk factors in hypertensive with MAU

The renal damage (figure 8) in controlled and uncontrolled hypertension were:(1) mild renal impairment with eGFR 60-30ml/min/m² in 72 cases (20%) of controlled and 154 (28%) of

uncontrolled hypertensive patients; (2) macroscopic proteinuria, present in 8 cases (2.22%) of uncontrolled and 6 (1.09%) controlled hypertensive patients; (3) total renal damage with eGFR <30 ml/min/m² and/or UACR ≥300 mg/g, present in 35 cases (9.7%) of uncontrolled and 22 patients (4%) of controlled hypertension.

Of the 61 patients with MAU, 42 (68.8%) presented carotid vascular damage consisting in mean cIMT of 0.93±0.45 and atherosclerotic plaques in 28%. Carotid injury was statistical greater in MAU patients in comparison with hypertensive patients without MAU (mean cIMT 0.82±0.39 and 15 % plaques, $p < 0.05$).

The comparison of the hypertensive group with microalbuminuria and subclinical carotid damage with a hypertensive control group of 96 patientswithout MAU and subclinical carotid damage demonstrated greater mean IMT, age, weight, BMI, total cholesterol, LDLcholesterol, systolic and diastolic blood pressure in the first study group, as presented in figures 10, 11 and 12.

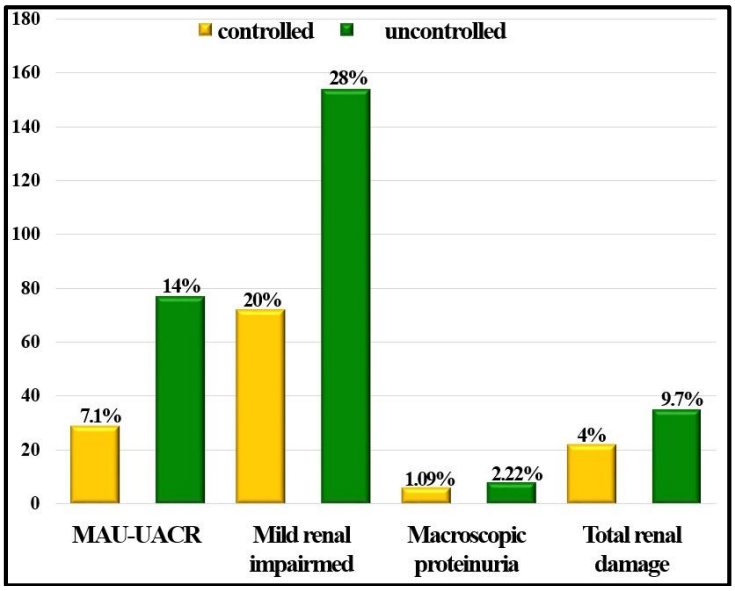

Figure 8. Subclinical and clinical renal damage in hypertension

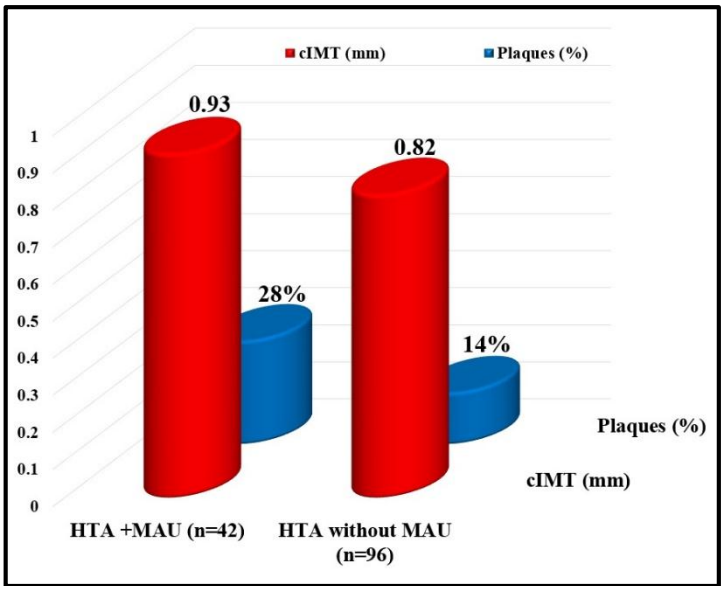

Figure 9. cIMT and plaques in HT with MAU and normal albuminuria

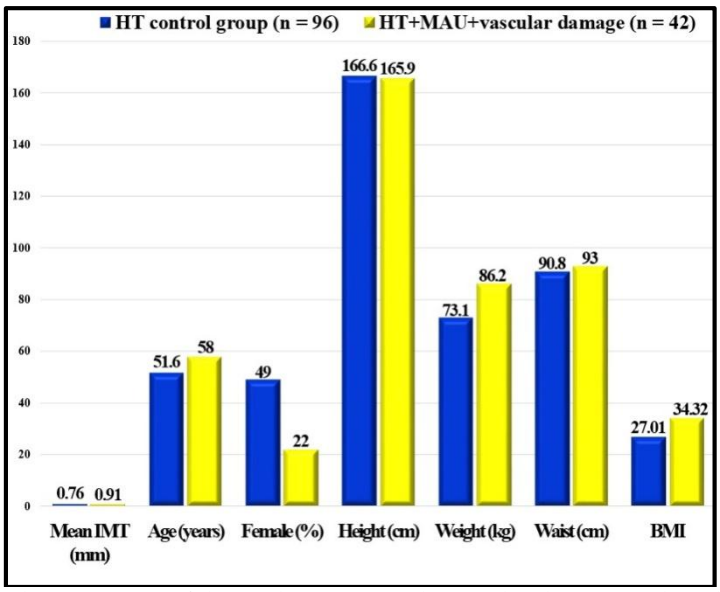

Figure 10. Baseline characteristics of the study groups with vascular damage with and without MAU (1)

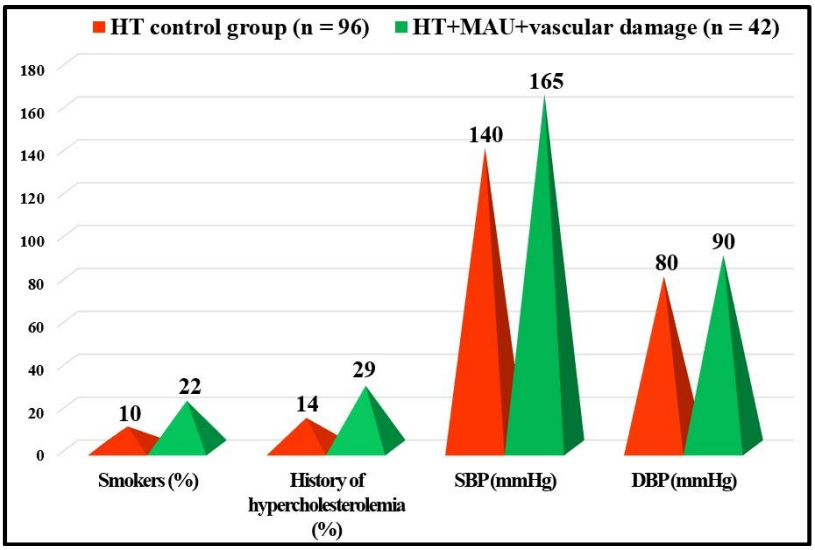

Figure 11. Baseline characteristics of the study group swith vascular damage with and without MAU (2)

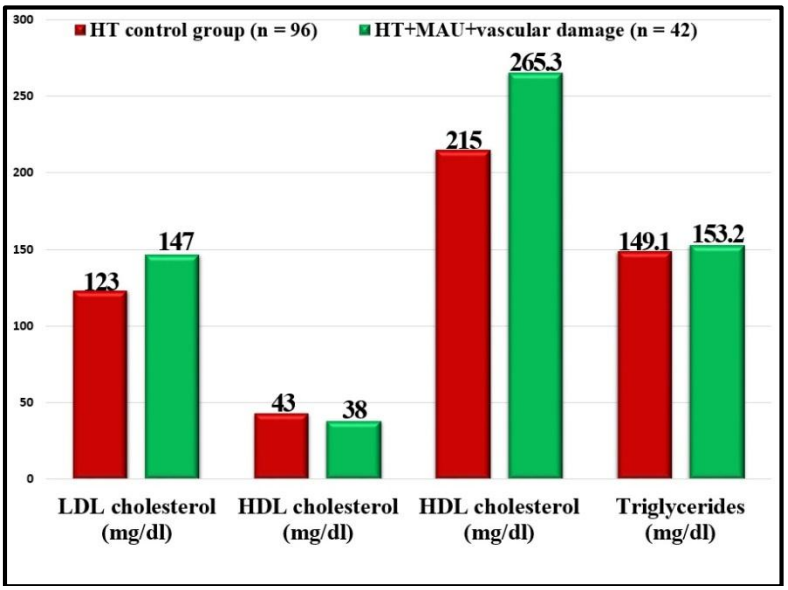

Figure 12. Baseline characteristics of the study groupswith vascular damage with and without MAU (3)

DISCUSSIONS

Microalbuminuria, in addition of being an early sign of kidney damage, is often found in patients with essential hypertension. Many studies have shown that even very low levels of MAU correlate with CV risk, independent of the presence of other risk factors (1, 9, 10). Increased microalbuminuria indicates endothelial dysfunction and predicts end-organ damage, cardio and cerebrovascular events and death. European guidelines recommend screening for microalbuminuria in patients with hypertension. Available tests for screening for microalbuminuria as dip sticks are sensitive, accessible and reliable. Early identification of high-risk patients through detection of MAU allows selection of aggressive treatment to slow disease progression. Antihypertensive agents providing angiotensin II blockade are recommended for the treatment of hypertensive patients with MAU, as they assure effective reduction of MAU, blood pressure and long-term prevention of CV events beyond blood pressure reduction. In this way a substantially reduced burden on healthcare resources can be obtained. The rapid testing by dip sticks has facilitated the screening for microalbuminuria in essential hypertension.

Moderate to severe renal dysfunction has been shown to be an independent risk factor for atherosclerosis. There are studies that demonstrated an association between IMT and MAU (11). Our results showed that subclinical atherosclerosis, evaluated by carotid IMT, increased significantly in subjects with MAU.Estimated glomerular filtration rate was not independently associated with increased IMT after adjusting for traditional cardiovascular disease risk factors. A slight elevation of albuminuria is a significant determinant of carotid IMT, independent of traditional risk factors (4). It is important to search to detect atherosclerosis through carotid ultrasound when MAU is found. Possible mechanisms for the relationship between microalbuminuria and IMT aredamages of the vascular endothelial wall that may cause atherosclerosis and albuminuria.

CONCLUSIONS

1. Microalbuminuria reflects a state of pathophysiologic vascular dysfunction that makes an individual susceptible to organ damage.
2. In patients with essential hypertension microabuminuria was correlated with systolic and diastolic blood pressure values, obesity, left ventricular mass and carotid injury.
3. Increased carotid IMT is associated directly with MAU and plaques, fact that highlights the importance of evaluating the progression of silent, asymptomatic systemic vascular disease in these hypertensive patients.
4. It is important to search to detect atherosclerosis through carotid ultrasound when microalbuminuria is found.

Further studies are needed to elucidate the underlying pathophysiological links between MAU and injuries of the carotid vascular structure.

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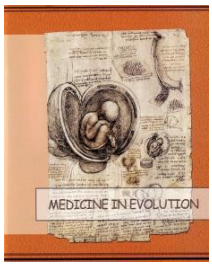

CONCOMITANT TREATMENT AND 2 YEARS FOLLOW UP OF A RECTAL ADENOCARCINOMA ASSOCIATED WITH A VOLUMINOUS ABDOMINAL AORTIC ANEURYSM IN A KRAS MUTATED PATIENT

ANDREAS ELIESCU[1, 2]

[1]Department of Vascular Surgery, Interbalkan Medical Center, Thessaloniki, Greece
[2]Department I of Surgical Oncology,"Prof.Dr.Al.Trestioreanu"Institute of Oncology, Bucharest, Romania

ABSTRACT

The concomitant presence of a rectal neoplasm and of an AAA is a rare encountered case. Speciality literature reports a frequency of 0,5%-1% till 2% of whole amount of cases (1, 2) Usually are met at older ages, at Western type populations with a diet regimen rich in animal fat and cholesterol(3, 4). The delay of treatment of these diseases may have a lethal outcome (5). The simultaneous management andthe follow- up, represents a challenge for the practitioner and necesitate a good interdisciplinary team of general surgeon, vascular surgeon and clinician. This double pathologic entity arise multiple dilemmas concerning the necessity of applying the curative surgical treatment in one or multiple stages, the order of operational stages as well as the gravity that could have the adjuvant radio or chemoterapeutic treatment in the final outcome. In this paper is presented a case report of adenocarcinoma of upper rect, stage IIA TNM, moderately differentiated, KRAS mutated. The following imagistic investigation revealed a 6,9 cm AAA with a neck angulation over 60^0 and calcifications, which made an intravascular (EVAR) abord inappropriate(4). The diagnosis of the patient with a KRAS mutation on codon 12, as well as the presence of a voluminous AAA, both restrictive factors, made us to avoid the administration of radiotherapy(in neoadjuvant or adjuvant regimen)(6, 7).

This case of double pathogenity, with a favorable evolution, being followed for up to 22 months, is presented with the intent to bring a contribution in solving the dilemmas arising from similar situations.

Key words: *rectal neoplasia, abdominal aortic aneurysm, KRAS mutation, adjuvant chemotherapy, radiotherapy*

Correspondence to:

Eliescu Andreas
M.D
Address: Vascular Surgery Center, Tsalopoulou 2, P.O.62123, Serres, Greece
E-mail address: andreas_eliescu@yahoo.com

INTRODUCTION

Concomitant with the society aging, are encountered clinical cases with more and more complex pathology. Such is the colorectal cancer(CRC) in association with an abdominal aortic aneurysm (AAA). Diseases with a high rate of mortality if aren't early treated, in the incipients stages are asymptomatics and are frequently revealed by routinely imagistic exams.(8)

In advanced stages these diseases encount for a more intense symptomatology. A CRC can manifest with hemorrhagies on digestive tract, perforations, occlusive phenomena or distant metastasis. Regarding the AAA, a voluminous one can cause compression on adjacent organs as duodenum or on urinary tract and in worse situations a cataclysmic haemorrhage can be the result of its rupture with a high mortality rate (9).

There is presented a case of rectal carcinoma(RC) complicated by the presence of an AAA. The particularities of the AAA imposed an open procedure. The one stage treatment which is preffered due to the urgency of both diseases, presents the danger of cross contamination of AAA. Performing a low anterior resection(LAR) in presence of a voluminous AAA as well as the treatment of the last one, is a solicitant situation due to the possible exchanges of the anatomical sites and the risk of injury of the aneurysmal sac. Also the use of neo or adjuvant RT for the cure of RC may have unexpected consequences, imprevisible in the evolution of an untreated or respectively of an operated AAA. The reverse situation of staged therapy with first the abord of the AAA would delay the cure of RC with possible aggravation(1).

CASE PRESENTATION

A 76 years old male wasdiagnosted with RC after a colonoscopy examination due to the presence of blood in stool and constipation for about 10 months. He suffered from hemorrhoids. The colonoscopy revealed in the upper rect a tumor mass 8/7/7cm with hemorrhagic areas and partial obstruction of the intestinal lumen.[FIG.1] Tumor markers were: CEA=12,4ng/ml (N.R.<5-10), CA19-9=15,3ng/ml (N.R.<37). The bioptic material showed adenocarcinoma of medium differentiation (G2), the abdominal and pelvic MRA attest the presence of neoplasia–TNM stage IIA (T3N0M0) and revealed a AAA of 6,9 cm who begining below the renal arteries, with angulated neck>60^0 and calcifications[FIG.2]. Chest CT was normal. PCR diagnosed KRAS mutation of codon 12. The laboratory test HT-36,8%, Hb-12,3g%, RBC-3,2x $10^6/mm^3$, biochemichal test of normal range.

We decided to performan one stage open surgery procedure with resection of both the AAA and the rectal adenocarcinoma. In first step it was resected the AAA and replaced by a Dacron bifurcated prosthesis of 24-8-8mm.The retroperitoneum was suturated over the prosthesis and covered carefully with the omental wrap[FIG 3].After that we proceed at a low anterior resection (LAR) of the upper rectum with total mesorectal excision(TME), termino-terminal (T-T) anastomosis with circular stappler and protection ileostoma. There was administered antibiotics for 7 days (Metronidazol and Cefalosporine 2^{nd} generation).

The ileostoma functioned satisfactory from the 2^{nd}

daypostsurgery. There was a pyretic oscilation of $37,5^0$ -38^0. The patient was discharged in the 10th day. He began chemotherapy (CHT) one month later with Capecitabine and Oxaliplatin for 6 Cicles, 2+1 weeks per cycle. Seven months after the 1st operation he underwent the resection of the ileostoma and Termino-Terminal anastomosis of the ileon with satisfactory intestinal function. The CEA at 1 month postsurgery decreased at 9ng/ml, after 3 months at 8ng/ml and in 6th month at 4,5ng/ml. In 12th month 4,7ng/ml and 10 months later at 4,2ng/ml. Abdominal MRA, chest CT and colonoscopy were normal. 22 months after the first operation the patient is under control being part of a prospective study thatmonitorises the oscillations of CEA and CA19-9 markers in Rectal Cancer in connection with KRAS genotype in patients treated in Oncologic Institutes of Bucharest and Thessaloniki.

Figure 1. The partial occlusion of intestinal lumen by the tumor

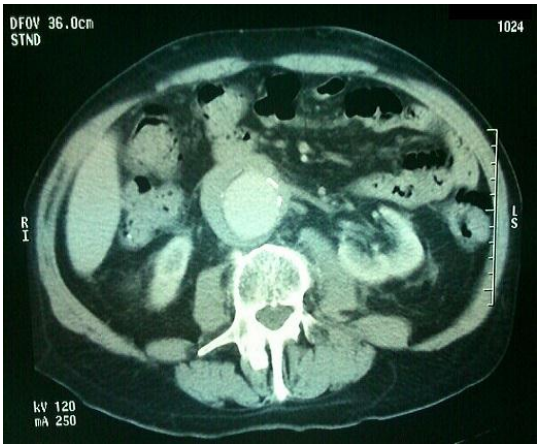

Figure 2. The abdominal aortic aneurysm

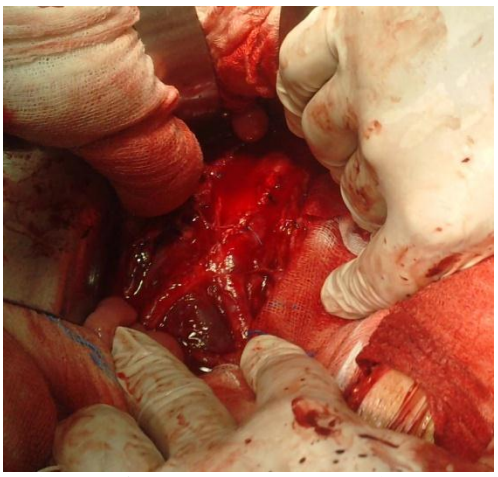

Figure 3. Closure of retroperitoneum over the aortic prosthesis

DISCUSSIONS

Important issues arise concerning the best decision in the management of a rectal cancer concomitant with an AAA, both potential lethal diseases(1,5,9). Which procedure would be optimal to perform, a one stage or a secvential therapy? Which disease must be treated first? How safe is and how beneficial would be a neo/adjuvant radiotherapy and how soon to administrate the CHT in order not to periclitatethe heal of resected AAA? Does the age of the patient permit the exposure to a procedure of a

such magnitude? EVAR was not an option due to the angulated over $60^{0.}$ and calcificated aortic neck(4,9).

There is a consensus that the age, the stage and the prognosis of CRC in terms of life expectancy and the volume of AAA are major factors in the decision of performing a therapy for both diseases (8,10). The volume of the AAA and the stage of RC do mandatory an operation without delay (9,10,11,12). An one stage therapy in several situations has a lower morbidity and mortality because a second surgical trauma isn't necessary and the patient will not be exposed to a subsequent anaestheticstress(13). Also a two stages operation is more problematic because on the second procedural step you will confront a 'hostile' abdomen. Veraldy and co. report that 30 days postoperative morbidity and mortality for single stage therapy in a 102 cases analysis is 8% and respectively 4,5% while for secvential therapy is 21,3% and respectively 6% in 118 cases (13). Also a laborious intervention in the abdominal area as a LAR of rectum can periclitate the integrity of AAA in the perioperative period by activating some proteolytic enzymes and may increase the risk of rupture especially for aneurysms larger than 6 cm (4,11,14,15). Also if AAA is resected first, this could lead to a significant delay in algorithm of surgical and adjuvant therapy of RC (2,11).

Disadvantages of one step therapy is that dealing in one stage with both disease, exposes the patient to a potential risk of cross contamination and infection of vascular prosthesis, a high mortality complication. Also a long lasting operational time is more demanding for the patient.

Most authors agree that the disease that most periclitate the life should be treated first(11,14). In our case the AAA>6cm and the preocclusive RC stage IIA made us to follow an one step procedure.

Great concern was if it must be done a neo or an adjuvant radiotherapy (RT). Could post RT reaction damage the aneurismal wall or an adjuvant RT could damage the anastomosis and lead to a haemorrhage. Previous RT for pelvic malignancy is an important predisposing factor for the appearance of ischemic colitis, a rare but devastating complication of AAA repair(16,17). Also there was an increased risk of paraplegia due to ischemic injury of the spinal cord (18). To avoid such an outcome it must preserve the circulation in inferior mesenteric artery or at least in one internal iliac artery. A KRAS mutation in primary CRC occur in 30-40% of cases(6,19,20,21,22). KRAS mutated in codon 12 was unfavorable prognostic factor and literature admit that RT has less efficacy on KRAS mutated in codon 12 RC, with a low response rate (7,4% mutated vs 19,2% Wild tipe) (7,21). The incremental benefit of RT is likely to be small in a proximal T3N0M0 with clear margins after LAR and TME. Also the low preoperatoryincreament of CEA with decrease after surgical treatment and the advanced age made us to avoid the exposure of the patient to RT. The studied case till now had a satisfactory evolution with normal imagistic exams and CEA in normal range. The favourable outcome made us believe it was a correct theurapeutic approach.

CONCLUSIONS

It is important to take decision after a good evaluation of each case particularity.

One stage procedure in some cases is preferable, dealing first with the AAA and next with RC. The risk of infection of the vascular prosthesis can

be minimized following rigourous aseptic rules, adequate antibiotics, careful retroperitoneal suture and covering with the omental wrap.

To avoid rectocolonic ischemia after AAA repair is indicate to verify the patency of at least one intern iliac artery or to reimplantate the inferior mesenteric artery. In our case both iliac arteries were patent.

The RT therapy was considered too risky and of low benefit for the concrete case KRAS mutant in codon 12.

The good interdisciplinary collaboration between general surgeon, vascular surgeon and pathologist oncologist was essential for the up to now favorable outcome.

More prospective studies with a larger number of subjects are necessary for a more concludent data analysis.

CONFLICT OF INTEREST: none declared

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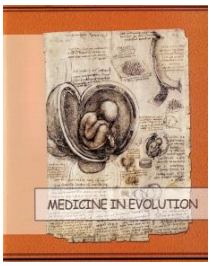
PRURIGO NODULARIS AND PSYCHOLOGICAL STATUS: A CASE REPORT



SIMONA-ROXANA GEORGESCU[1], CRISTINA MITRAN[2], MADALINA MITRAN[2], ISABELA SARBU[2], CLARA MATEI[1], ADRIAN DUMITRU[1], VASILE BENEA[2], MIRCEA TAMPA[1]

[1]"Carol Davila"University of Medicine and Pharmacy, Bucharest
[2]Dermatology Department,"Victor Babes"Hospital of Infectious and Tropical Diseases

ABSTRACT

Prurigo nodularis (PN) is a rarely encountered disorder of undetermined and still debatable cause. The condition is characterized by the presence of intensely itchy hyperkeratotic nodules bearing a hyperpigmented halo, located especially on the limbs and trunk. PN is often resistant to treatment. We present the case of a patient who was admitted to our clinic for the occurrence of pruritic nodular lesions; following investigations it was found that the patient did not present any underlying disease, however she suffered from obsessive-compulsive disorder. It is important that patients with chronic pruritus also be evaluated according to their psychological status; psychiatric evaluation and treatment is of a paramount importance to patient's response to dermatological treatment.

Key words: *chronic pruritus, prurigo nodularis, psychogenic dermatosis*

Correspondence to:

Clara Matei
MD, PhD
Address: Dionisie Lupu 37, Bucharest
Phone: +40314143571
E-mail address: matei_clara@yahoo.com

INTRODUCTION

Chronic pruritus is a common symptom affecting more women than men. Etiologically, chronic pruritus can be divided into four categories: dermatological causes, systemic causes, neuropathic causes, psychogenic causes. Chronic pruritus is the main symptom in prurigo nodularis (PN) (1).

PN is a dermatological disorder of unknown etiology described for the first time by Hardaway in 1880. In 1909, the condition was coined as „prurigo nodularis"by Hyde. PN is a benign neurodermatitis, with chronic evolution (2). Clinical features are the occurrence of pruritic nodules and papules, localized especially over the extensor surfaces of extremities. The nodules, in general, embrace a symmetrical distribution. The palms, soles and face are rarely involved. There is a vicious circle, scratching stimulating the occurrence of the lesions. The skin between the lesions can be normal, xerodermic or lichenified (3).

PN was more frequently reported in middle aged females. However, there are also studies about occurring PN in children (4,5). According to the new classification of chronic pruritus, published in 2007, PN was included in group III (Pruritus with chronic secondary scratch lesions) (6). Diagnosis is in most cases clinical, but the biopsy is necesary for the certification. Microscopically, a typical PN lesion may show achantosis, parakeratosis and papilomatosis. These histopathological features are nonspecific, also being visualised in other dermatological diseases such as psoriasis and ichthyosis. In addition, nerve fibers thickening has been obseved (2). There are studies which have revealed increased amounts of histamine containing cells in the lesions (7).

CASE PRESENTATION

A 51 year-old woman presented to our clinic with very pruritic nodular cutaneous lesions. The lesions had developed 17 years before. The onset was on her left calf. She was diagnosed with PN. The disease evolved with remissions and exacerbations. The remissions lasted about 4 months. Pruritus was exacerbated by warm water and sun exposure. The patient was a current smoker (10 cigarettes/day), with no relevant history of medical or surgical conditions. The patient had been diagnosed with obsessive compulsive disorder one year beforehand.

A physical examination revealed grouped and scattered erythematous nodular lesions rounded by a hyperpigmented halo, on the limbs, lower back and superior thorax (*Fig. 1,2*). Multiple excoriations were noticed. The patient was underweight, with a body mass index BMI of 17. Otherwise, the physical examination was unremarkable. Laboratory findings were within the normal range. A skin biopsy was performed from the left thigh. The skin biopsy revealed hyperkeratosis (ortokeratosis), epidermal hyperplasia and focal hypergranulosis. A dense dermal lymphohistiocytic infiltrate with numerous eosinophils was also found (*Fig.3*). These histopathological findings suggested PN. In the past, the patient was treated with topic and intralesional steroids. The patient had not undergone any treatment for the last three months. Treatment with oral doxepin and topical steroids (betamethasone) and sulfadiazine was initiated. The outcome was favorable, characterized by the marked alleviation of the pruritus and major improvement of the cutaneous lesions.

Figure 1. Multiple nodules surrounded by a hyperpigmented halo and excoriations on the lower back

Figure 2. Nodules and excoriations on the right thigh. Hypertrichosis due to corticosteroid treatment. Atrophic scar following the resolution of former nodules

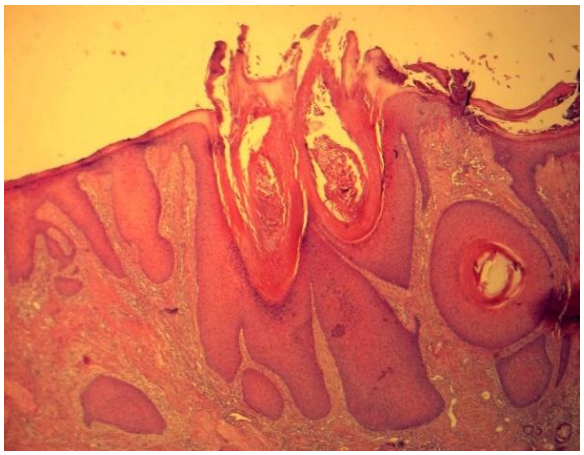


Figure 3. Hyperkeratosis with ortokeratosis, epidermal hyperplasia and focal hypergranulosis. Important lymphohistiocytic infiltrate with numerous eosinophils in the dermis

DISCUSSIONS

Pruritus is a frequent complaint. A study performed in Norway found that 8,4% of the questioned individuals had presented pruritus at least once. A slight female predominance was noticed (1,2:1) (8).

There are two forms of PN: atopic and non-atopic. Atopic PN is characterized by an early onset and associated with atopic dermatitis. Non-atopic PN is more common in the elderly (4). In our reported case, the patient had developed PN at the age of 34. Her PN was not associated with atopic dermatitis.

The origin of PN is unknown, but there are many conditions associated with prurigo nodularis (e. g. depression, hyperthyroidism, hepatitis B and C, leukemia, lymphoma, gluten-sensitive enteropathy, etc) (2). Some studies have shown that PN is more common in HIV (human immunodeficiency virus) infected individuals. Hence, in underdeveloped countries, where diagnosis tools are not available, PN may represent a severe immunodeficiency index (9). In our case, the tests of hepatic, renal, and thyroid function were within the

normal range. The medical history revealed that the patient had lost about 15 kg over the last 10 years. No other cause was identified. The patient stated that she had an appropriate diet. However, the patient had been diagnosed with obsessive compulsive disorder one year before. Sometimes, the management of these patients may be difficult. Patients are often not aware of the psychogenic origin of their pruritus. An optimal therapeutic approach needs a multidisciplinary team (a dermatologist, a psychologist and a psychiatrist) (10).

In a study conducted by Sommer F et al. including 263 patients, it was found that chronic pruritus was not associated with underlying diseases in almost 50% of investigated patients. Among the patients with pruritus of undetermined origin, 76.9% had PN (8). Tests in patients with chronic pruritus should include complete blood count, a chest radiography, and tests of hepatic, renal, and thyroid function. In the case of our patient all these investigations were normal. In addition, the physical examination did not reveal signs of underlying diseases and the patient did not accuse other symptoms apart from itching (1).

PN is often resistant to therapy. First, the cause should be treated. Mostly, the underlying cause remains unknown and the treatment is only symptomatic. Emollients play an important role considering that xerosis produces pruritus. Topical and intralesional steroids may be used. We should avoid superpotent steroids to prevent skin atrophy (4). Occlusive dressings are preferred because they offer the advantage of preventing patients from scratching (11). Capsaicin, which acts locally by desensitizing peripheral nerve fibers, may be used as antipruritic agent (1). Localized phototherapy and photochemotherapy (PUVA) is in some cases indicated. Recently a case of PN after PUVA therapy was reported in a patient with vitiligo (12).

Oral antihistamines, especially sedative antihistamines, play an important role in the therapy of PN. A recent study has found that ketotifen combined with a topical antibiotic may be useful in the management of PN. Systemic therapy also includes anti-depressants, oral steroids, ciclosporin, thalidomide (13).

Beside the medical therapy, psychotherapy is sometimes necessary. The emotional status of the patient may represent the key in the pathogenesis of PN. Psychiatric evaluation should be considered, mainly when the cause has not been found. Several studies have shown that social, psychological factors and dermatological conditions are interconnected. Even more numerous studies have highlighted the positive role of psychotherapy in PN approach (14). Several studies tried to demonstrate the relation between emotional stress and cutaneous lesions. Kiecolt-Glaser et al. found that in stressed individuals, bullous lesions healed more slowly than in unstressed individuals (15).Therefore, cognitive behavioral therapy may be helpful. Likewise biofeedback with or without associated hypnosis may be a strategy in treating these patients (14).

CONCLUSIONS

The management of patients with PN is difficult; first we should rule out the associated disorders. Notably when the cause remains unknown, a possible psychogenic condition should be evaluated. Numerous studies have shown the connection between emotional status and cutaneous manifestations. Therefore the management of these patients may require a multidisciplinary team consisting of a dermatologist, a psychologist and a psychiatrist. The pathogenesis of this disease is currently

not fully understood, therefore further studies regarding this aspect are still needed.

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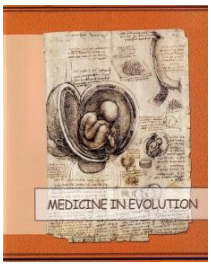
SPONTANEOUS CLOSURE OF TRAUMATIC CSF FOLLOWING CONSERVATIVE MANAGEMENT



COSTEA DANIEL[1,2], COSTEA CONSTANTIN, ANDREEA ALBOTA[2], ANGELESCU MARCEL[2]

[1] Neurosurgery Department of County Emergency Hospital Timisoara, University of Medicine and Pharmacy"Victor Babes"Timisoara, Romania
[2]Department of Neurosurgery, Emergency County Hospital"Pius Branzeu"Timisoara

ABSTRACT

CSF leaks represent a breach of the dura mater, resulting an abnormal communication between the intracranial compartment and the exterior. Middle fossa skull base fractures often are associated with otorrhoea. The treatment of otorrhoea usually is a conservative treatment, rarely surgical. This particular case was managed with the"wait and see policy"without any invasive treatment only drug administration and postural.

Key words: *Otorrhoea, CSF leak, skull base fractures*

Correspondence to:

Dr. Costea Daniel
Address: Str. Zurobara Nr.8, Timisoara, Romania
Phone: +40722692996
E-mail address: drcostea@yahoo.com

INTRODUCTION

The commonest cause of CSF fistulae is trauma to the skull base. Head trauma accounts for 50-80% of all cases of CSF leak, and up to 16% are iatrogenic. CSF otorrhoea complicates 6% - 30% of basilar skull fractures [1, 2]. The management is dependent on history, confirmation of CSF and identification of bony defect. The treatment may be conservative or surgical; the goal is repair of meningeal tears and underlying bone defects [1-4]. Spontaneous healing of CSF otorrhoea has been reported in 90% of cases following conservative management [1-4]. We hereby report a case of spontaneous closure following conservative management of traumatic CSF otorrhoea in a young male.

We present a 30 year-old male who sustained a head injury with right cerebrospinal fluid otorrhoea following a work accident. Plain radiograph revealed a defect in the temporal bone extending in to the tympanomastoid area. Patient was managed conservatively with closure of the fistula and resolution of the leakage within 7 days after injury.

CASE REPORT

A 30 years old male, right handed forest woodcutter presented to the Accident and Emergency Department of local hospital with a 1 day history of right otorrhoea and hearing impairment following a working accident. He was hit by a stub in the right temporal area; this was followed by a bloody right otorrhoea and loss of consciousness. He regained consciousness within1 hour and bloody otorrhoea ceased, later turned to clear, colourless fluid. There was no evidence of bleeding from other craniofacial orifices. He was referred to our centre 3 days of injury.

Examination revealed a conscious and well- oriented adult with a Glasgow Coma Scale score of 15/15. The right ear showed clear colourless fluid, filling the right concha. The audiometric exam showed a right conductive hearing loss. The facial nerves were intact; and the left ear; nose and throat were normal. There were healing abrasions and lacerations on the face and a sutured right temporo-parietal injury. He presented a right retro auricular bruise-like hematoma.

Computer tomography reveals fracture line extending through the lateral wall of the petrous bone extending through the tegmen tympani into the middle ear. MR investigation with CISS protocol reveals the trajectory of CSF leak to the middle ear

Patient was started on antibiotic and acetazolamide. A clean piece of gauze was left in the concha area of the right pinna to soak the effluent. He was nursed head – up position and advised to stop or minimize action that can increase intravenous pressure such as coughing, sneezing or bearing down. Gradual reduction in the volume of the effluent was observed on day 1 and 2, until day 3 (5 days post trauma) when the concha was largely dry with only a drop found around the opening of the external auditory canal. No lumbar punctures or drainage of CSF was performed.

DISCUSSIONS

Traumatic CSF fistulae have been described since the middle ages. Willis was reported to be the first to record instance of CSF fistula in 1676 [3] and

Walter Dandy was credited with the first successful repair of traumatic dural laceration secondary to basilar skull fracture [4]. Leakage through an enlarged labyrinthine facial nerve canal and enlarged geniculate fossa has been reported [5]. Other sites include fractures of the petrous temporal bone, developmental defects of the tegmen tympani or petrous apex with meningocele formation and spontaneous or posttraumatic meningeal laceration, translabyrinthine fistula due to the Mondini developmental defect of the cochlear modiolus and/or lamina cribrosa, wide cochlear aqueduct syndrome and perilymphatic fistula from trauma with stapes fracture and torn round or oval window membrane [6-8]. Diagnosis of CSF otorrhoea is dependent on good history, physical examination and radiologic investigations.

In the background of head injury, a clear colourless fluid in the ear is suggestive of CSF otorrhoea. However beta-2 transferrin assay is the test of choice because of its high sensitivity and specificity [5, 6-9]. Radiology plays an important role in confirming the site, size and etiology of the leakage. CT Scan demonstrates the fracture site that overlies the traumatic leak and provides information about the adjacent brain parenchyma.

Intrathecal Fluorescein is the most accurate method of localizing site of leak, however it is associated with complications like transverse myelitis and allergic reactions [10]. Digital subtraction cisternography is useful when the conventional methods fail to identify the site of leak. Diagnostic yield may be improved by injection of metrizamide or omnipague [7-10]. A brisk CSF leakage can be best demonstrated by MR with CISS,. The management could be conservative or surgical. Fifty to eighty-five percent of traumatic CSF leaks resolve conservatively within 7 days

Conservative treatment aims to reduce CSF pressure to allow for approximation of dural tear and induction of healing by primary intention. This includes bed rest with patient in head - up position and avoidance of coughing, sneezing and heavy lifting. Therapeutic reduction of the spinal fluid production using agents such as acetazolamide and furosemide and repeated removal of CSF via lumbar tap or an indwelling catheter has also been tried.

Spontaneous closure observed with CSF otorrhoea has been explained to be due to the rich arachnoid mesh in the middle and posterior fossa area. A waiting period between 5 days and 8 days has been reported [7-12]. Posttraumatic CSF fistulas persisting beyond 7 days, spontaneous CSF leaks with skull-base defects, increasing pneumocephalus, and meningitis are positive indications for surgical intervention. Extracranial and endoscopic repair by the neurosurgeon can be performed; however, open craniotomy with intradural repair is necessary for large skull-base defects [10-13]. Prophylactic antibiotics used in this patient seem to be the widely accepted practice. This is to prevent the occurrence of infection, particularly meningitis. Meningitis has been reported in 25-50% of untreated traumatic CSF fistulas and in 10% of patients in the first week after trauma with head injury [10-12]. However, we were able to prevent this in the patient and hence successfully discharged for follow – up in the outpatient clinic.

CONCLUSIONS

This case has further reinforced the success of conservative management of CSF fistula, particularly otorrhoea, however, meticulous care is needed with proper application of guidelines in the management of skullbase defect.

Post-traumatic CSF leaks are usually resolved without surgical intervention. Successful management in refractory cases often involves a combination of observation, CSF diversion, and/or extracranial and intracranial procedures. The factors that had a critical influence on outcome are level of Glasgow Comma Scale on admission and presence of additional intracranial pathology associated with CSF leakage within cases of traumatic CSF fistulae due to skull base fractures. Patients with CSF leaks that persist greater than 7days are at risk for meningitis, and may require surgical intervention. Prophylactic antibiotics may be effective and should be considered in this group of patients. Treatment decisions should be dictated by the severity of neurological decline during the emergency period and the presence/absence of associated intracranial lesions. The timing for surgery and CSF drainage procedures must be decided with great care and with a clear strategy.

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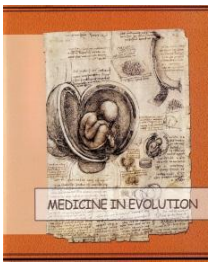

THE ROLE OF CELLULAR AND MOLECULAR BIOMARKERS IN ASSESSING PROGNOSIS OF PRECANCEROUS CERVICAL LESIONS

DEMETRA SOCOLOV[1], CORALIA BLEOTU[2], GABRIELA ANTON[2], LIANA GRIGORESCU[3], RAZVAN SOCOLOV[1], ALEXANDRU CARAULEANU[1]

[1]University of Medicine"Gr.T.Popa", Iasi
[2]Institute of Virusology"St.Nicolau", Bucharest
[3]University of Medicine"Carol Davila", Bucharest

ABSTRACT

Limited histologic and cytologic diagnosis accuracy may have consequences either in overtreatment of patients with less severe lesions or in under-treatment of patients with significant high-grade lesions. Therefore biomarkers and surrogate ones are need to identify patients at risk of developing cervical cancer. This paper aims to review potential biomarkers that may improve the diagnostic accuracy or more accurately assess prognosis in patients with cervical disease.

Key words: *biomarkers, cervical cancer, precancerous*

Correspondence to:

Demetra Socolov
Md Phd Associated Profesor
Address: Univeristy of Medicine"Gr.T.Popa", Iasi, Str.Universitatii nr.16 700115, Iasi, Romania
Phone: +40721481747
E-mail address: socolov@hotmail.com

INTRODUCTION

Cervical cancer is the third cause of cancer death in women worldwide. Despite the efforts to prevent this malignity through screening programs and immunization, approximately 500,000 new cases are diagnosed each year. Clinical, epidemiological and molecular data confirmed high-risk HPV (human papillomavirus) as etiological agent of cervical cancer [1]. While more than 90% of HPV infections regress spontaneously, a small minority progress to high grade lesions, from which a fraction evolve to cervical cancer. Typically, cervical cancer develops from precancerous lesions over 10 -20 years in a multi step process which include hyperexpression of high risk HPV E6 and E7 oncogenes accompanied by p53 and pRB inactivation, the integration of viral genom into the host cell ones and the alteration of cellular regulatory mechanisms. In order to have a better estimation of the risk a cervical lesion has to progress to cancer there is a great need of specific biomarkers in the routine practice. Recently, several studies proposed many options linked to the critical role of HPV E6 and E7 oncogenes in the induction and maintenance of the malignant phenotype and some of them are currently used in medical practice. The biomarkers currently used address to cell cycle regulation, proliferation, differentiation, apoptosis and chromosomal stability.

According to a 2007 study [2], the biomarkers must meet several requirements like: allowing early detection of cancer or the risk assessment of lesions, predicting the progression, allowing the detection of post-treatment recurrences. Although histological investigation of colposcopy -guided biopsies is recognized as the"gold standard"method for cervical lesions assessment, the method has limitations related to variations in interpretation as well as related to the impossibility of predicting the risk of progression or regression [3]. In gynecology area, the need for biomarkers is claimed by the necessity to discriminate between normal and CIN lesions and between low grade CIN from high grade ones [4] or to overcame diagnostic inaccuracies due to CIN mimics (like immature squamous metaplasia). Therefore, the biomarkers must be differently expressed in normal and high-risk tissues, must be synthetized in defined stage of carcinogenesis and its assay must be sensitive, specific and accurate [5].

To induce an environment conducive to their life cycle, papillomaviruses (through oncoproteins expression) induce loss of cell cycle control, interacting with different host cell genes and leading to aberrant expression of these cell genes along with cervical oncogenesis. The control of cell cycle is realized by cyclin dependent kinases (CDKs) whose activity is regulated by cyclins and inhibitory proteins (p15, p16, p21, p27). The inactivation of p53 by E6 HPV oncoprotein leads to G1/S checkpoint overcoming and to the downregulation of p53 targets, p21 and p27, which in turn, regulate cyclin E pathway. Also E7 oncoprotein can directly regulate cyclin E expression through binding to pRB. E2F released from hyperphosphorylated pRB induce an increase level of cyclin E in HPV infected lesions. Therefore, the aberrant expression of cyclin E might be as an early event in carcinogenesis can be used for early diagnostic and for monitoring patients with cervical dysplasia. Reports on the expression patterns of p21 and p27 in cervical carcinoma progression are still contradictory [6]. On the other hand, p53 was proposed as a molecular marker in estimating the risk of progression of HPV-associated LSIL lesions. The expression of p53 is

correlated with lesion severity, being higher in HGSIL and cancer than in LGSIL.

p16INK4a is a tumor-suppressor protein that normally acts like a negative regulator of cellular proliferation. Overexpression of p16INK4a in cervical tissue as a result of pRb inactivation by high-risk HPV E7 made this protein a surrogate marker of HPV E7-mediated pRb catabolism. Most studies provide evidence of p16 hyperexpression in HSIL lesions but not in cervicitis or metaplastic epithelium (in the absence of CIN lesions). p16 expression increased during progression from CIN 1 to carcinoma. p16 negativity in CIN-I and CIN-II biopsies does not always suggest the regression of the lesion and the diagnosis of CIN-II should not be based solely on p16 results [7]. The addition of p16INK4a immunostaining to the hematoxilin-eosin staining, improve significantly the interpretation of biopsies (both punch and cone ones). In order to improve diagnostic accuracy and reproducibility, p16INK4a immunohistochemistry may help in identifying high-risk HPV types CIN1 lesions that are prone to progress to severe dysplasia or carcinoma. Moreover, p16INK4a in immunocytochemistry provides better specificity than viral testing for the triage of ASC-US and LSIL cases.

HPV genome integration that often accompanies malignant progression, induce genomic instability. Genomic instability in cervical enables tumor cells to acquire genetic alterations that are necessary for clonal expansion. Most notably aberration is aneuploidy and tripolar mitotic figures might be considered a hallmark for high-risk-HPV-positive lesions. Among markers of chromosomal instability is the expression of telomerase, mainly of hTERT, the catalytic subunit of this enzyme. In normal cells, the number of cell divisions is set to limited number and is determined by telomers (TTAGG repetitive DNA sequences which protect each chromosome termini) progressively shorten. Through this process, characterized by no telomerase activity, normal cell enter senescence and loss proliferative capacity, despite the fact it retains viability and metabolic activity [8**].** In tumor cells, the telomerase is activated and elongate telomers. Therefore, tumor cells overcome the senescence barrier and have an increasing proliferative capacity. In cervical neoplasia, the expression of telomerase is suggested to be a consequence of high-risk HPV infection, high levels of E6 viral oncogene triggering the enzyme activation [9]. Immunohystochemical studies, using hTERT as marker, showed negative expression in normal epithelium, positive nuclear immunoreactivity in all the layers of epithelium and occasional in cytoplasm in CIN lesions and more cytoplasmic staining with less intra-nuclear signal in squamous carcinomas [10].

An increased proliferative activity of the cells is generally associated with premalignant and malignant phenotype. The estimation of proliferation might be assessed by different techniques but the mitotic count in histological and cytological samples are currently in use. Ki-67, a non-histone protein is a proliferation marker expressed in the stratified squamous epithelium of CIN lesions. Ki-67 expression correlates positively with histologic grade and distinguishes non-diagnostic atypia from SIL. Ki-67 immunostaining alone does not discriminate between HPV induce-dysplasia and benign reactive processes, this limiting its use in cytologic specimens. P16 and Ki-67 complete each other to reach an accurate diagnosis, being useful markers in establishing the risk of CIN lesions [11]. However, association between the presence of high-risk-HPV infection and the p16(INK4a) and Ki67 expression is even stronger among

women with HSIL [12]. Comparing the performance of p16/Ki67 dual-staining and human papillomavirus (HPV) testing in 1123 women referred to colposcopy, it was established that morphological evaluation of p16/Ki67-positive cells has higher specificity than HC2 tests for HSIL or CC and may have some benefits in women younger than 30 years or with low-grade squamous intraepithelial lesion [13]. Also, a study on total of 27,349 women 18 years or older attending routine cervical cancer screening, prospectively enrolled in five European countries, established that the p16/Ki-67 dual-stained cytology combines superior sensitivity and non-inferior specificity over Pap cytology for detecting CIN2+ [14]. Commercial available assays targeting cellular biomarkers are available. CINtec from MTM Laboratories can be used on the basis of a single dual stained epithelial cell, independent of cell morphology, resulting in sensitivity comparative to HPV testing and a greater specificity that hrHPV testing.

Few studies correlated the highly conserved minichromosome maintenance proteins (MCM), an important tool in the initiation of DNA replication, with the progression of cancer. MCM are members of the DNA factor family required for DNA replication and are overexpressed in cervical high-grade dysplasia and carcinoma. Some studies suggest that HPV-infected cells strongly express MCM2, but MCM2 is not a good biomarker when comparing the different clinical stages of cervical cancer [15]. ProExC test (BD TriPath Imaging, USA) is a immunocytochemical assay which exploit the ability of MCM2 proteins and topoisomerase 2α (a marker of aberrant S-phase induction) to identify the high-grade dysplasia. When comparing with p16 immunostaining, ProExC seems to have similar specificity for CIN 2+ and higher specificity for CIN 3+ but lower sensitivity for CIN 2+ and CIN 3+. Although ProExC provides better results for HSIL lesions (the test is negative in normal cytology) it is not intensively used, due to MCM expression in some benign squamous and glandular cells.

HPVs life cycle is closely related to host epithelial differentiation program and this is reflected in the expression profile of cell cytokeratins and transmembrane proteins. Among these proteins, E-cadherin expression in relation with cervical cancer was investigated. E-cadherin (a transmembrane protein connected to the actin cytoskeleton) down-regulation correlated positive with CIN progression and cell proliferation. Decreased E-cadherin expression seems to be a useful marker of malignant potential of cervical lesions, providing an additional criterion in correlation with cyto- and histomorphology and colposcopy to define high grade CIN lesions. Impairment of E-cadherin expression seems to be an early event in cervical carcinogenesis [16].

Evaluating the gene expression changes involved in neoplastic progression of cervical intraepithelial neoplasia using microarray analysis, Rotondo et al., (2014) identified 37 candidate genes with continuously increasing or decreasing expression during CIN progression. Characterization of phosphoglycerate dehydrogenase, by quantitative reverse transcription-polymerase chain reaction and immunohistochemical analysis confirmed that expression of phosphoglycerate dehydrogenase consistently increases during progression of CIN toward cancer, and may be a potential prognostic marker for CIN progression [17]. Some other markers showed statistically significant association with CIN stage: GLUT1in CIN II and CIN III; hPygopus2 in CIN III; and beta-catenin, in CIN III and cancer [18].

Apoptosis is program with genetic determinism, which leads to high molecular weight DNA cleavage. The apoptosis of HPV infected cells includes also the cleavage of viral DNA, thus affecting the virus spread. In order to elude this cellular mechanism, viral oncoproteins modulate the apoptotic proteins like Bcl2, Bak and Bax. Among these proteins, Bcl2 expression might be used in detecting CIN3 lesions with low risk to progression. Also, Bcl2 increased expression seems to correlate with poor survival rate following radiotherapy.

Recent studies revealed the contribution of epigenetic mechanisms to cervical lesions progression. Epigenetic mechanisms encompass three types of changes: chromatin modifications, DNA methylation and microRNA each of them being altered in cancer cell. The reversible character of epigenetic changes makes them attractive in developing new therapies. The most studied epigenetic change is DNA methylation. Generally, tumor cells are characterized by general DNA hypomethylation and focal DNA hypermethylation. DNA methylation is performed by DNA methytransferases (DNMT), mainly by DNMT1 and it takes place at cytosine bases within the CpG dinucleotide (named CpG islands). CpG islands are short DNA stretches situated mainly in the promoter region of the genes. CpG islands are usually unmethylated, and their methylation lead to gene inactivation. As a consequence, in cervical oncogenesis some important tumor supresors cannot exert specific anti-oncogenic function; therefore the methylation of tumor supresors' promoters are considered as a mark of cell deregulation. Specific DNA methylation patterns might be associated with a specific pathology. [19].

Recent studies have shown that methylation of both viral and cellular DNA is a potential biomarker for the improved accuracy of cervical screening and for the triage of abnormal cytology or high-risk HPV-positive women. Analyzing the literature focused on the utility of methylation markers in cervical cancer, from 68 different cellular genes investigated, three might be used as markers (DAPK1, CADM1 and RARB) as showed elevated methylation pattern in cervical cancers and CIN3 lesions. Taking into account that molecular techniques might detect the aberrant methylation pattern in cervical smears many years prior to the diagnosis of cervical cancer, host gene methylation investigation may have great value for the triage of women positive for high risk HPV.

Regarding the contribution of viral oncogenes to chromatin remodeling, it was shown that E7 oncogenes associate with histone deacetylases and histone acetyl transferases. These interactions may contribute to transforming activities of high-risk HPV E7 proteins. On the other hand, a recent study indicates deregulation of the chromatin remodeling complex components and its influencing factors in the development of CIN lesions. The increase in SWI/SNF stabilizing molecule *SMARCC1* as event in the early stages of dysplasia development provides novel candidate markers for screening [20].

Several biomarkers have been recognized to identify specific stages in the natural history of HPV infection and cervical cancer progression. Nevertheless, there is an increasing need for novel biomarkers that use small sample size and new assays with true clinical value in discriminating women at risk for cervical lesion progression.

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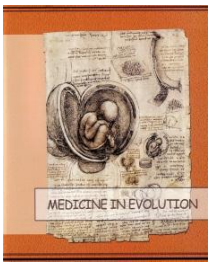

THE EFFECTS OF DEEP BRAIN STIMULATION IN A PACIENT WITH PRIMARY GENERALIZED DYSTONIA WITH EARLY ONSET- CASE REPORT

RUXANDA DANA CHIRILEANU[1,2], MIHAELA SIMU[1,2], RALUCA TUDOR[1,2], CECILIA ROŞCA[1,2], PATRICIA JURCA[2]

[1]University of Medicine and Pharmacy „Victor Babeş"Timişoara, România
[2]Department of Neurology, Emergency County Hospital of Timisoara

ABSTRACT

Dystonia is a rare neurological disorder, with unknown etiology and no etiological treatment, which can lead to a major disability especially in the case of generalized dystonia. At the moment deep brain stimulation, mainly stimulation of the palid globe is considered to be the best treatment option, particularly in generalized dystonia which do not respond to other therapies used in the case of dystonia. This intervention can lead to a control of the disease. We present the case of a patient with primary generalized dystonia with early onset at the age of 11 and the effect of deep brain stimulation surgery on this pacient's disabilities.

Key words: *generalied dystonia, deep brain stimulation, rare neurological disorder, involuntary movements*

Correspondence to:

Assoc. Prof. Ruxanda Dana Chirileanu
MD.PhD. Senior neurologist
Address: Emergency Clinic Hospital 2nd Department of Neurology, I Bulbuca Street, Number 10, code 300736, Timisoara
Phone: +40722743356
Fax: +40256487890
E-mail address: dana.chirileanu@gmail.com

INTRODUCTION

Dystonia is a rare neurological disorder, that presents with sustained muscular contractions and involuntary movements, which lead to abnormal positions, frequent torsions and sometimes pain in the affected areas [1]. Usually, the intellect is not affected. Focal dystonia with adult onset, affects a specific body part, but generalized dystonia can cause a major disability.

Dystonia can be primary (idiopathic), when it is believed that a genetic mutation is involved [2], or secondary when it appears as a symptom in other neurological disorders. Patients with generalized dystonia (musculorumdeformans dystonia) seem to have a genetic mutation involving DYT 1 gene, the pathophysiology is unclear, but it involves a simultaneous contraction of agonists and antagonists muscles [1], the onset is usually early, during childhood, symptoms begin in the lower limbs [2] and at least 3 body parts are affected. The diagnosis of dystonia is mostly a clinical diagnosis, due to the fact that there are no specific diagnostic tests [3].

As treatment options anticholinergic drugs are used, botulinum toxin injections are used in focal dystonia, and for generalized primary dystonia the best treatment option is deep brain stimulation of the pale globe, that through the modulation of nervous impulses can improve the symptoms, especially gait [1], and even if it doesn't cure dystonia, it can control most of the involuntary movements.

CASE REPORT

I. Anamnesis

A 29 year old patient presents to our clinic for diagnosis and treatment, with dyskinesias, non-kinesigenic dystonic contractions mostly of the right lower limb- varusequin leg and internal rotation, kinesigenic dystonic contractions of the right upper limb, dysarthria and swallowing disorder for both liquid and solid food.

From the patient's history we found out that the symptoms started at the age of 11 with walking disorder and difficulty in realizing right leg dorsiflexion.

In the couple of years that followed involuntary movements, dystonic positions, a speech disorderand a swallowing disorder for both liquid and solid food appeared.Due to the daily fluctuations of these symptoms, the patient underwent multiple evaluations in Universitary Neurology Clinics because the diagnosis of myasthenia gravis was suspected, however this diagnosis wasinvalidated through the absence of anti-acetylcholine receptor antibodies and the lack of response to anticolinesterase.

II. Clinical examination data

The neurological examination was limited by the presence of dystonia. The patient was conscious, cooperative, and temporo-spatial oriented. No parkinsonian syndrome was present and she had dyskinesias, non-kinesigenic dystonic contractions mostly of the right lower limb(varusequin leg and internal rotation) and kinesigenic dystonic contractions of the right upper limb. Gait was possible without support, asymmetrical and parasitized by dystonic positions, Romberg test was negative, lower limb deep tendon reflexes were absent and a normal cutaneous plantar reflex was present; there were no sensation disorders, she had orofacial dyskinesia with deviation of the tongue to the right, as well as

dysarthria and swallowing disorder for both liquid and solid food. Mini Mental Examination revealed a scor of 30.The physical examination was without abnormalities and there were no signs of neurovegetativedisautonomy.

III. Laboratory data: The biological findings were all normal.

IV. Additional paraclinical investigations

An electrophysiological study was performed although without results that would suggest the diagnosis of primary axonal polyneuropathy. Magnetic Resonance Imaging showed no abnormalities, electroencephalography was without hyperexcitability modifications, tests involving the thyroid gland were normal, there were no arguments for Huntington's disease, Wilson's disease was also excluded and the Levo-Dopa test which was performed to exclude dopa responsive dystonia was negative - the medicine brought no benefits and the patient had a digestive intolerance to the medicine.In the end the patient was diagnosed with primary generalized dystonia with early onset localized mostly in the right hemibody, probably of genetic etiology, but the financial possibilities did not allow genetic testing. Since there is no etiological treatment for generalized dystonia, genetic testing wasn't absolutely necessary.

V. Treatment and evolution

The patient underwent treatment with Trihexiphenidyl in gradually increased dosages up to 2 milligrams per day, Tetrabenazine 25 milligrams per day, Viregyt K 100 milligrams per day and Rivotril 0,5 milligrams per day. The treatment led to a suboptimal improvement of the symptoms, since an important disability persisted. Deep brain stimulation was recommended, at the moment this being the best treatment option in generalized dystonia. The patient was informed about the benefits and side-effects of this therapy and also about the fact that there was no possibility of performing this surgery in Romania. The patient was referred to an European center of treatment and underwent deep brain stimulation surgery in Istanbul at Florence Nightingale Hospital.At about a month after the surgery the patient showed improvements in walking, swallowing and talking. During that period of time the patient underwent a series of stimulation adjustments which were performed according to her clinical presentation. The adjustments were performed during the time span of fivedays in Florence Nightingale Hospital-where a physical therapy rehabilitation program was commenced and ankle-foot orthosis were prescribed. Afterstimulation adjustments and physiotherapy, walking and balance continued to improve, but there weren't any more changes regarding talking and swallowing. The patient underwent neurosurgical and neurological evaluations and a rehabilitation program was recommended; this program included physiotherapy, periodical evaluations for stimulation adjustments at every 2 months in the beginning, afterwards at every 6 months and periodical neurological controls. The battery of the stimulator was recommended to be changed after 4 or 5 years from the surgery, and the patient is not allowed near magnetic fields.

DISCUSSIONS

After the surgery a good control of the dystonia symptoms was obtained and walking, balance and non-kinesigenic dystonic contractions were improved.

Deep brain stimulation led to about 50% reduction of disabilities in

this patient considering the fact that half of the symptoms improved significantly.

No significant improvement was observed regarding talking and swallowing, but gait became relatively normal, only slightly parasitized by involuntary movements; physiotherapy and ankle-foot orthosis also contributed to gait improvement. After the surgery a rehabilitation and stimulation adjustment plan was established in order to continue the amelioration of the symptoms over time.Although the final positive effects of the deep brain stimulation surgery will be observed later in time and the amelioration physical therapy program is a long one, the immediate results of this treatment option proved to be encouraging for this patient. So, deep brain stimulation had a benefic effect on this patient with generalized dystonia who did not respond to medication, significantly improving the quality of her life.

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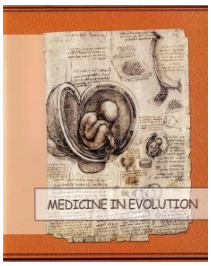
THE INVOLVEMENT OF EXECUTIVE FUNCTIONS IN THE OCCURENCE OF PSYCHOMOTOR AGITATION IN CHILDREN AND ADOLESCENTS



ANDA MARIA JURMA[1], DAIANA LAVINIA MORARIU[2], AMALIA LUISA MITRULESCU PĂIȘEANU[2], LAVINIA MARIA HOGEA[1]

[1]University of Medicine and Pharmacy"Victor Babes"Timișoara, România
[2] Child and Adolescent Neurology and Psychiatry Clinic Timișoara, România

ABSTRACT

Aim. To analyse the relationship between psychomotor agitation, deficits in executive functions and functional impairments in children and adolescents.

Material and Method. Participantswere 43 children and adolescents aged between 11-18 years consulted / hospitalized for psychomotor agitation in 2014, with externalizing problems (23) and with internalizing problems (20). We have used: Barkley Deficits in Executive Function Scale - Children and Adolescents (BDEFS-CA) and Barkley Functional Impairment Scale - Children and Adolescents (BFIS-CA).

Results.Children and adolescents with psychomotor agitation have affected executive functionsin the areas of emotional regulation and self-restraint, whether with externalizing or internalizing problems. In children with externalizing problems the executive function of self-restraint affects the functioning in almost all situations of the home and community.

Conclusions. The skills of self-control can be an important goal of therapy in aggressive children who present internalizing and externalizing problems.

Key words: *executive functions, psychomotor agitation, children, adolescents*

Correspondence to:

Jurma Anda
Address: Corbului 7, Timișoara, România, Child and Adolescent Neurology and Psychiatry Clinic
Phone: +4 0745514741
E-mail address: andamaria.jurma@gmail.com

INTRODUCTION

The psycho-motor agitation is a psychical syndrome which manifests itself through the following symptoms: psycho-motor anxiety, clastic fits, raptuses, explosive reactions, pronounced auto and hetero-aggression, erratic, incoherent and bizarre activity. It can constitute a psychiatric emergency in the context of bipolar disorders (maniacal fury, agitated depression - suicidal risk), psychoses, acute reactions to stress, dissociative disorders, anxiety, disruptive behavioural disorders in the case of children [1].

Executive functions are neuropsychological processes which interfere with auto-adjustment and allow the subject to react and adapt accordingly to the environmental factors. The inhibition and adjustment of motor and verbal actions, working memory, organization and planning, detachment of emotions from thoughts are executive functions which allow the subject to be capable of picking the correct answer for a given task, to maintain the answer or change it if such an action is requested. Thus, actions are deemed executive if they imply the"when"and"if"aspects, which are related to behaviour [2,3,4].

Executive functions are indispensable for human behaviour. Their destruction could lead to numerous behavioural disorders as well as psychiatric disorders such as ADHD, learning problems, autism, depression, anxiety, schizophrenia.

Aggressive behaviour is a frequent reason for psychiatric hospitalization during childhood and is associated with a series of psychiatric disorders: behavioural, attention and emotional deviations [5]. The presence of such behaviours creates significant difficulties for the family, school and community [6]. The executive functions identified as predictors for violent behaviour are: weak performance in spatial working memory, inhibition difficulties, continuous memorizing and rememorizing information, solving problems associated with decreased self-control. [6,7]

Among his studies, Denson (2011) presents the angry rumination as a mechanism which overwhelms self-control and increases aggression.Angry rumination implies two components: the thought of or reliving an event which generates anger and repeated thoughts of revenge. Starting from these thoughts, the reactivation of the neuronal network which strengthens these associations occurs. In fact, angry rumination determines negative emotion, which increases and maintains anger as well as the cognitive accessibility of the anger constituents, thus creating a state which could always trigger an access of rage. [8]

Aim and Objectives

The publication has followed the analysis of the relationship between the fits of psycho-motor agitation, deficits of the executive functions and the functional disorders in the case of adolescents.

MATERIAL AND METHODS

The study has included 43 participants, both children and adolescents, with ages raging between 11 and 18 years, which were consulted and hospitalized due to psycho-motor agitation during 2014, with externalizing issues (can be situated in the diagnosis of hyperkinetic disorder with lack of attention - ADHD, or conduct disorders) and with internalizing issues (can be situated in the diagnosis of anxiety disorders,

affective disorders and emotional disorders) (Table I)

Table I. Study participants characteristics

	Average age (Standard Deviation)	Sex		Family			Diagnosis				
		F	B	N	D	I	ADHD	Conduct disorders	Anxiety disorders	Affective disorders	Emotional disorders
Internalizing group	13.55 (1.6)	9	11	12	2	6	-	-	3	4	13
Externalizing group	14.26 (1.88)	8	15	10	3	10	3	20	-	-	-

F = girls, B = boys, N = normal family, D = disorganized family, I = institution

This study has made use of: the Barkley Deficits in Executive Functioning Scale – Children and Adolescents (BDEFS-CA) and the Barkley Functional Impairment Scale – Children and Adolescents (BFIS-CA).

The Barkley Deficits in Executive Functioning Scale – Children and Adolescents, BDEFS-CA, evaluates the dimensions of executive functioning in the case of children and adolescents: emotion control, motivation, self-control, regulation and time management.

The Barkley Functional Impairment Scale – Children and Adolescents, BFIS-CA, evaluates possible deficiencies from 15 different fields of day to day activity in the case of children and adolescents. More specifically, home circumstances: interaction with their mother, interaction with their father, school performances, interaction with brothers or sisters, money administration, daily care, carrying out daily chores, doing homework, respecting rules and community circumstances: playing with children from the neighbourhood, society, behaviour during visits, playing with children at school, interaction with other adults and sports.

All the children who participated in the study have filled in both scales after signing the form which acknowledges their consent for this study.

The results obtained after applying the working tools have been processed and analysed using statistics tests from the SPSS statistics processing software package. This study has analysed the average scores of the 5 executive functions, the average scores of functional disorders in the 15 cases of home and community circumstances as well as the possible correlations between the disorders of executive functions and the functioning of children.

RESULTS

The results obtained from the analysis of the scale for the executive functions have shown that subjects with internalizing symptoms have affected, in particular, emotional regulation and self-control, while in those with externalizing symptoms are affected self-control, emotional regulation but also the organization (Fig 1).

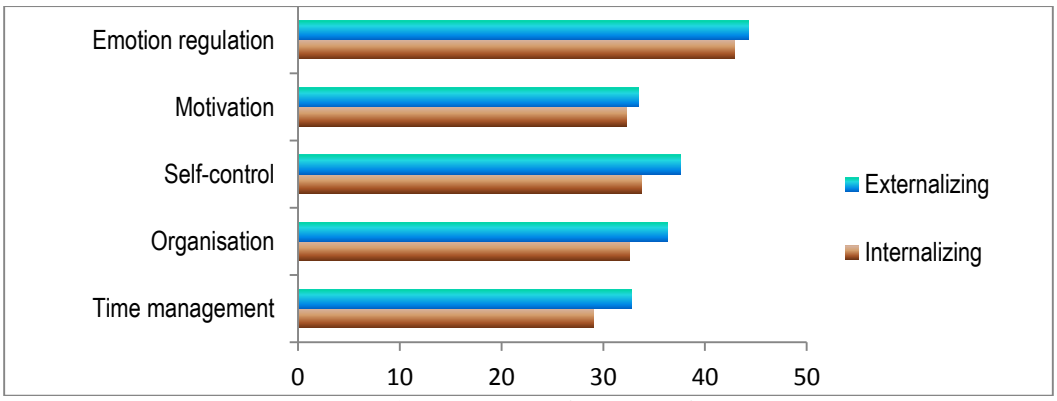


Figure 1. The mean score of executive functions

Regarding the degree of clinical impairment of executive functions in children with internalizing symptoms results showed that 15% had a severe degree of impairment of motivation, 20% have a severe degree of damage to the organization, the same percentage meeting in the self-control moderate impairment, and 25% had a moderate degree of impairment in emotional regulation. In children with externalizing symptoms, a severe degree of damage to the organization and to the self-control was found in 17.4% of subjects, while 26.1% had a moderate degree of impairment in emotional regulation and organization.

The results obtained from the analysis of functional impairment scale showed that all children have a large degree of impaired functioning in home situations, children with externalizing disorders obtaining mean scores (M) higher in all cases (Fig. 2).

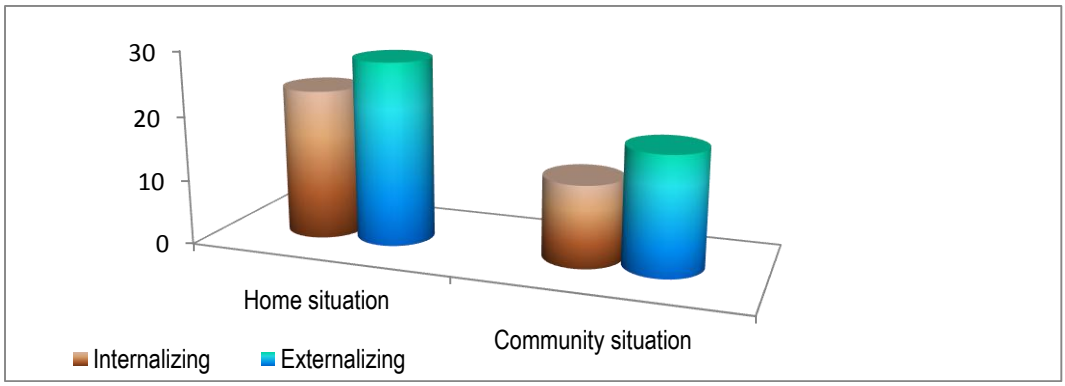


Figure 2. The mean score of home and community situation

In children with externalizing disorders the impairment degree is higher related to interaction with the father (M = 6.06; SD = 3.02), school performance (M = 5.37; SD = 2.47), interaction with brothers / sisters (M = 4.29; SD = 3.19) and following rules (M = 4.57; SD = 3.02) but also the interaction with other adults (M = 4.04; SD = 3.06), whereas children with internalizing disorders have a more pronounced degree of involvement in the interaction with the mother (M = 4, SD = 3.22) but also interaction with Dad (M = 3.53; SD = 3.27) and school performance (M = 3.68; SD = 3.25).

Internalizing symptoms in subjects with a moderate degree of functional impairment was found in terms of playing with the kids (22.2%) and following rules (15.8%), while severely impaired highlighted in interaction with the mother (18.8%) and money management (17.6%). Frequencies were higher in children with externalizing problems, so a moderate degree of functional impairment was found in interaction with the father (23.5%), school

performance (21.1%), playing with the kids in school (25%) and daily care (30.4%), severely affected being the interaction with the father (29.4%) and visiting behavior (36.4%).

Spearman Correlation analysis between executive functions and functional impairment showed that in children with internalizing symptoms, self-control is closely correlated with the interaction with other adults ($p = 0.005$), while in children with externalizing symptoms, self-control is closely correlated with interaction with other adults ($p = 0.005$) but also with community situations ($p = 0.000$), interaction with brothers / sisters ($p = 0.005$), playing with the kids in the neighborhood ($p = 0.000$), playing with the kids in school($p = 0.006$), behavior in society ($p = 0.000$), visiting behavior ($p = 0.003$), money management ($p = 0.000$), daily care ($p = 0.002$) and perform tasks ($p = 0.001$). Less significant correlations ($p <0.05$) were found between emotional regulation and interaction with the father, between time management and home situations, between motivation and homework, and between the organization and school performance, sports and interaction with other adults.

Significant correlations were also found in subjects with externalizing disorders, so, time management was correlated with situations in the community ($p = 0.002$), playing with the kids in school ($p = 0.000$), money management ($p = 0.005$), playing with the neighborhood children and behavior in society. Motivation was closely corelated to the situations in the community ($p = 0.000$), playing with the kids in the neighborhood and playing with the kids in the school ($p = 0.000$), behavior in society ($p = 0.001$), performing tasks and daily care. Also have been found correlations between organization and community situations, behavior in society and playing with the kids in the school.

DISCUSSIONS

Studies show that deficits in executive functions are involved in anxiety disorders, conduct disorders, substance abuse, eating disorders and borderline personality disorders which are characterized by difficulties in regulating emotions. [9]

Although made on a small number of subjects, this study identified the presence of executive function impairment in children who experienced psychomotor agitation crisis, whether they experienced externalizing problems (integrated diagnosis in ADHD or in conduct disorder) or internalizing problems (integrated in anxiety disorders, affective or emotional disorders).

Effect on clinical level of self-control and emotional regulation in children with externalising problems correlate with other studies. ADHD associated with symptoms such as irritability, nervousness, low frustration tolerance and sudden, unforeseen changes into negative emotions such as anger, dysphoria and sadness involves modifications and alterations of emotional reactions causing difficulties in emotional self-regulation.[10,11]

Other studies show that in affective disorders may occur deficits in several aspects of executive functions as difficulties in inhibiting distractibility attention to external stimulus identification problems of facial features and emotional recognition, which contribute to increased suicide risk. [12,13]. The present study also found impairment of executive functions at the clinical level in the emotional regulation and organization.

The presence in the study of many children from institution and who exhibit psychomotor agitation crisis correlates with the literature

dates that highlights impaired executive functions in children undergoing various adversities.[5,14,15]

Numerous studies show that self-control deficits involving behavioral adjustment and metacognition (planning, organizing, working memory) can influence the relationship between aggressive behaviour and externalizing and internalizing problems, as the present study shows [16]. Also, in the current study a significant percentage of children (30%), both with internalizing and externalizing problems have affected at clinically level executive function such as organisation or time management in severe or moderate degree.

The limits of this study are given mainly by the absence of a control group of children who did not manifest psychomotor agitation crisis and the small number of children in the study who did not allow the analysis of executive functions and the degree of functional impairment of children by sex, psychiatric diagnosis and type of family.

CONCLUSIONS

The model of exhausting FE resources, involved in auto-adjustment explains the mechanisms which trigger aggressive behaviour [8]. Engaging in tasks and activities which require a certain form of inhibition or self-control will trigger the temporary depletion of executive function resources, even when the source of depletion is not correlated with anger. Thus, having these resources depleted temporarily, individuals exhibit difficulties in controlling their behaviour and emotions, as well as the inability of having adequate social interaction.[8]

This paper has attempted to highlight the involvement of the executive functions of psychiatric pathology in children and adolescents, improving them can help preventing aggravated symptoms and even suicide. In recent years a particular interest in how the executive functions can be improved through repeated training in a healthy population, and clinical risk was shown. Thus, self-control skills may be an important objective of therapy in aggressive children, who present internalizing and externalizing problems. Interventions should emphasize the ability of self-control, including handling negative emotions and behavioural impulses in a friendly and flexible manner.

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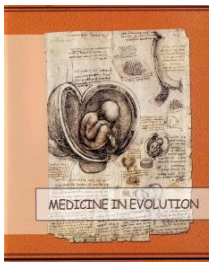

ASSOCIATION BETWEEN HIGH-DOSE STATIN TREATMENT, PHYSICAL ACTIVITY, ADP PLATELET AGGREGATION RESPONSE AND INFLAMMATORY MARKERS IN CARDIAC REHABILITATION AND RECOVERY OF CORONARY PATIENTS

VIRGIL M. LUCA[1], ARDELEANU ELENA[2], CONSTANTIN T. LUCA[3,4], ADRIANA POTRA[5], DAN GAITA[3,4]

[1]Medical Semiology Discipline, University of Medicine and Pharmacy"Victor Babes"Timisoara
[2]Family Medicine Discipline, University of Medicine and Pharmacy"Victor Babes"Timisoara
[3]University of Medicine and Pharmacy"Victor Babes"Timisoara
[4]Institute of Cardiovascular Diseases, Timisoara
[5]Former Pfizer Sales Agent

ABSTRACT

Background:Recent studies showed that inflammatory parameters such as high sensitive C-reactive protein (hs-CRP) and fibrinogen (Fib) are associated with cardiovascular morbidity and mortality.A lot of studies demonstrated that a high dose of statins and a physically active lifestyle are associated with a reduction in coronary events. Furthermore, the patients with impaired platelet aggregation to ADPlabelled –as"low- respondersto clopidogrel"with history of acute coronary syndrome are at high risk for development of new ischemic events.

Material and Methods:In this retrospective study we investigated the association between LDL-cholesterol (LDL-C), statins therapy (ST), hs-CRP, fibrinogen and the aggregation response to ADP in 236 subjects (age 67,2 ± 4.1 yr, BMI 28,6 ± 3,8 kg/m2) with coronary heart disease, participating in outpatient exercise groups for cardiac rehabilitation.

Results:The hs-CRP level in the whole group was0,34±0,4 mg/dl andthe Fib level 391 ± 96 mg/dl. Compared to patients treated with low doses of statins those with high doses of statins showed lower concentrations forhs-CRP(–10%, p<0.05, respectively). In patients with an LDL-C < 100 mg/dl, hs-CRP (–12 %) and Fib (–8%) were significantly lower (p<0.05, respectively) than in patients with LDL-C > 100 mg/dl. High level of physical activity patients exhibited significantly lower values for hs-CRP (–18%), Fib (–10%) than the low-physical activitypatients. The combination of a high response to ADP and high statin doses intake reduced hs -CRP (–33 %), p<0.01, respectively- compared to patients with a low level of ADP aggregation response and taking low doses of statins.

Conclusions: The data from this study suggest that factors such as high doses of statins, LDL-C (< 100 mg/dl),normal and high ADP aggregation response and a high level of physical activity are associated with lower levels of inflammatory markers in patients with coronary heart disease. The proof of causality of these findings should further be investigated in randomized controlled trials.

Key words: *coronary heart desease; atherosclerosis; inflammation*

Correspondence to:

Dr. Luca Constantin Octavian
Address: str. Saligny nr 6A ap 5
Phone: +40722207101
E-mail address: lucaconstantinoctavian@yahoo.com

INTRODUCTION

There is important evidences that inflammatory processes play an integral role in the formation of atherosclerotic plaques [1,2,3]. Therefore, it is possible that an improvement of cardiovascular risk factors by statins therapy, increased physical activity or weight reduction would also reduce inflammatory processes, and hence, atherogenesis in the vessel wall [4, 5, 6, 7,8]. Intensive lipid intervention with high- dose statins produce clinical events reduction and is especially powerful in the setting of acute coronary syndrome (9-11), including the setting of percutaneous coronary intervention(12,13). Patients with high platelet reactivity are at increased risk of adverse cardiovascular events after PCI. High- dose statins improve prognosis in high-risk patients by lipid and nonlipid-related mechanisms, including antithrombotic effects (14). Clopidogrel"low- responders"patients with non-ST segment elevation acute coronary syndrome (NSTE ACS) are at high risk for development of new ischemic events. The assessment of"low- or non-responder"status to dual antiplatelet therapy (aspirin and clopidogrel) in patients undergoing percutaneous coronary intervention (PCI) with coronary stent placement for NSTE ACS analyzed by platelet aggregometry using both arachidonic acid (AA) and ADP as agonists to detect the responder or non-responder status to aspirin and clopidogrel, respectively.(15) We used the multiple electrode aggregometry (MEA) performed with Multiplate® analyzer by Dynabyte, Munich, Germany in order to assess the post- treatment platelet reactivity.

The role of LDL cholesterol (LDL-C) in the pathogenesis of atherothrombotic diseases is wellknown (16, 17, 18, 19). Accumulating evidence indicates that lowering LDL-C reduces the risk for fatal and non-fatal myocardial infarction in both primary and secondary prevention of coronary heart disease (CHD) (20,21,22). Recommendations for optimal LDL-C concentrations were published by the Joint European Societies in 1994 and 1998 or the Adult Treatment Panel III of the United States National Cholesterol Education Program (23,24). There is general agreement that LDL-C in secondary prevention of CHD should be lower than 100 mg/dl, and that in high risk patients, the target level for LDL-C should be < 70 mg/dl. In many cases, this goal can only be achieved by lipid-lowering drugs, which are well known to reduce morbidity and mortality associated with CHD [25 26].

Furthermore, guidelines from the National Cholesterol Education Program(8), theAmerican Heart Association/American College of Cardiology (9), and the American Heart Association/American Stroke Association (10) all support recommendation to lower low-density lipoprotein cholesterol (LDL-C) to at least under 100 mg/dl, with recommendation to lower LDL-C<70 mg/dl as a"therapeutic option". These level of LDL-C (<70 mg/dl) are especially applicable in patients following acute coronary syndrome.

High on-tratment platelet reactivity has been shown to be an independent risk factor for recurrent ischemic events, particularly in patients undergoing percutaneous coronary intervention (PCI). The clinical benefit of statins is attributed to multiple mechanisms that go beyond their lipid-lowering effects and include antithrombotic properties. In fact, statins inhibit adenosine diphosphate (ADP) and thrombin-induced platelet aggregation in healthy subjects and also in patiens with coronary artery disease.

Statin therapy is effective in reducing markers of inflammation [24,28]. So far, it is currently unknown whether patients with CHD and LDL < 100 mg/dl exhibit lower plasma levels of inflammatory parameters than patients with LDL > 100 mg/dl. Furthermore, few data are available concerning whether subjects taking statins present a more favourable inflammatory state than patients without statin therapy. In addition, few studies have investigated the effect of physical activity on inflammatory markers in patients with CHD [30], and the results are not consistent [31].

Therefore, in the present study we looked at the association between hs-CRP, Fib and the LDL-level, the intake of statins therapy (low or high-dose), the platelet aggregation to ADP and the level of physical activity in a sample of patients participating in outpatient exercise groups for cardiac rehabilitation.

MATERIAL AND METHODS

Patients (n= 236) with diagnosed coronary heart disease during 2010-2012 were retrospectively enrolled in the study group. All subjects participated regularly in prescribed outpatient exercise groups for cardiac rehabilitation. All subjects were on statin therapy in different doses (2 subgroups were identified- low and high-doses of statins).

The patients of the whole group were divideddepending of their physical activity status. Information was obtained about their physical activity (≤ 1time/week, 2 times/week or ≥ 3 times/week) during the cardiac rehabilitation program. Leisure time physical activity (> 45 min. duration) of ≤ 1 time/week was labelled as a low physical activity status and ≥ 3 times/week as a high physical activity status.

The blood samples were collected fasting and plasma concentrations of hs-CRP, Fib, total cholesterol (TCho), triglycerides (TG), LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C) were determined. Hs-CRP*and Fib were quantified nephelometrically using reagents from Dade Behring (Dade Behring, Schwalbach, Germany). Lipoprotein levels were determined by lipid electrophoresis (REP, Greiner, Limburg, Germany).*The multiple electrode aggregometry (MEA) method was used to assess the platelet reactivity to ADP. These ADP aggregation tests were performed with Multiplate® analyzer by Dynabyte, Munich, Germany. In all patients we determined induced aggregation (ADPtest). The result of the test is represented by a curve of aggregation (AU) /time (minutes).The most important parameter is area under the curve AUC expressed in AU*min or U (10 AU*min= 1U).

Groups were tested for significant differences using the Mann-Whitney U-Test for unpaired samples. P-values < 0.05 were considered significant.

RESULTS

Subject characteristics regarding age, body mass index (BMI) and lipoprotein lev-els are shown in Table 1.

Table 1. Age, BMI and lipoprotein levels of patients investigated. Physical activity=PA; LPA = low physical activity status, HPA = high physical activity status; ST = statin therapy.

Values are mean ± SD.

§ = p<0.01 within groups

	Whole group	Statin therapy		LDL < 100 mg/dl		Physical activity (PA) status		Physical activity status/low/high statin therapy	
		Low doses	High doses	No	Yes	Low	High	LPA+ST Low dose	HPA+ High dose
	n=236	n=143	n=93	n=132	n= 104	n=137	n=99	n=138	n=98
Age (yr)	64,2 ± 4,1	64,9 ± 3,2	63.8 ± 2.9	64,4 ±3,3	63.3 ± 3.8	64,5 ± 3,6	65,0 ± 2,9	64,5 ± 3,2	64,2 ± 3,4
BMI (kg/m2)	27,6 ± 3,9	27,4 ± 4,6	27,5 ± 3,41	27,8 ± 4,1	27,1 ± 3,4	27,4 ± 3,8	26,9 ± 4,0	27,3 ± 3,7	26,5 ± 3,9
TChol (mg/dl)	224 ± 42	242 ± 42	214 ± 40§	242 ± 35	186 ± 27§	233 ± 47	214 ± 37	218 ± 42	209 ± 34
TG (mg/dl)	242 ± 153	231 ± 123	250 ± 170	232 ± 123	264 ± 202	257 ± 19	213 ± 107	256 ± 198	229 ± 109
LDL-C (mg/dl)	120 ± 32	134 ± 32	112,3 ± 29§	136 ± 24	86,0 ± 10§	126 ± 34	113 ± 31	114 ± 30	109 ± 28
HDL-C (mg/dl)	49,8 ± 16	53,8 ±18	47,2 ± 14	51,2 ± 16	47,2 ± 16	51,0 ± 17	50,1 ± 6,3	46,9 ± 14	48,1 ± 15

There were no significant differences within and between groups for age, BMI, HDL-C and TG levels. Due to the selection criteria, significant differences were observed for TChol and LDL-C. More than half (60%) of patients took high doses of statins, but only 55 % achieved the desired level of LDL-C < 100mg/dl.

Compared to patients with low-dose statin therapy, those with high-dose statin therapy showed lower concentrations for CRP (–7%) ($p<0.05$).(Fig.1)

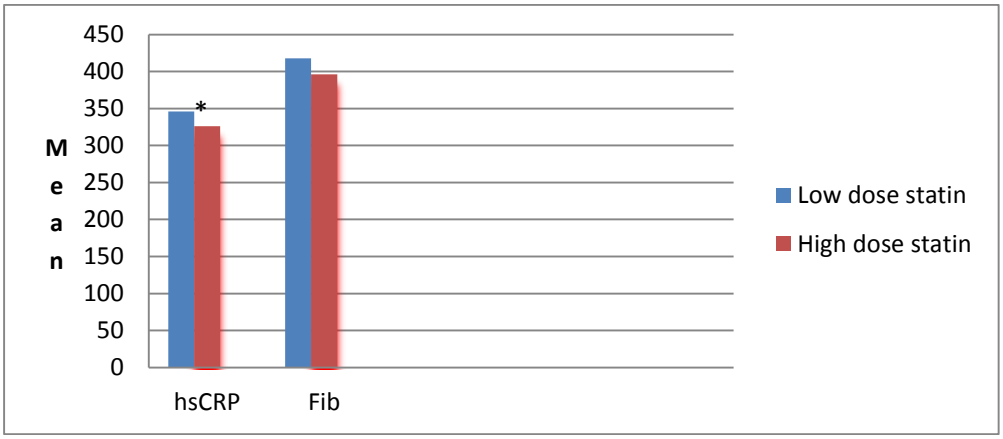

*Figure 1. Mean values for hs-C-reactive protein (hs-CRP), fibrinogen (Fib) in dependence on the statin doses intake. For better visualisation, the concentrations of hs-CRP were multiplied *1000.The value are showed in mg/dl.*

In patients with an LDL-C<100 mg/dl, hs-CRP(-2%) and Fib (-8%) were significantly lower ($p<0.05$, respectively) than in patients with LDL-C > 100 mg/dl.(Fig.2)

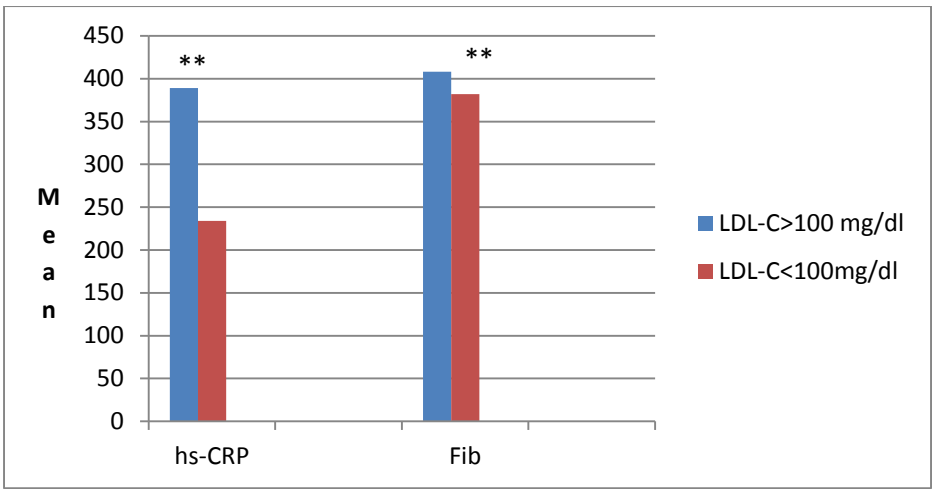

*Figure 2. Mean values for hs-C-reactive protein (hs-CRP), Fibrinogen (Fib) in dependence on LDL-C lower than 100 mg/dl.**= p<0.01. For a better visualization, the concentration of hs-CRP was multiplied *1000*

More than a half of patients (58%) - 137p. showed a low physical activity level, the rest of 99 (32% of the patients) were included in the high level physical activity status. In patients with a high level physical activity status significantly lower values for hs-CRP were identified (-19%), Fib (-14%), p<0.01, respectively, comparing with those with a low level of physical activity status. (Fig.3)

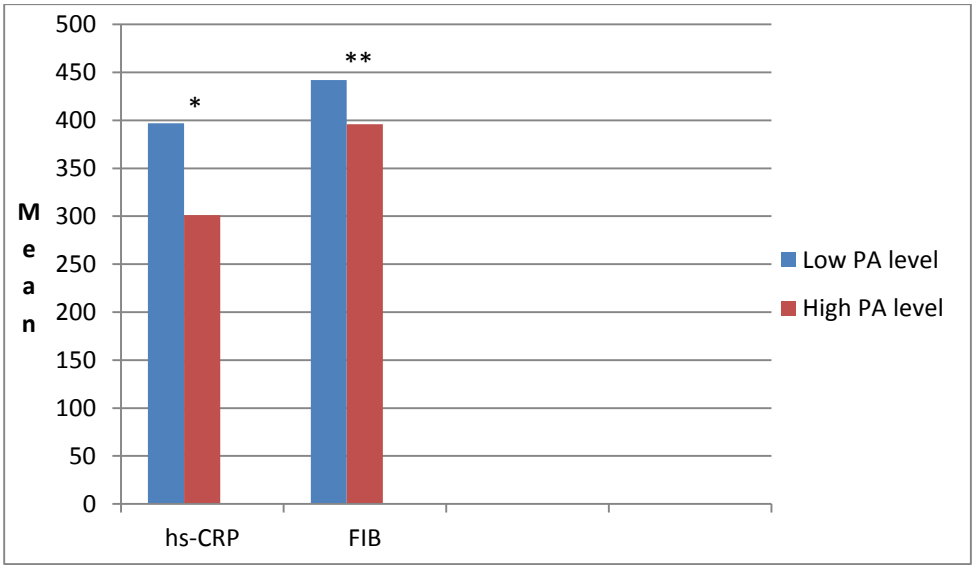

*Figure 3. Mean values for hs-C-reactive protein (CRP), fibrinogen (Fib) in dependence on the level of physical activity (PA); **=P<0.01. For better visualization, the concentration of hs-CRP was multiplied *1000*

The combination of a high level of physical activity and the high dose of statin therapy (41 % of patients) further reduced hs-CRP (-37%) with no supplementary decreasing in the Fib value (-10%), p<0.01, respectively compared to those patients with a low level of physical activity status and treated with high doses of statins. (Fig.4).

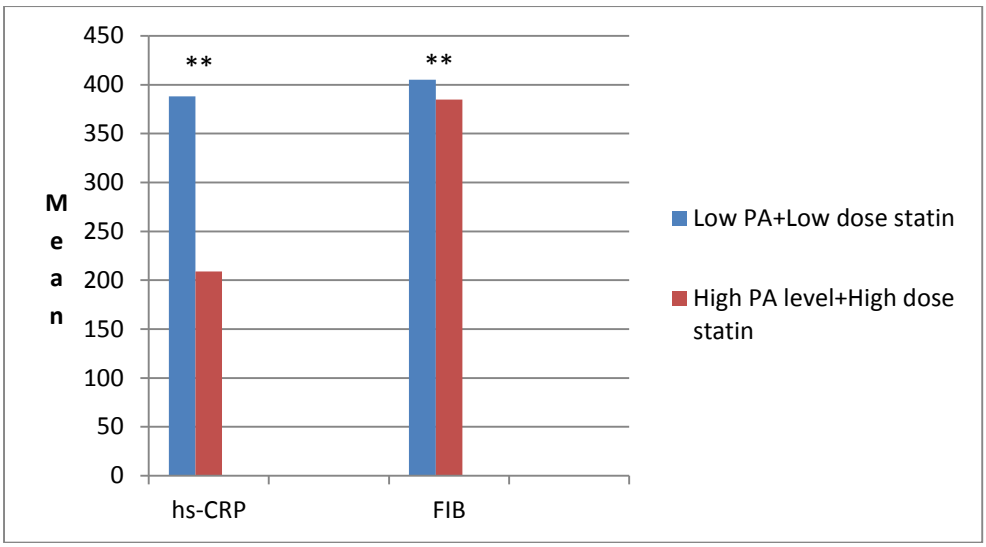

*Figure 4. Mean values (in mg/dl) for hs-C-reactive protein (CRP), fibrinogen (Fib) in dependence on the level of physical activity (PA)and intake of statin in low or high doses; **=P<0.01. For better visualization, the concentration of hs-CRP was multiplied *1000*

In all patients were determined ADP- induced aggregation (ADPtest) using the multiple electrodes agreggometry performed with Multiplate® analyzer. The result of the test is represented by a curve of aggregation (AU) /time (minutes).The most important parameter is area under the curve AUC expressed in AU*min or U (10 AU*min= 1U), which is in accordance with an platelet aggregation to ADP effect, respectively.The mean ADP- induced intensity of platelet aggregation response (represented by mean AUC value done by ADPtest) in the described groups was: 599.3 AU*min in the first group (low dose of statin + low level physical activity), 158.5 AU*min (high doses statin+ high level physical activity), respectively. Comparing the group of the patients with high level physical activity and high doses of statin therapy with those of low level physical activity and low statin dose regarding the platelet aggregation response performed with ADP test (performed with Multiplate® analyzer multiple electrodes aggregation methodMEA) we find significantly lower values for AUC in the second group, p<0.01.(Fig.5)

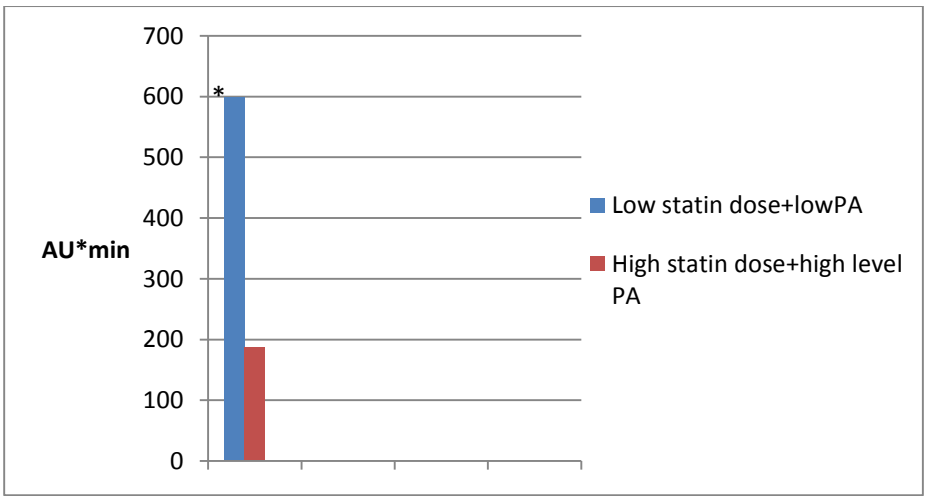

*Figure 5. Mean values for platelet aggregation ADP test performed by multiple electrodes agreggometry expressed in AU*min in the groups with low statin doses therapy and low physical activity compared with the group with high statin doses therapy and high level of physical activity; **=p<0.01*

DISCUSSIONS

The results obtained in the present study showed that factors such as the therapy with high doses of statins, attainment of LDL-C levels below 100 mg/dl or a high level of physical activity status, particularly in combination with high doses of statin therapy were associated with a reduction in hs-CRP, the wellknown inflammatory parameter. Therefore, our findings are in keeping with most previous studies that an improvement of cardiovascular risk factors reduces systemic inflammation in patients with CHD [3,20,15,18,32].

Furthermore, statins are known to have multiple nonlipid-lowering (pleiotropic) effects- they have anti-inflammatory, vasoprotective, antioxidant, immunomodulating pro**pertie**s, and also inhibit platelet function (2). Additionally, statins have been shown to reduce platelet aggregation, dense granule release, and platelet-mediated thrombus formation in a dose-dependent manner (3,4,22,23).

The established coronary risk factors such as hypertension, dyslipoproteinemia, smoking or hyperglycemia greatly enhance proinflammatory potential- evidence suggests that. A large series of authors have designated various inflammatory markers as an independent risk factor for atherosclerosis (9,20,32). Therefore, measurement of hs-CRP the parameter that has been most consistently associated with atherosclerosis, has been recommended (level of evidence B) by the American Heart Association to identify high risk patients with coronary heart disease [42].

Elevated levels of hs-CRP and fibrinogen in subjects with atherosclerosis or acute coronary syndromes are commonly observed, and are often associated with the severity of atherosclerosis and prognosis of coronary heart disease [20,24,28].

Therefore, measurement of hs-CRP the parameter that has been most consistently associated with atherosclerosis, has been recommended (level of evi-dence B) by the American Heart Association to identify high risk patients with coronary heart disease [42].

In our study group, hs-CRP was the only inflammatory marker that was significantly lowered by LDL-C < 100 mg/dl, and physical activity. For the whole group hs-CRP concentration were 0.31 mg/dl. According to the American Heart Association scientific statement (42), these patients were included in the high risk population.The lower hs-CRP values by high doses statin therapy, LDL-C levels below 100 mg/dl oror a high level of PA, downgrades these patients into a population with an lower risk.

Increasing evidence demonstrated that a physically active lifestyle is associated with a reduction in cardiovascular risk factors and coronary events (17,19). The selection of patients in the present study who were participating in outpatient exercise groups for cardiac rehabilitation have resulted in a sample of well-guided and informed patients. The fact that only 59% showed LDL-C levels below 100 mg/dl is alarming. Nevertheless, our results have clearly demonstrated that a high level of physical activity was associated with markedly lower levels of hs-CRP.

A meta–analysis (Cochrane Library) stated that inclusion of exercise programs in cardiac rehabilitation was associated with a significant benefit regarding total mortality and fatal cardiovascular events [23].

Furthermore, studies not dealing specifically with patients in secondary prevention of CHD have reported that the direct effect of physical activity and exercise on LDL-C is relatively small [6,15]. Beyond doubt, the influence of

statin therapy on LDL-C levels is decisively stronger than the effect of physical activity.

The present the results suggest that reduced pro-inflammatory markers may contribute to the beneficial effect of physical activity on the reduced cardiovascular event rate [8].

CONCLUSIONS

In conclusion, the data from this investigation suggest that factors such as statin therapy, especially in high dose, LDL-C < 100 mg/dl and a high level of physical activity are associated with reduced levels of inflammatory markers and a a low platelet aggregation response to ADP in patients with coronary heart disease. Particularly with respect to hs-CRP values, a high level of physical activityin combination with high doses of statin therapy, showed the most pronounced effects. In the future, studies should investigate the amount and intensity of physical needed to induce favourable changes within the inflammatory and platelet aggregation profile in CHD patients.

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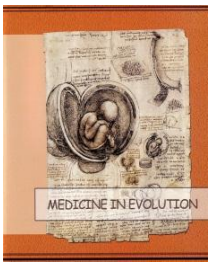
BAALC, WT1 AND FLT3 MOLECULAR DIAGNOSTIC ASSAYS IN CANCER



MIHALA ADRIAN[1], TAMAS LIVIU[1], IONITA HORTENSIA[2], SECLAMAN EDWARD[1], ANGHEL ANDREI[1]

[1]Department of Biochemistry and Pharmacology, University of Medicine and Pharmacy"Victor Babes"Timişoara, Romania
[2]Department of Internal Medicine I, University of Medicine and Pharmacy"Victor Babes"Timişoara, Romania

ABSTRACT

Acute myeloid leukemia (AML) is a heterogeneous group of clonal hematopoietic stem cell disorders characterized by accumulation of non-functional myeloblasts. Currently, cytogenetic abnormalities are the most important factors predicting clinical outcome in AML. However, half of adult AML patients younger than 60 have normal cytogenetics and very few markers have been consistently revealed to be predictive. The present study evaluates the expression of brain and acute leukemia, cytoplasmic (BAALC) and Wilms' tumor gene (WT1) and mutations of fms-like tyrosine kinase 3 (FLT3) in patients with AML. Patients selected from newly diagnosed cases of AML with normal cytogenetics and a control group of healthy individuals were studied. Total RNA and DNA from pretreatment blood samples of 6 AML patients were extracted. Quantitative Revers Transcription Polymerase Chain Reaction (RT-PCR) with gene-specific, intron spanning primers for BAALC, WT1 and glyceraldehyde-3-phosphate dehydrogenase (GPDH) as housekeeping gene was performed. Multiplex PCR reactions with primers for internal tandem duplication (ITD) and aspartic acid 835 (D835) were used to identify mutations of FLT3. One sample with modest BAALC overexpression and one sample with high WT1 overexpression were found. FLT3 mutations were detected in 2 leukemia samples (one sample with ITD and one sample with D835). BAALC, WT1 and FLT3 molecular diagnostic assays are fast and reliable method for evaluation of disease aggressiveness in AML patients with normal cytogenetics.

Key words: *AML, BAALC, WT1, FLT3-ITD, FLT3-D835*

Correspondence to:

Anghel Andrei
PhD, Professor
Address: 2 Eftimie Murgu, Timisoara, Romania
Phone: +40723603233
E-mail address: biochim@umft.ro

INTRODUCTION

Leukemias, a challenge to society and a cost factor because of their frequency, are usually divided into four major categories: acute lymphocytic leukemia, chronic lymphocytic leukemia, acute myeloid leukemia (AML) and chronic myeloid leukemia. The annual incidence rate of leukemias in Romania is 7.3 cases per 100000/year (both sexes, 2012) (1).

AML is a clinically and molecularly heterogeneous disease, mainly an adult's disease with a median age at presentation of 64 years. It accounts for 30 % of all adult leukemias (2). Currently, cytogenetic abnormalities are the most important factors predicting clinical outcome in AML. Patients with t (8; 21) and abn16q22 have cure rates of over 60 % with cytarabine but those with t (6;9) and abn3q26 have very poor outcomes after chemotherapy (3).

Approximately half of adult AML patients younger than 60 have normal cytogenetics. Although this group has an intermediate prognosis less than half are long-term survivors (4). A variety of molecular markers have been investigated to improve risk profile characterization of AML patients with normal cytogenetics (5,6).

Brain and acute leukemia, cytoplasmic (BAALC) is a gene implicated in normal hematopoiesis and the protein is almost exclusively expressed in neuroectoderm-derived tissues, hematopoiectic progenitor cells and acute leukemia. The gene is located at chromosome 8q22.3 and the protein sequence showed no homology to any known proteins or functional domains (7,8). In hematopoiesis, BAALC is aberrantly expressed in a subset of acute leukemias (9) and overexpression of BAALC has been shown to be an adverse risk factor in newly diagnosed AML patients with normal cytogenetics (10).

The Wilms' tumor gene (WT1) encodes a zinc finger protein, originally identified as a gene involved in genetic predisposition to the childhood kidney cancer (11). WT1 appears to be useful as molecular marker in AML patients especially for monitoring minimal residual disease (12). The expression of the WT1 gene in leukemic cells is higher than in normal bone marrow or peripheral blood cells (13).

Fms-like tyrosine kinase 3 (FLT3) gene belongs to the subfamily of class III tyrosine kinase receptors. FLT3 is recognized as one of the most frequently mutated gene in AML. About one third of patients have small internal tandem duplications (ITD) in the juxtamembrane region and one tenth of AML patients have point mutations that most frequently involve aspartic acid 835 (D835) of the kinase domain (14). Both mutations are involved in leukemogenesis by activating the FLT3 receptor (15).

MATERIAL AND METHODS

This study included 6 patients with a primary diagnosis of AML. Patients were classified as having normal cytogenetics on the basis of analysis of peripheral blood metaphases.

All leukemic samples (peripheral blood) were obtained at the time of diagnosis. The blood samples were collected into EDTA K3 tubes and immediately treated with RNA-later (Qiagen) and stored at -20°C. Total RNA and DNA were extracted from thawed samples using RiboPure - blood kit (Ambion) and QIAamp DNA Blood Mini Kit (Qiagen) following the manufacturer's directions. Total RNA from 16 healthy samples were used for

the controls. The quantity and quality of isolated total RNA and DNA were verified with NanoDrop ND-1000 (Thermo Scientific).

FLT3 mutations. Multiplex PCR reactions were composed of 1X Master Mix (Promega) and 10 ng DNA. Reactions were run using the PXE0.2 Thermal Cycler (Thermo) in a final reaction volume of 50 µl. Primers for the ITD mutation were 11F, 5′-GCAATTTAGGTATGAAAGCCAGC-3′ and 12R, 5′-CTTTCAGCATTTTGACGGCAACC-3′ and primers for the D835 mutation were D835F: 5′-GTAAAACGACGGCCAGCCGCCAG GAACGTGCTTG-3′ and D835R: 5-CAGGAAACAGCTATGACGATATC AGCCTCACATTGCCCC-3′ previously described labeled [16]. Thermocycling conditions were: initial denaturation step at 95°C for 2 minutes, 30 cycles of 95°C for 30 seconds, 56°C for 1 minute, 72°C for 2 minutes, with a final extension at 72°C for 10 minutes.

A volume of 10 µl of PCR product was digested with 20 U EcoRV (NEB) at 37°C for 30 minutes. After restriction enzyme heat inactivation, 10 µl of product was run on 2 % NuSieve GTC agarose (Lonza) along with a 50 bp DNA Ladder (NEB). The DNA fragments were visualized by ethidium bromide staining.

BAALC and WT1 expression. Quantitative RT-PCR used QuantiTect SYBR Green one step RT-PCR (Qiagen), with gene-specific, intron spanning primers for BAALC, WT1 and glyceraldehyde-3-phosphate dehydrogenase (GPDH) as housekeeping gene. Comparative real-time Revers Transciption Polymerase Chain Reaction (RT-PCR) assays were performed in triplicate (leukemia samples) or duplicate (control samples) with 5 ng of total RNA. Reactions were run using the LightCycler 1.5 (Roche) in a final reaction volume of 20 µl. Amplification was carried out at 50°C for 30 minutes (revers transcription step) and 95°C for 15 minutes (DNA polymerase activation step), followed by 45 PCR cycles at 95°C for 15 seconds, 55°C for 30 seconds and 72°C for 30 seconds. Positive and negative controls were included in all assays.

RESULTS

The FLT3-ITD mutations were analyzed using 11F and 12R primers designed to amplify exon 11 and 12 of gene. A wild-type PCR fragment generates a 330 bp band on gel-electrophoresis whereas ITD mutations yield PCR products greater than wild type.

D835 mutation was detected using D835F and D835R primers. The revers primer contains an EcoRV restriction site in order to discriminate the undigested PCR product (150 bp) from digested D835 mutant (129 bp). The wild-type PCR product digestion generates a small 80 bp fragment while D835 abolishes the EcoRV restriction site (Fig. 1).

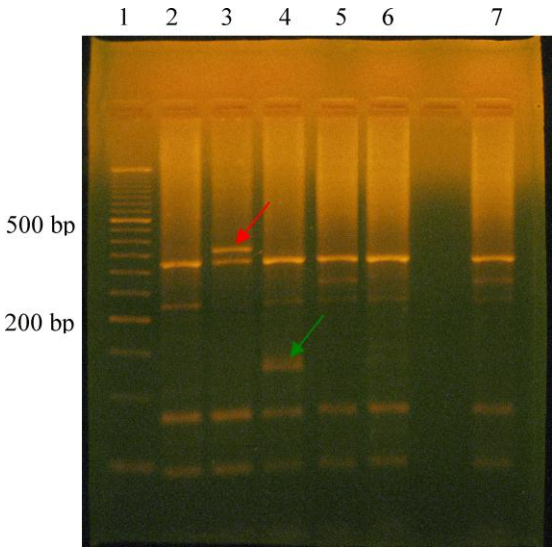


Figure 1. FLT3 mutations. Line 1: 50 bp DNA ladder, line 2-7: AML samples. Line 3: ITD mutant (red arrow) ~350 bp. Line 4: D835 mutant (green arrow) 129 bp. Line 2, 5, 6 and 7: no mutations. In line 3 and 4, both mutant and wild-type fragments are visible on gel-electrophoresis because DNA was extracted from o mixture of leukemic and normal cells.

The comparative cycle threshold (C_T) method was used to determine the relative expression levels of BAALC and WT1. This involves comparing the C_T values of the leukemia samples of interest with a control (RNA from healthy donor). The C_T values of both the control and the leukemia samples are normalized to an endogenous housekeeping gene (GPDH). The comparative C_T method is also known as the $2^{\Delta\Delta CT}$ method, where

$\Delta\Delta C_T$ = ΔC_T healthy control - ΔC_T leukemia sample

Here, ΔC_T healthy control is the C_T value for any sample normalized to the endogenous housekeeping gene and ΔC_T leukemia sample is the C_T value for the control also normalized to the endogenous housekeeping gene.

Relative levels of BAALC and WT1 expression were analyzed in 6 patients. Cycle threshold for BAALC, WT1 and housekeeping gene in healthy control and leukemia sample are presented in Table 1 and Table 2.

Table1. Cycle threshold for BAALC, WT1 and housekeeping gene in healthy control expressed as mean ± standard deviation (SD)

Healthy control (n = 16)	C_T		
	BAALC	WT1	GPDH
Mean ± SD	28.10 ± 0.74	35.89 ± 0.75	23.09 ± 0.51

Table 2. Cycle threshold for BAALC, WT1 and housekeeping gene in leukemia samples. Results expressed as mean

Leukemia sample	C_T		
	BAALC	WT1	GPDH
1	27.27	36.44	22.18
2	27.04	28.83	21.08
3	29.36	32.45	19.95
4	28.58	34.16	22.55
5	29.74	35.75	25.12
6	30.33	34.83	22.97

The relative expression levels of BAALC and WT1 calculated according to comparative C_T method are presented in Fig. 2.

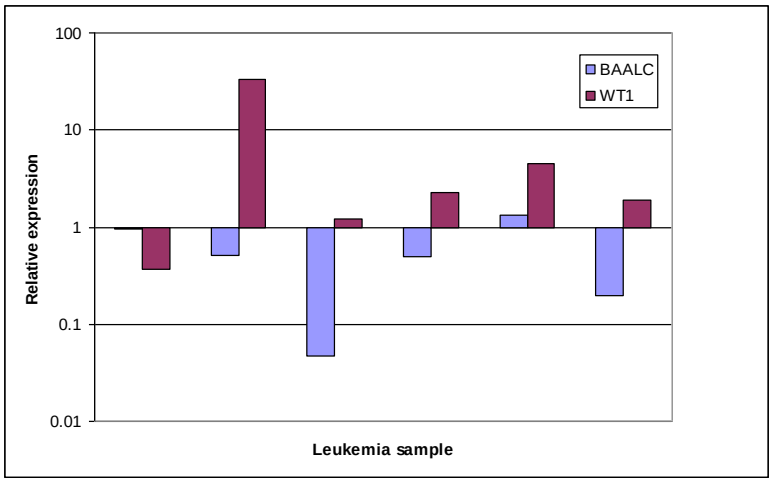


Figure 2. Relative expression levels (logarithmic scales) in leukemia samples

DISCUSSIONS

At the time of diagnosis approximately half of AML adult patients lack clonal chromosome aberrations. Although this group has an intermediate prognosis, less than half are long-term survivors. For AML patients with normal cytogenetics, the identification of novel molecular markers is necessary to design new risk-adapted treatment. BAALC overexpression is recognized as one of the most important risk factors associated with reduced survival.

DNA was extracted from peripheral whole blood which contains o mixture of normal and leukemic cells. FLT3 mutants (if present) are visible on gel-electrophoresis together with wild-type fragments. Moreover, it is possible that ITD mutations produce a new EcoRV restriction site. This event is unlikely, but if it happens, EcoRV digestion could generate products with smaller size.

European LeukemiaNet consortium validated molecular kits aimed at standardizing, detecting and quantifying WT1 and BAALC gene transcripts (17). FLT3 mutation assays are also available for in vitro diagnostic in patients with AML (18).

CONCLUSIONS

In this small study of AML patients with a normal karyotype, we have identified one modest BAALC overexpression in sample 5 and one high WT1 overexpression (33 times) in sample 2. We found BAALC underexpression in sample 3 while modest WT1 overexpression was established in sample 3, 4, 5 and 6**.** FLT3 mutations were detected in 2 leukemia samples (one sample with ITD and one sample with D835).

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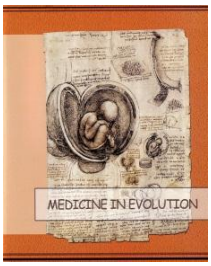

RISK FACTORS INVOLVED IN NOSOCOMIAL INFECTIONS REGISTERED TO ORTHOPEDIC PATIENTS FROM THE COUNTY CLINIC EMERGENCY HOSPITAL OF BRASOV, ROMANIA

RAUTIA CALIN[1], NEMET CODRUTA[2]

[1] Faculty of Medicine, Transilvania University of Brasov, County Clinic Emergency Hospital of Brasov, Romania
[2] Faculty of Medicine, Transilvania University of Brasov, Romania

ABSTRACT

Aim and objectives: This paper aims to rank the involved risk factors in nosocomial infections recorded in an orthopedic ward.

Material and Method: This paper is an analytical epidemiological case-control retrospective study carried out in two groups of patients during January 2009 - December 2010.

Results: 37 cases of nosocomial infections have been identified. Of all nosocomial infections detected, 91.89% are of postsurgical wound origin. The duration of surgery exceeded 2 hours in 43.24% of cases. The average number of hospitalization days for both groups was 22.22. Antibiotic prophylaxis was performed in 86.49% of patients. The average number of hospitalization days for both groups was 22.22.

Conclusions: The following risk factors were identified: the duration of surgery an hour or above, over 10 days of hospitalization, the presence of a contaminated wound, the presence of a medical-surgical emergency; antibiotic prophylaxis; urban environment and comorbidities.

Key words: *nosocomial infections, ortopedics, risk factors, case-control study*

Correspondence to:

Dr. Nemet Codruta
Address: 28 Faget Street, Block 1, 6th flat, Brasov, Romania
Phone: +40723611488
E-mail address: codruta_nemet@yahoo.com

INTRODUCTION

Nosocomial infections can cause prolonged hospitalization, long term disability, increased resistance to microbial agents, high costs for health-care systems, high costs for patients and their families, and a considerable number of deaths [1].

AIM

This paper aims to rank the involved risk factors in nosocomial infections recorded in an orthopedic ward.

OBJECTIVES

The research objectives were: identifying risk factors for nosocomial infections in the orthopedic ward; highlighting the most common germs associations and their degree of resistance to antibiotics; discussing the antibiotic schemes used in the current practice; identifying additional costs for the nosocomial infections detected through the pinpointing of the number of days of hospitalization.

MATERIAL AND METHODS

This analytical epidemiological case-control retrospective study was carried out in two groups of patients, out of 5907 patients admitted to the orthopedic ward of County Clinic Emergency Hospital of Brasov, during the period January 2009 - December 2010. According to the case definition, a group of 37 patients was identified that developed nosocomial infections during inpatient care. A control group of 37 was selected that displayed similar characteristics, but who did not develop nosocomial infections.

The data was collected from the patients' observation charts and for statistical processing Microsoft Excel and EpiInfo were used.

The applied nosocomial infection case definition was obtained from the Order of the Ministry of Public Health, number 916 of 2006.

RESULTS

The gender distribution of the group that developed nosocomial infections during hospitalization, in the orthopedic ward, did show a higher frequency in males (81.08%) compared to females. Furthermore, the frequency proved higher in patients from urban areas (78.38%) higher than those from rural areas.

The average age at which nosocomial infections were developed was 50.78 years. The minimum age was 24 and the maximum 83 years. Age groups between 40-49 and 50-59 years old were equally affected by nosocomial infections, 21.62%, closely followed by patients over 70 (18.92 %). The least affected was the age group 20-29 (10.81%).

Most nosocomial infections in patients admitted to the orthopedic ward were those relating to a postoperative wound (91.89%), followed by urinary infections, 5.41%. Nosocomial pneumonia (2.70%) was recorded in patients whose length of hospitalization exceeded 15 days.

In 67.57% of patients with nosocomial infections fever was recorded for a minimum of 3 days after the surgery, compared to 13.51% in the control group which presented fever for maximum a day. It can be inferred that fever lasting more than three days after the surgery is the result of nosocomial infections. We calculated relative risk, RR = 2.89 (1.73 to 4.81), OD = 1.76 (3.15 to 3.80), chi square = 20.04, p-value = 0.0000076. Therefore, the data is statistically significant.

Regarding the duration of surgery, in 43.24% of cases it exceeded

2 hours (we considered a risk factors the duration of surgery for over an hour). It was confirmed that the duration of surgery longer than an hour is a risk factor for the occurrence of nosocomial infections (RR = 6.93 (1.83 to 26.28), OD = 18.47 (3.51 to 129.57), chi square = 19.21, p-value = 0.000017). The data is once again statistically significant.

A total of 78.38% out of the 37 patients selected for the group, were admitted to the hospital by the emergency services as a result of road accidents or several other serious injuries. Emergency is a risk factor in the development of nosocomial infections. The data is statistically significant, we calculated: RR = 2.08 (1.12 to 3.88), OD = 3.83 (1.25 to 12.04), chi square = 7.06 and p-value = 0, 0079002.

Antibiotic prophylaxis was performed in 86.49% of patients within the 2 groups. Of the 37 patients who developed nosocomial infections, 32 received antibiotic prophylaxis. However, only one person received it correctly.

Prophylactic antibiotic was performed as monotherapy in 56.25% of the cases. We calculated: relative risk: RR = 1.36 (0.66-2.82), OD = 1.77 (0.45-7.13), chi square = 0.84 and p = 0.3594401.

For perioperative prophylaxis the class of antibiotics most often used, was that of cephalosporin, in a percentage of 77.78%. Prophylactic administration of cephalosporin as monotherapy consisted in mostly those medications from the third generation, such as Cefort or Cefotaxime, (64.29%), compared to those of second generation, such as Axetin (35.71%).

For the prevention of nosocomial infections a combination of two antibiotics was also used. The highest percentage of 78.57% was oxacillin combined with gentamicin. Equally, we can also highlight the use of cephalosporins in combination with gentamicin or another glycopeptide antibiotic in lower percentages of 7.14%.

The pathological products investigated (88.89%) were extracted from the wound secretion, followed by urine analysis, periprosthetic fluid and the fluid present in the hematoma. There were patients that were infected with a single microorganism (25%), but three quarters of them presented associations of two or three germs.

A total of 52.46% of patients were infected with Gram-positive bacteria, of which Staphylococcus was identified most frequently, followed by Enterococcus. Staphylococcus epidermidis was found in a percentage of 3.13%. From the Staphylococcus species, we encountered during the two years of study, a rate of 47.36% for methicillin-resistant Staphylococcus aureus (MRSA). In 47.54% of patients, were found Gram negative bacteria, most frequently was Enterobacter, followed by Escherichia coli and Acinetobacter baumanii.

All the isolated organisms were resistant to multiple antibiotics.

The average number of hospitalization days for both groups was 22.22, with a minimum of 2 days and a maximum of 76 days. The average number of hospitalization days for the nosocomial infection was 34.02 days, with a minimum of 8 days and a maximum of 76 days. The risk of those hospitalized more than 10 days to develop a nosocomial infection is 4.80 higher than of those hospitalized less than 10 days. We calculated: relative risk: RR = 4.80 (1.31 to 17.53), OD = 13.13 (2.33 to 96.82), chi square = 13.23 and p-value = 0, 0002757. The data is statistically significant.

In our study, the average cost of hospitalization, in patients with nosocomial infections, is EUR 2042.01, with a minimum of EUR 719.22 and a maximum of EUR 2237.98. In patients that did not develop a nosocomial infection the cost is EUR 768.18 with a minimum of EUR 281.08 and a maximum of EUR 1438.70.

Most patients diagnosed with nosocomial infections (94.59%) presented wound secretion during the first 2 weeks after surgery.

In 48.65% of studied patients with nosocomial infections systemic comorbidities were not detected. A total of 40.54% had a mild systemic disease, 8.11% had severe systemic disease and one person (2.70%) had a disabling systemic disease. The comorbidity is a risk factor for nosocomial infections. From the control group 72.97% of patients were without medical history, 24.32% with a mild systemic disease and 2.70% with major systemic diseases.

According to the contamination class, 64.87% of patients who developed nosocomial infections were with contaminated wound, followed by those with possibly contaminated wound (24.32%) and by those with clean wound (10.81%). The presence of contaminated wounds is a risk factor for nosocomial infections in our study. Relative risk was calculated RR = 2.42 (1.48-3.97), OD = 6.69 (2.14-21.73), chi square = 14.10 and p-value = 0.0001738. Hence, the data is statistically significant.

DISCUSSIONS

Nosocomial infections produced prolonged hospitalization, long term disability, and increased resistance to microbial agents. Furthermore, they triggered higher costs for healthcare systems, high costs for patients and their families and a large number of deaths,[1,2]. In Europe, every year, more than four million people suffer at least one nosocomial infection and 37.000 die from complications thereof,[1].

The incidence of nosocomial infections in the group of patients hospitalized in 2009-2010, in the orthopedic ward, was at 0.62%. According to the case definition this amounted to a total of 37 patients with nosocomial infections.

The study's limitation was the poor compliance of the physicians declaring the nososcomial infections.

Patients from urban areas (81.08%) are most likely prepossessed to severe accidents and injuries at work or at home than those from rural areas.

Age groups between 40-49 and 50-59 years old were equally affected by nosocomial infections (21.62%). Those groups containing the active population are frequently at risk of injury, followed closely by patients over 70 years (18.92%), who, because of the age are considered more exposed to falls and serious injury.

Of all nosocomial infections detected, 91.89% are of postsurgical wound origin.

The surgery duration depends on the severity of trauma. Therefore those that exceed one hour are considered a risk factor for the appearance of nosocomial infection, with a relative risk 6.93 times higher than those lasting less than one hour. The risk of the development of a nosocomial infection, in patients that were admitted in the hospital with contaminated wounds, generally those with trauma from car accidents or broken bones, is 2.42 times higher than those who had a clean wound or a possibly contaminated one.

The class of preoperative antibiotics most commonly used was that of cephalosporins, in a percentage of 77.78%, to the detriment of narrow-spectrum antibiotics. According to the Guidelines issued by the Romanian Society of Intensive Care on perioperative antibiotic prophylaxis, conditions related to dosage or time of administration were not strictly followed.

The Romanian Guide of preoperative antibiotic prophylaxis specifies that the antibiotic should be administered intravenously. Other

means can be used, such as the oral antibiotic. In artificial prosthesis is recommended the impregnation of the cement with antibiotic, in addition to intravenous antibiotic,[3]. In our research, most of the patients received intravenous antibiotic. However, in two of the cases the antibiotic was administered orally as pills, despite not fitting necessarily in the conditions listed above. We could not encounter any cases of joint prosthesis, in which there was an evidence of cement impregnated with antibiotic.

Although this guide has been implemented in our country in 2009, when our study began, its directions were not complied with, regarding the monotherapy. Mainly broad-spectrum antibiotics were used at the disadvantage of narrow-spectrum antibiotics; time exceeded the recommended duration of administration (87.5% of cases), and the route of administration was not always the recommended one. In this context, the antibiotic prophylaxis becomes a risk factor in the development of nosocomial infection, and not a protective one, which is highly worrying.

A total of 75% of patients were infected with two or three microorganisms with more than half of the patients infected with Gram-positive bacteria which are circulated by staff. In our study 47.54% of patients were infected with Gram-negative bacteria that are circulated through hospital environment elements.

Staphylococcus epidermidis, a major agent responsible for bacteremia from intravenous catheter or surgical prosthetic material infection, and orthopedic or cardiovascular was found in 3.13%.

Nosocomial infections increased hospital costs by about 265%.

The patients were informed that data from the case would be submitted for publication, and gave their consent.

CONCLUSIONS

1. There is a lack of compliance with the indications of preoperative antibiotic Guide, regarding the use of monotherapy; the use of cephalosporins, in the detriment of narrow-spectrum antibiotics, time and the form of administration. The antibiotic prophylaxis was also not in full compliance with the guide. Therefore, it has become a risk factor for the development of nosocomial infections.
2. Nosocomial infection increases the cost of hospitalization.
3. Our research has identified the following risk factors for the development of a nosocomial infection, in patients admitted to the orthopedic ward: the duration of surgery of over an hour, days of inpatient stay over 10, the presence of contaminated wounds, and the presence of a medical-surgical emergency; antibiotic prophylaxis; urban environment and comorbidities.

CONFLICT OF INTEREST STATEMENT

No financial or personal relationships are maintained with other people or organizations that could inappropriately influence or bias this paper.

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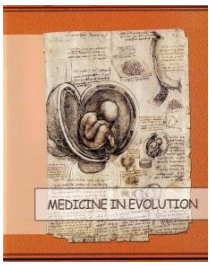
OCCUPATIONAL POSTURAL DEFICIENCIES INDENTISTS, REVEALED BY THE THE PLANTARPRESSURE STUDY



FARKAS ANDREI ZOLTAN[1], SORIN PENTA[2], MIRELLA ANGHEL[3], ARGESANU VERONICA[4], LIGIA ADRIANA STANCA MUNTIANU[5]

[1]IOSUD, Mechanics Department, UPT
[2] 2nd Department Ergonomics and Scientific Research Methodology, Faculty M.D., UMF Carol Davila Bucharest
[3] 1st Department Oral Diagnosis and Ergonomics, University of Medecine and Pharmacy"Victor Babes"Timisoara
[4] Mechanics Department, UPT
[5]1st Department Removable Denture, Faculty M.D., UMF Carol Davila Bucharest

ABSTRACT

Aim: Dental treatments are stressful for both the patient and the dentist. For the dentist the profession leaves deep impressions on his mind as well on his body. In the present study we focused on physical long term influences of every day practice on dentist's physical body, and the consecutive adaptations of the body trying to cope with the stress.

Materials and method: Because plantar pressure study reveals interesting facts about the state of the physical body, we studied the plantar pressure of three female dentists having worked in the field for: 5. 20 and 34 years, in order to evaluate the profession's impact over different periods of time. The results were compared for normality with the results of three groups of young sportsmen, and for confirmation of the adaptative changes to the results of two male dentists with 35 years of experience.

Results and discussions: adaptative changes (in response to professional stress) were found in all dentists and similar adaptations were found in the sportsmen, depending on the sport they practiced.

Key words: *dentist professional stress, body adaptative changes, plantar pressure*

Correspondence to:

Penta Sorin
UMF Carol Davila Bucuresti, Cat. Diagnoswtic Orodentar, Ergonomie si Metodologia Cercetarii Stiintifice
Phone: +40766632249/+40785263440
E-mail address: sorinpenta@yahoo.com

INTRODUCTION

Posture (stance) is a process of unstable equilibrium that requires correction by continuous muscle activity[1]. This permanent balance around equilibrium position, although unconscious, triggers, according to Galozzi (2005)[2] a whole system, the tonic postural system, which\ has multiple roles, from counterbalancing gravity to external forces annihilation and posture correction.

The tonic postural system comprises several"muscle chains"discovered by Françoise Mézières[3], composed of muscles, tendons and fascia[4]. Their anatomy and functions was described by Valentino[5], presenting two main classifications: - Stuyf Godelieve Denys[6] and Busquet[7].

Generally they are described as running straight on the body, or crossing from one side to the other. The fundamental concept is a functional cooperation[8] of all the components and the capacity to transfer information[9] between them[10] in a mutual process[11], both ways, upwards as well as downwards. This is why any (and every) malfunction in the human body has a corresponding change in the pattern of plantar pressure. So, the study of plantar pressure reveals the existing problems of the whole human organism, at a holistic level.

Aim and objectives

Human body responds to the physical stress by an adaptative process, both mentally[12] and physically[13]. Our aim was to determine the long term adaptative changes in dentist's body caused by professional stress, and our objectives were to identify the changes in dentist's posture using a noninvasive method, plantar pressure study.

MATERIAL AND METHODS

In this study we analyzed the plantar pressure of three female dentists having a work experience of 5, 20 and 34 years, looking for the impact of professional stress on their physical body (adaptative changes).

The results were compared to those of two male dentists with 35 years of experience (to verify the existence of similar adaptative changes), and to those obtained by three groups of young sportsmen practicing different sports (as normality pattern), in search for specific adaptative changes.

Plantar pressure was studied using a mat with barosensors to record it, and a dedicated software, Milletrix 2.0 produced by"Diagnostic Support Postural Biomedicine SRL"Italy.

This device allows us to measure plantar pressure and ground reaction force during static and dynamic activity. During static recordings information were gathered about the characteristics of the movements needed to acquire the balance of human body (figure number 2), as well as the value of plantar pressure for each point of the feet surface (figure number 1).

The acquisition of static information was completed by a MTX scan (figure number 3).

During dynamics, were recorded the characteristics of the plantar pressure of a complete gate cycle (figure number 4).

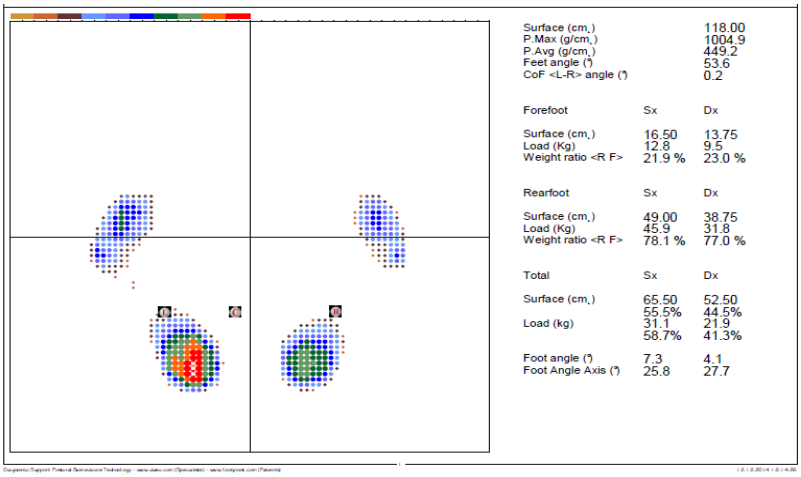


Figure 1.

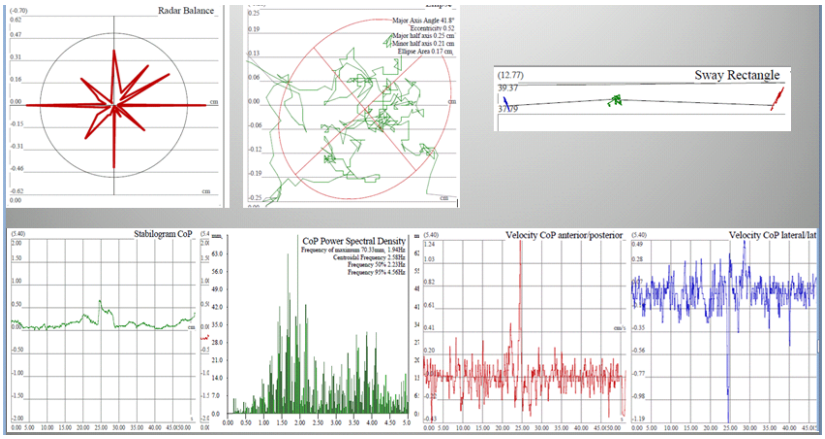


Figure 2.

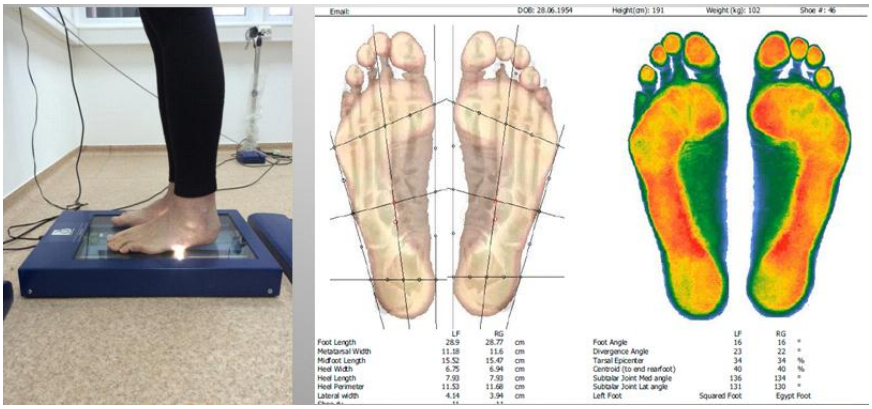


Figure 3.

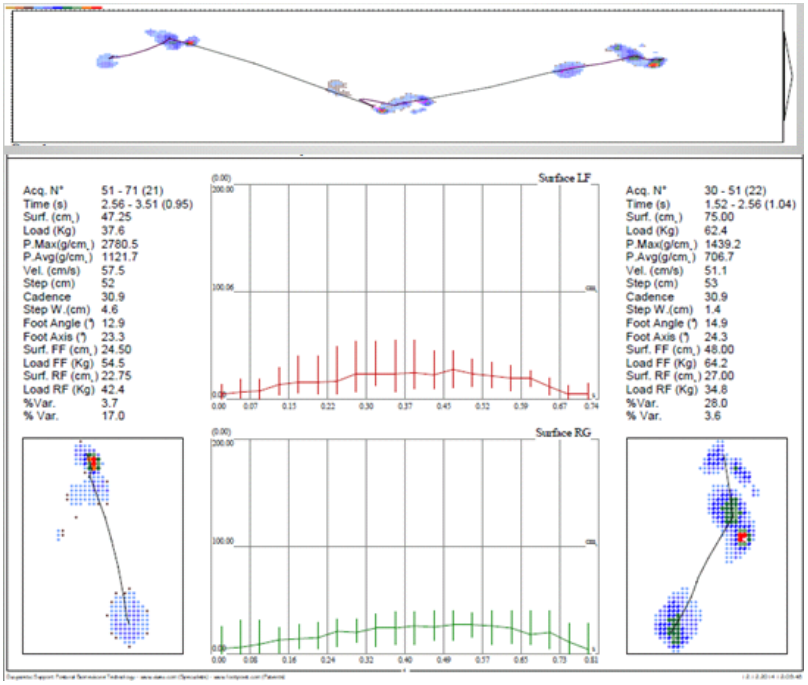
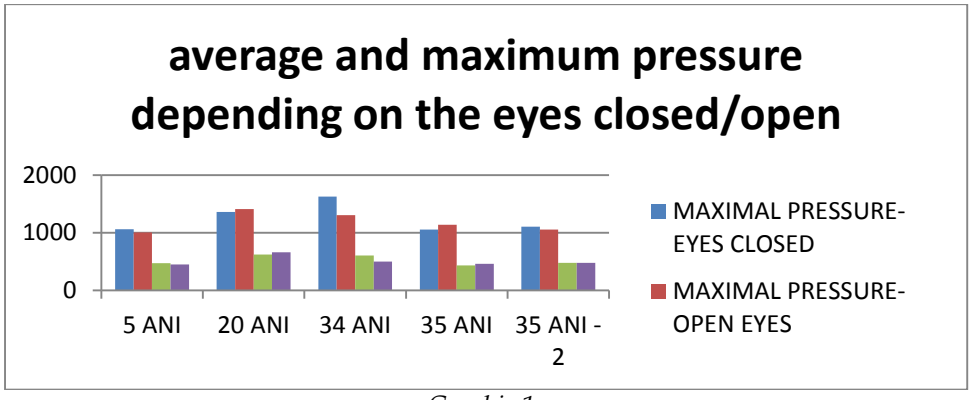


Figure 4.

RESULTS AND DISCUSSIONS

The results obtained for each of the five dentists and for the three groups of sportsmen are too elaborated to be presented in extenso in this article, so we will present only the most interesting findings.

The plantar pressure shows a difference between the static position with eyes closed compared to the one with open eyes due to the increased importance of plantar baroceptors for the stability of the body:



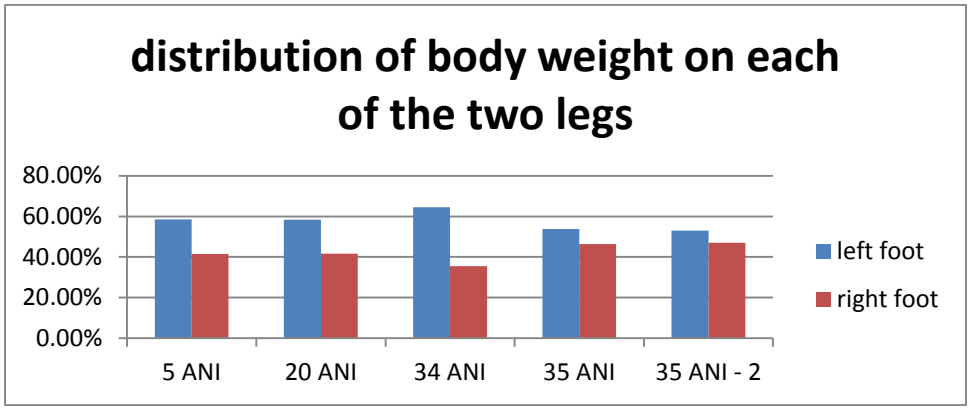
Graphic 1.

The posture of all the dentists shows a pattern of adaptative changes of the body to the professional stress, both in static posture as well as in dynamics.

A. Static posture analysis showed adaptative changes:

- The distribution of body weight on each of the two legs is different, the left foot bearing a major part of the weight
- Amplitude of the movements to gain equilibrium is decreasing, but their number increasing, the total amount of these movements, given by the area surrounded with the red line of radar chart shows an increase, parallel with increasing length of the profession, as an adaptative process to the necessity of body stability providing a steady support for precise movements

needed during medical treatment (figure number 5):



Graphic 2.

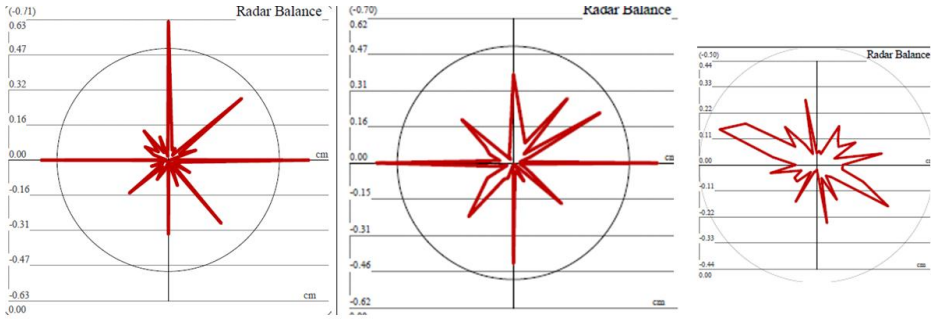
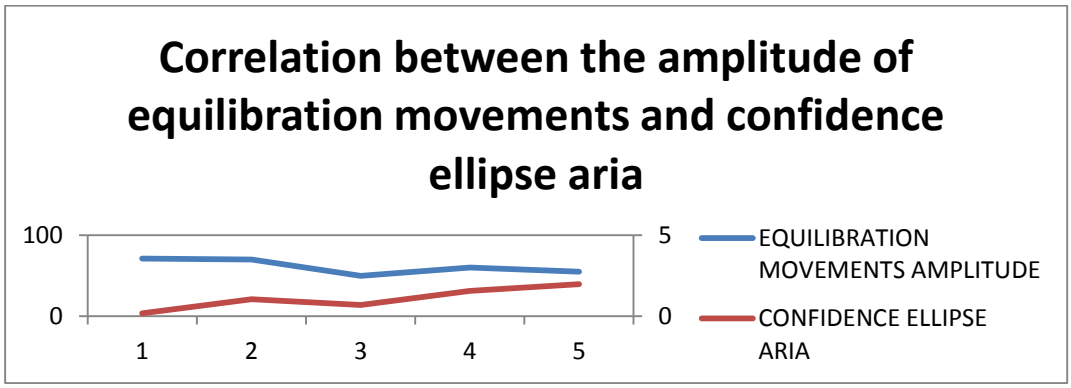


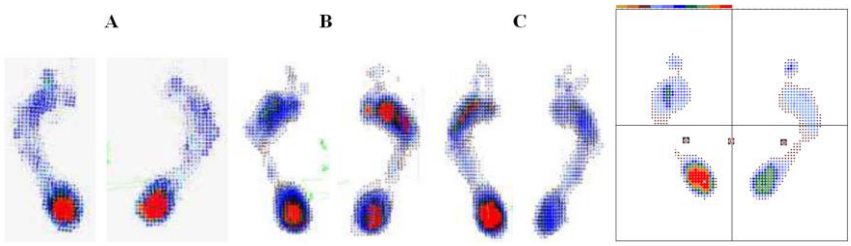
Figure 5.

This increased stability is proved also by graphic number 3 showing a relevant negative correlation between the amplitude of equilibration movements and confidence ellipse aria (correl. coefficient: -0,42197).



Graphic 3.

The results of the plantar pressure for the three groups of sportsmen were different in function of the sport performed –A- volleyball; B- rugby; C- football (typical dentist on the right side):

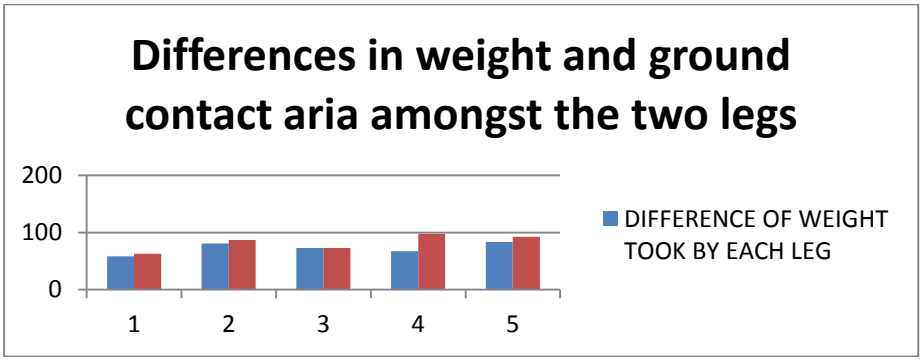


B. Dynamic analisis showed also adaptative changes:

Gate indicates similarities between the five examined, dentists, meaning that all of them have an unbalanced gait with different weight taken by each of the the two legs, with different surfaces with which the legs touch and press the ground.

In order to simplify the graphical presentation we:

- considered to be 100% the surface area of the foot that has greater ground contact and it is not plotted,
- plotted in the graphic only the foot surface which has a smaller ground contact area, which is represented as a percentage of the surface of the foot with greater contact area
- the same was done for the difference in weight taken by the legs see graphic number 4:



Graphic 4.

All five examined dentists had a preponderant left heel support. If we perform a cross-examination with the results of the three groups of sportsmen we see that only the sportsmen who need the right foot for dribbling have the same plantar pressure pattern.

CONCLUSIONS

All five dentists have adaptative changes residing in an unbalanced posture that follow the same pattern, with:

- The body center of gravity deviated towards back and to the left. This is an adaptative change which offers to the right leg, the necessary freedom to control the commands of the equipment (being relieved partially from weight bearing). It is interesting to note that after only five years of practice this adaptative imbalance is already installed and then remains constant throughout life. This postural adaptative change to practice is similar to the adaptative change of the football (soccer) players who need the right foot for dribbling. This adaptation is specific, since the other sportsmen which do not need a"free"foot (volleyball or rugby) do not have it.
- The presence of a rotation of the upper half of the body, an adaptative change which makes the right foot to be placed more advanced than the left. It is a postural consequence of practice, since the torsion motion is one of the most common movements in the profession, especially in the recommended sitting position.
- The modified plantar surfaces, with the body weight placed more on the left leg, and toward the back, an adaptative change facilitating rotational movements of the whole body on the left heel.

- During gate the adaptative changes are also evident, the posture remains unbalanced, existing a difference in:
  - The surface of ground contact of each leg.
  - The weight supported by each foot in dynamics.

This is inevitable when the profession has already left its mark on our physical body.

The movements to maintain balance show also adaptative changes to the profession, because, with the passing years of practice these movements tend to become smoother, of a lower amplitude, but faster, satisfying the need for a stable support to ensure maximum precision of hand movements required during dental treatment. This adaptative change is not checked by all measured indicators (at all the examined dentists), so it can be a feature influenced also by other extra-professional factors.

PATERNITY

We hereby declare that each author has equally contributed to take public responsibility for the content.

The key person responsible for the article: Farkas Andrei Zoltan (Email: farkasandreiz@gmail.com)

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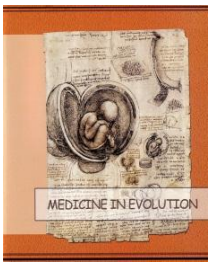
PHOTODYNAMIC THERAPY FOR THE TREATMENT OF PSORIASIS



MIRCEA TAMPA[1], MARIA ISABELA SARBU[2], ALEXANDRA ELENA SARBU[3], IOAN BULESCU[1], CLARA MATEI[1], ADRIAN DUMITRU[4], SIMONA ROXANA GEORGESCU[1]

[1]"Carol Davila"University of Medicine and Pharmacy, Bucharest
[2]"Victor Babes"Hospital for Infectious and Tropical Diseases, Bucharest
[3]Emergency Eye Hospital, Bucharest
[4]Department of Pathology, Universitary Hospital Bucharest

ABSTRACT

Psoriasis vulgaris is an immune mediated polygenic, chronic, recurrent dermatosis with a worldwide distribution. Currently available treatments are either messy or difficult to apply or associated with serious adverse reactions. Photodynamic therapy (PDT) is a minimally invasive therapeutic method which has been more extensively used for the treatment of various dermatological disorders in the last years. It has shown promising results in the treatment of localized psoriasis. However, currently used photosensitizers have unfavorable adverse event profiles and therefore the identification of new sensitizers is mandatory. The present article aims to look over the medical literature in this field.

Key words: *photodynamic therapy, psoriasis, photosensitizer*

Correspondence to:

Mircea Tampa
MD, PhD
Address: Mihai Bravu 281, 030303, Bucharest
Phone: +40726382600
E-mail address: tampa_mircea@yahoo.com

INTRODUCTION

Psoriasis vulgaris is an immune mediated polygenic, chronic, recurrent dermatosis with a worldwide distribution. It is characterized by the occurrence of well demarcated erythematous papules and plaques, covered by thick, silvery-white scales, most commonly involving the knees, elbows, sacral area, scalp, but also hands, feet, trunk and nails. It has a prevalence of 1-2% of the world's population. It can occur at any age but it usually appears between the ages of 15 and 30 years. (1,2)

A whole repertoire of therapies is available for the treatment of psoriasis, both topical and systemic. Topical treatments are associated with low risk of systemic side effects but patients find them messy and their administration is time consuming. The use of systemic treatments is limited by serious side effects and demanding schedules. Biological therapies are efficacious treatments for psoriasis but they are very expensive and are not recommended for localized forms of the disease. Phototherapy and photochemotherapy using psoralen and ultraviolet radiation are first-line treatments for moderate to severe psoriasis. They are highly effective but are associated with an important risk for non-melanoma skin cancers and melanomas, but also epithelial malignancies in other organs. Photodynamic therapy was introduced in the treatment of psoriasis as an alternative photochemotherapeutic method with lower carcinogenic risk. (1,3,4)

Photodynamic therapy (PDT) is a minimally invasive therapeutic method which has been more extensively used for the treatment of various dermatological disorders in the last years. In the US it has been approved for the treatment of actinic keratoses. Nevertheless it has been used, off label, for the treatment of basal cell carcinoma, squamous cell carcinoma, Bowen's disease, acne vulgaris, rosacea, hidradenitis suppurativa, psoriasis vulgaris, microbial diseases, alopecia areata, photoageing and rejuvenation- among others, with some good results (5). PDT requires the simultaneous presence in the target area of three essential components: a photosensitizer - a light activated chemical compound for selective cell killing-, light and oxygen. (6,7)

The pathogenesis of psoriasis is very complex and involves interactions of multiple cytokines, chemokines and growth factors. Psoriatic lesions have large amounts of $CD4^+$ and $CD8^+$ T-cells. $CD4^+$TH1 cells produce INF- γ which amplifies the production of IL-23 in the dendritic cells which expand $CD4^+$-T cells subsets (Th 17 and Th 22). $CD4^+$-T cells produce interleukin IL-17, IL-21 and IL-22 which have an important role in maintaining inflammation. IL-17 produces neutrophil chemotaxis and angiogenesis, IL-21 is involved in naive T-cell differentiation to TH17 while IL-22 leads to keratinocytes hyperproliferation. TNF-α is produced by activated T-cells and dendritic cells. $CD8^+$ cells produce IL-17, IL-22 and INF-γ, thus promoting the production of chemokines, antimicrobial peptides, growth factors thereby promoting epidermal hyperplasia and activation of the keratinocytes which produce IL-8 which increases INF-γ production by T-cells and attracts neutrophils to the epidermis and IL-17 and IL-5 which influence the turnover of $CD8^+$ cells. (1,3)

PDT mechanism: the photosensitizer accumulates in selected cells and is activated by luminous radiation of an adequate wavelength, therefore switching from a *ground singlet state* to an *excited singlet state*. This state is not stable and can either result in desexcitation by energy release or in transition to a triplet state which allows the participation in type I

photodynamic reactions (oxidoreduction reactions) and type II reactions (energy transfer reactions) resulting in the occurrence of reactive oxygen species such as hydroxyl radical (OH), singlet oxygen ($^1O_2$) and superoxide anion (O_2^-). Singlet oxygen is very aggressive and is responsible for some of the destructive effects of PDT, together with the induction of apoptosis. (5,8)

On the other hand PDT has a direct action on immune cells in general and T-cells in particular. Therefore it has been shown that activated T-cells incubated with photosensitizers were killed selectively after light exposure, as compared to their not-activated equivalents and it was thereby hypothesized that PDT may have an important role in the treatment of T-cell mediated diseases such as psoriasis (4,9).

Boehncke et al. showed in a study performed in 1994 on six patients with psoriasis that PDT with red light decreases the release of TNF-α, IL-1 β and IL-6 in mononuclear cells in a similar, yet less potent, fashion as PUVA therapy. (9,10)

A study performed by Smits et. al in 2006 on 8 patients with psoriasis showed that the lesions treated with PDT showed a decrease in the $CD4^+$, $CD8^+$ and CD450RO cells, as well as $Ki67^+$ nuclei (non-histone nuclear protein closely connected with cell proliferation) and an increase in the K10 expression, thus showing that PDT induces normalization of differentiation and infiltration of relevant T-cell subsets.(9,11).

PHOTODYNAMIC THERAPY AND PSORIASIS

The first reports regarding the use of photodynamic therapy date back to year 1900 when Oscar Raab, a medical student from Munich, discovered that when paramecia cells (*Paramecium caudatum*) were exposed to either acridine orange or a light source, they did not die, but when they were exposed to both acridine orange and the light in the same time the cells gradually lost motion and were unable to divide and died within two hours. (12) In 1937 H. Silver reported the first case of psoriasis vulgaris treated with hematoporphyrin and UV light. However it was only in 1989 that the real PDT was used in the treatment of psoriasis when Meffert et. al. described the treatment of psoriasis with topical hematoporphyrin and a halogen lamp emitting light with a wavelength of 375 to 750 nm. (4)

Numerous studies have been performed since the early 1990s to assess the efficacy of photodynamic therapy in psoriasis, especially with topical δ-aminolevulinic acid (ALA). Thereby, in 1990 Kennedy et al reported that topical ALA determined selective accumulation of protoporphyrin IX (PpIX) in the psoriatic lesion and in 2002 Bissonette et al reported that oral administration of ALA also resulted in selective accumulation of PpIX in the psoriatic plaque, with a tenfold increase in fluorescence 3-5 hours after administration, as compared to normal skin. (13-16)

In 1999 Dominic J. et al performed a study which included 10 patients with psoriasis who were treated with topical 5-ALA and exposure to a broadband visible radiation and from whom they took biopsies after treatment. Their study showed that PpIX was localized throughout the epidermis and stratum corneum but the level of fluorescence was not consistent between sections within the same biopsy. Heterogeneity of plaques in fluorescence values was considered an obstacle for the successful treatment of this disorder. (13,14,17,18)

In 2006 Kleinpenning et. al treated 14 patients with stable plaque psoriasis with 20% 5-ALA ointment after keratolitic treatment and biopsies were taken from high and low fluorescent psoriatic lesions. Their study showed that fluorescence intensity was negatively correlated with the thickness of the stratum corneum and they concluded that the heterogeneity of plaques in fluorescence may result from differences in penetration of ALA and light as a result of differences in the thickness in stratum corneum. They also emphasize on the importance of optimal desquamation prior to PDT.(19)

A study performed by Radakovic et al in 2005 which included 29 patients with psoriasis who were randomly allocated to 1% ALA and a light dose of 5 J cm(-2), 10 J cm(-2) or 20 J cm(-2), after previous treatment with keratolytics, showed that PDT with 20 J cm(-2) decreased the baseline PSI with 59%, PDT with 10 J cm(-2) decreased PSI with 46% and PDT with 5 J cm(-2) decreased PSI with 49%. However, they also note that keratolitic treatment alone reduced the baseline PSI with about 25% in all groups. They conclude that ALA-PDT is an inadequate therapy for psoriasis. (20)

Most studies concluded that PDT with topical ALA had suboptimal efficacy, slow pace and unfavorable adverse event profile. Most patients experienced painful sensations in a dose dependent manner and the therapy was not considered a valid option for the treatment of psoriasis vulgaris.(18,21) However, Bissonnette et al showed in their study that the overall tolerability of systemic ALA-PDT was excellent and the patients did not complain from any pain during light exposure, erythema and mild edema being the only signs observed in these patients. With regard to the systemic symptoms, they report one case of nausea. Systemic symptoms reported by other authors include nausea, vomiting and decreased blood pressure. (13,15,16)

Since most authors considered that PDT is a promising method for the treatment of psoriasis, its use, however, being limited by the negative adverse reaction profile, the overall conclusion was that new agents needed to be studied. (22)

In 2009 Salah et al conducted a study on patients with resistant psoriasis who were treated with repeated sessions of PDT with topical methylene blue activated with a light with a wavelength of 670 nm. Sixteen patients had complete remission of the treated lesions and the authors concluded that methylene blue might be a safe, effective therapy for selected cases of localized psoriasis. (23)

In 2010 Rook et al conducted a placebo-controlled study on PDT with topical hypericin and visible light on patients with psoriasis or cutaneous T-cell lymphoma and concluded that hypericin treatment was an effective, well tolerated treatment and a good alternative for PUVA. (24)

Other studies showed that systemic treatment with ALA-PDT and verteporfin are also well tolerated and might be efficacious photosensitizers for the treatment of psoriasis. (16,21).

CONCLUSIONS

Psoriasis is a chronic, recurrent disorder with a worldwide distribution, affecting 1-2% of the global population. Even though there is a whole repertoire of treatments available for this disease, they are often associated with serious adverse reactions, they are difficult to take or very expensive. Therefore, the search for new therapies with a better safety profile continues. Photodynamic therapy is a novel therapy which showed some promising results in the treatment of localized psoriasis.

However, since the currently used photosensitizers had unfavorable adverse event profiles, the identification of new sensitizers is mandatory.

Acknowledgement

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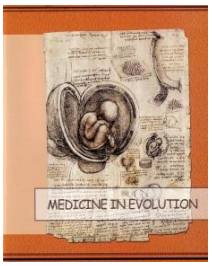
THE PSYCHOSOCIAL TREATMENT OF HEMOPHILIA PATIENTS



LAVINIA MARIA HOGEA[1], ANDA MARIA JURMA[2]

[1] Neuroscience Department, University of Medicine and Pharmacy"Victor Babes"Timişoara, România,
[2] Neuroscience Department, University of Medicine and Pharmacy"Victor Babes"Timişoara, România; Child and Adolescents Psychiatry

ABSTRACT

Hemophilia affects each person beyond the physical problems. In order to optimize the efforts to facilitate the patients' health, it is important to provide them with psychosocial care alongside medical treatment, as an integral part a multidisciplinary approach.

People with bleeding disorders live all around the world. The professional people from the hemophilia centers play an important role in the critical moments from the patient life, giving them essential tools to help them improve their lives, to actively participate in the treatment and to be able to cope with the disease.

Key words: *hemophilia, psychosocial treatment, quality of life*

Correspondence to:

Lavinia Hogea
PhD
Address: Department of Psychology, University of Medicine and Pharmacy"Victor Babes"Timisoara, P-ta Eftimie Murgu, 2, Timisoara, Romania
Phone: +40745042035
E-mail address: laviniahogea@yahoo.com

INTRODUCTION

Psychosocial support is an important part of the treatment for people with hemophilia. Helping both patients and their families in order to learn to face reality must come from a multidisciplinary team of which specialists from various fields, psychologists social workers should be part of. Although this is not always possible due to limited financial resources, healthcare professionals should be aware of all the problems that may occur to patients with hemophilia [1,2,3,4,5].

Beside medical problems, patients with hemophilia face many social and economic challenges. And as it can be seen from this study, medical treatment is not sufficient to improve the quality of life the interventions for various psychosocial problems are needed. The bleeding disorder is a chronic condition that imposes limitations, but can also lead to positive changes such as the abilities of learning and self-awareness. Psychotherapies, alternative therapies and social services can help people cope with hemophilia limitations. The strategies to cope physically, mentally, emotionally and socially must include individual education, family counseling, resources, support from the community, support from others hemophiliacs, accessibility to prophylactic treatment, products for the replacement of the coagulation factor, access to physiotherapy and corrective surgery [idem].

It was found that accessibility to medical treatment has a big impact on the quality of life for both the patient and his family. In one study it has been noted that those living in the areas where the consumption of the coagulation factor is high, the quality of life is better compared to those who live in an area where the consumption of the coagulation factor is low [6,7].

The psychosocial functioning and well-being are specific to their age and cognitive development. And in the families that have hemophiliacs genetic counseling must be used in order to make the parents understand the test results and the options that they have [6].

A hemophilic diagnostic may cause emotions that may vary from negation to acceptance, confusion, anger, guilt and fear for the future. These feelings may complicate or contradict the happiness caused by the baby's arrival. The hemophilia diagnostic may be traumatizing because a coagulation disorder increases the anxiety level regarding the imminent danger. Furthermore, besides anxiety, depression may occur, and the level of stress may grow [5].

The disease can be frightening for those in the family who have never before faced such diagnostic and tends to have a major impact over the financial and emotional aspect of the family. Literature underlines the fact that the disease diagnostic has a life changing effect, the parents living in a state of shock, having feelings of guilt [2]. Studies have shown that in the initial stages of diagnostic the quality of life is the most depreciated [9]. Most literature studies suggest that mothers have a key role in the treatment of their sons, thinking of themselves the main ones responsible for their son disease [6].

Parental guidance is needed regarding the negative consequences that child overprotection may have, parents must learn to encourage normal activities, recreational activities, taking into account the possible risks, of course, for a healthy development [idem]. Inactivity and excessive protection may often lead to isolation, to problems due to poor social interaction, weight problems due to lack of exercise, emotional disorders, mental disorders, etc. [idem]. Psycho-social staff can help parents to develop a balance between autonomy and

putting certain limits to their impulses [10]. A major challenge for a haemophiliac patient lies in the ability to learn to manage stress due to both chronic pain and joint dysfunctions. Many of the psychological issues faced by people with hemophilia come from pain and from the impact of physical condition. Arthropathy, repeated bleeding, arthritis and many orthopedic surgeries reduce the quality of life, which result in frustration and lack of independence, due to the reduced mobility reduse [1,2]

Protective measures and devices help to protect joints from injury and bleeding. At the same time, a child must learn to be able to feel his body, to trust the senses, and learn how to manage his movements in the best way [5]. Social interactions are important for learning and cognitive development of children. Social skills are developed through relationships with colleagues, friends and family, thus developing his self-confidence and self-esteem [2].

Physical or functional limitations can distort the body image that is formed with the onset of puberty, which can be the cause of shyness and shame, of feelings of inferiority and decrease self-confidence. [idem]. Psycho-social professionals can help hemophiliac patients learn how to visualize their status more easily and be better prepared to handle this situation. They must identify the feelings towards the physical changes and learn to take responsibility for their own health and person. School programs and professional orientation and counseling are required, these will help them make the best decisions about schooling and finding a job. [5].

Hemophiliac patients often face social stigma mostly when they suffer from other infections like hepatitis, HIV/AIDS infections. Psychosocial staff can also provide important support for physical and mental challenges of HIV-infected patients, providing information on sexual education, preventive security measures, genetic counseling, taking into account the individual, culture and religion.

The family role in the patient's moral support is essential for successful treatment depends on family involvement.

The family must also have good communication with the doctors who treat the child.

Having a child with hemophilia is considered to be a highly stressful experience that a family may go through.

All negative emotions that a parent will face are normal, but it is necessary to use active methods to control them.

Agitation, anxiety, sadness, fear or guilt, have a negative influence on the child. It is therefore important to maintain a tranquil atmosphere, calm and encouraging that will determine the child"to move forward"and more easily to bear all the procedures by which he must pass. Parents must control their emotional states using the relaxation techniques, discussing with family, friends or other parents who are going through similar situations.

Psychosocial staff can help both parents and patients with hemophilia to develop techniques for encouragement, self-encouragement and positive thinking.

Techniques for stress reduction and to overcome difficult moments can be useful both for the parents and for the patient:

- **Distraction** - can be used during medical procedures. This technique consists in describing a story, or an event from his life. Anxiety and fear are the most common emotional responses that accompany painful behavior.

- **Guided imagery** - is a technique that aims to relax. It allows you to change the pathological mental representations about pain, switching the attention from suffering to optimism.

- **Relaxation techniques** - anxiety and fear amplifies pain because they activate the pain receptors from the brain. Relaxation is a useful method for pain relief, reducing muscle tension, increasing the level of control, decreasing arterial tension.

- **Explanations the patient understand** - information and explanations decrease the ambiguity and uncertainty.

- **Positive thinking** - it's a mental attitude, it anticipates happiness, joy, health and satisfying results for every situation and action.

- **The level of control during treatment** - the sensation of having control reduces anxiety thus, can offer the patient the opportunity of taking control of certain parts of the treatment.

Numerous literature studies show that there is a clear need for psychosocial support for both patients and their families. The aid must focus on helping the patient and his family to cope, manage and self-manage the disease state. All this must take account of the social and economic situation, and the cultural needs of patients with hemophilia.

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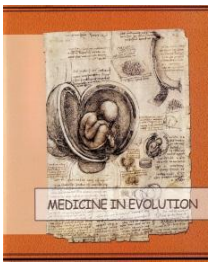
PNEUMOCOCCAL VACCINATION AMONG OLDER ADULTS AND PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE



IOVAN IONELA[1], VANCEA DORIN[1], TUDORACHE VOICU[2]

[1]Hospital of Infectious Diseases and Pneumology"Victor Babes", Timisoara
[2]University of Medicine and Pharmacy"Victor Babes", Timisoara

ABSTRACT

Respiratory infection with Streptococcus pneumoniae (SPN) remains a leading cause of morbidity and mortality worldwide, especially in older adults ≥ 65 years. The presence of chronic obstructive pulmonary disease (COPD) is a major risk factor for pneumonia, acute exacerbations, hospital admissionsand all-cause mortality related to SPN infection. Pneumococcal vaccinations can prevent some of theseevents and thereforeshould be administered to all patients ≥ 65 years, especially to those with COPD. There are currently three established approaches to antipneumococcal vaccination: pneumococcal capsular polysaccharide (PPV), pneumococcal protein-polysaccharide conjugate (PCV) and pneumococcal protein-based vaccines (under investigation). Although patients ≥ 65 years with COPD are commonly described as at-risk population for pneumococcal diseases (PD), studies on pneumococcal vaccination efficacy in such patients are very limited and vaccination effectiveness remains controversial. Further research efforts should focus on improving current vaccines and identifying novel targets for future vaccine development.

Key words: *chronic obstructive pulmonary disease, older adults, pneumococcal vaccination*

Correspondence to:

Ionela Iovan
Hospital of Infectious Disease and Pneumology"Victor Babes", Timisoara
Address: Bld. 3 August1919,nr. 4, ap.3, Timisoara,Timis,cod 300092
Phone: +40724817369
E-mail address: neagoe_ionela@yahoo.com

INTRODUCTION

Streptococcus pneumoniae is a bacterium that typically colonizes the respiratory tract and it's causing mucosal infections (e.g., bronchitis/COPD exacerbations), pneumonia or invasive pneumococcal diseases (IPD).

COPD is currently a leading cause of morbidity and the fourth cause of mortality worldwide. The prevalence of COPD is underestimated, being more common in older people, especially those aged 65 years and older.It is estimated that ≈ 50% of the European Union Countries (EU) population will be ≥ 65 years of age by 2050, the number of people with chronic pulmonary conditions will grow and the number of those at risk of PD will also increase. The incidence of all-cause pneumonia among people with COPD is 3-4 times higher than in the general population, SPN infection remaining the most common identified cause. The SPN-infected patients have more severe pneumonia, higher hospital admission and mortality rate.(1)

Acute exacerbations (AEs) are the most important cause of mortality and morbidity in patients with COPD. There is an increased risk of AEs in COPD patients, SPN being responsible for almost a thirdof bacterial exacerbations. Hospital admission rate, among COPD patients with pneumonia or AEs, increases with the intensity of airflow obstruction and severity of the disease. Immunization with pneumococcal vaccines is a strategy that may be effective in order to reduce the incidence of pneumonia, exacerbation rate and hospital admission in COPD patients.(2)

CURRENT STATUS OF KNOWLEDGE

1. Burden of Pneumococcal Diseases

From the 90 distinct SPN serotypes existing, ≈ 20 (contained in PPV23) are responsible for ≥ 85% of cases of IPD (meningitis, bacteraemia and invasive pneumonia) in all age groups, and 7 (contained in PCV7) are responsible for 70%of PD in infants. Each year, approximately 1.6 million people throughout the world die from PDs. In developed countries, the risk of death from IPD is highest in elderly adults (aged ≥ 65 years) and in those with underlying chronic diseases (e.g., COPD).(2,3,4) In at-risk adults compared with healthy adults, there is an increased risk of PD in patients with COPD (3-6-fold), asthma or tobacco smokers(3-4-fold).In adults atrisk for PDs all persons ≥ 65 years of age are also included.(5)

2. Epidemiology of Pneumococcal Disease among adults aged ≥65 years

In 2008 the rate for IPD in EU was 5.2 per 100,000 population, with the highest peak in adults ≥ 65 years of age (12.10 cases/100,000).(7) Community-acquired pneumonia (CAP) is more frequent in patients with underlying condition of age ≥ 65 years than those of age ≤ 64years (70.5% vs. 29.5%).(8,9)SPN remains the most common microorganism identified among patients with COPD and CAP.(10)The overall case-fatality rate reported for pneumococcal bacteraemia may reach 15-20% in young adults, but it increases to 30-40% among COPD adults despite appropriate antibiotic treatment.(11) Serotypes with low invasive potential (3, 6A, 6B, 8, 19F and 23F) behave as opportunistic pathogens in at-risk individuals, whereas serotypes with high invasive potential (1, 7F) can act as primary pathogens in previously healthy individuals.(12,13,14) The use of PCV7 since 2000 and PCV13 since

2010, among children in the United States (US) and EU, has reduced SPN infections directly among children, and indirectly ("herd protection") among adults. By 2013, the incidence of IPD caused by serotypes unique to PCV13 among adults aged ≥ 65 years had declined by approximately 50% compared with 2010, when PCV13 replaced PCV7. Approximately 20%-25% of IPD and 10% of CAP cases in adults aged ≥65 years are caused by PCV13 serotypes and are potentially preventable with the use of PCV13 in this population.(15,16)An increased incidence of IPD, caused by non-PCV serotypes ("serotype replacement") after PVC use, has also been reported.(17,18)

3. Types of antipneumococcal vaccines

Pneumococcal polysaccharide vaccine contains capsular polysaccharide antigens from the 23 serotypes, which have historically caused 85-90% of cases of PDin the adult population. It induces a host response that is T-cell independent, producing antibodies (ABs), but does not induce immunologic memory.(19) Within 2-3 weeks after vaccination,over 80% of healthy adults develop ABs versus vaccine-related serotypes. The ABs response is poor in elderly, persons with chronic illness and in children < 2years. Aftervaccination, Abs titre declines rapidly over a 1-2-year period, persisting at low levels for 10 or more years.(20,21) Revaccination with PPV23 induces persistent functional antibody response in healthy middle-aged and older adults.(22)Duration of immunity is ≈ 5 years in healthy adults andis 60-70% effective in preventing IPD, but does not offer protection against CAP.(23,24)

Recommendations for PPV23 vaccination in adults. The US Centers for Disease Control and Prevention (CDC) Advisory Committee on Immunization Practices (ACIP) recommendsPPV23 vaccination among:

- Adults at risk (e.g., smokers, asthma, COPD) aged 19-64 years: a single dose.
- Adults at high risk (immunocompromised) aged 19-64 years: maximum 2 doses separated by at least 5 years.
- All adults ≥ 65 years of age: 1 dose, if at least 5 years have passed since the previous dose.(25)

The European Union Geriatric Medicine Society (EUGMS) and the International Association of Geriatrics and Gerontology–European Region (IAGG–ER) recommend PPV23 vaccination for all adults ≥ 60 years of age followed by revaccination every 5 years.(26) In EU, 8 countries recommend routine PPV23 vaccination for all persons ≥ 65 years, 5 countries recommend vaccination starting at 60 years, 4 countries recommend PPV23 only for at-risk groups, which differ considerably from country to country.(27)

In the Pneumococcal conjugate vaccines, polysaccharide is conjugated to a carrier protein. The resulting antigen is recognized as T cell-dependent, inducing memory B cell response, stimulating a better and more persistent serum AB response, mucosal immunity, and immunologic memory in children (including those <2 years) and adults.The implementation of infants vaccination with PCV7 began since 2000.(28) In 2010, PCV13, which contains 6 additional serotypes, replaced PCV7. In 2011, PCV13 was approved for use among adults aged ≥ 50 years to prevent CAP and IPDs.In 2012, PCV13 began to be recommended for use in selected high-risk adults ≥19 years. Since 2014, the ACIP recommend PCV13 for all adults ≥ 65 years of age.(29) PCV13 is not recommended for healthy adults < 65 years of age who do not have a specific risk factor for pneumococcal infection. Randomized clinical studies among older adults, aged 60–64 and also ≥70 years, showed that PCV13 induced an

immune response as good as or better than that induced by PPV23.(30,31,32)

Recommendations for sequential PCV13+PPV23 vaccination among adults ≥65 years

Based on immunogenicity studies, ACIP recommends that both PCV13 and PPV23 should be administered routinely in series to all adults aged ≥ 65 years.(31,32, 33,34,35)

The pneumococcal vaccine-naive adults, should receive a dose of PCV13 first, followed by a dose of PPV23 (after8 weeks to 1 year). The two vaccines should not be coadministered.

The previous PPV23-vaccinated adults should also receive a dose of PCV13 ≥ 1 year after getting the most recent PPV23 dose. For those for whom an additional dose of PPV23 is indicated, this subsequent PPV23 dose should be given 6–12 months after PCV13 and ≥ 5 years after the most recent dose of PPV23.(25)

All adults aged ≥ 65 years should receive a dose of PPV23 even if they were vaccinated when they were < 65 years of age, with minimum interval of 5 years between PPV23 doses. The ACIP does not recommend routine revaccination of immunocompetent adults with PPV23.At the present time, revaccination of adults with PCV13 is not recommended.(25)

4. Vaccination effectiveness among older adults and patients with COPD

Despite many studies of PPV efficacy in different populations, few randomized controlledtrials (RCTs) to date were focused on COPDpatient and they have reportedinconclusive results. (1)

Observational studies demonstrate strong evidence that PPV protects healthy persons, elderly and COPD patients against IPD. In a retrospective cohort control study of 1898 elderly persons with COPD, Nichol et al, demonstrated that PPV vaccination results in a 43% reduction in hospitalization for pneumonia and a 29% reduction in mortality.With the regard to the non-randomized trials, PPV efficacy in the prevention of IPD ranged between 26-70% in immunocompetent adults and in subjects at risk (but not immunocompromised), with a global value of 52%. As a result, pneumococcal vaccination is now recommended by all guidelines as part of the routine management of COPD patients. (36)

In 2010 the Cochrane Review, which included in its meta-analysis 7 RCTs (1709 subjects),was specifically focused on COPD patients. The reduction in the risk of developing pneumonia (OR:0.72) and AEs of COPD (OR:0.58) among the PPV vaccinated compared to control did not achieve statistical significance. Regarding the secondary outcomes, there was no statistically significant effect for reduction in hospital admissions, emergency department visits and in the risk of all-cause death (OR:0.94), or death from cardiorespiratory causes (OR:1.07). The authors concluded thatit is possible that PPV may provide some protection against morbidity in persons with COPD.Those who benefited most of the positive effects were younger adults in good health conditions, while the evidence of a benefit in older patients is weaker.(37)

A 2013 Cochrane Review assessed the efficacy of PPV for preventing invasive pneumococcal disease, all-cause pneumonia, and all-cause mortality of adults in 16 randomized trials. The following results were observed: PPV significantly reduced the risk of IPDs (OR: 0.26) especially in low-income countries. There was no evidence of protective efficacy among individuals with COPD in high-income countries.PPV reduced both invasive (OR: 0.26) and noninvasive pneumococcal pneumonia (OR 0.46). There was efficacy against all-cause pneumonia among individuals in low-income countries (OR 0.54), but not

among individuals in high-income countries in either the general population or in adults with COPD.PPV did not reduce all-cause mortality.(38)

The 2014 ACIP decision to recommend PCV13 for all adults ≥ 65 years of age, came after the proof of its efficacy against IPD in adults. A randomized placebo-controlled trial (CAPiTA trial) was conducted in the Netherlands among approximately 85,000 adults aged ≥65 years during 5 years to verify and describe further the clinical benefit of PCV13 in the prevention of SPN pneumonia. The results of the CAPiTA trial demonstrated 45.6% efficacy of PCV13 against vaccine-type SPN, 45.0% efficacy against vaccine-type nonbacteremic SPN pneumonia, and 75.0% efficacy against vaccine-type IPD among adults aged ≥ 65 years.(39)

In one open-label randomized trial, administration of the PPV23 six months prior to the PCV7, resulted in attenuated AB concentrations compared with PCV7 alone.(31) Based on these considerations, the US ACIP recommends that patients with an indication for both vaccines should generally receive the PCV13 first, followed a minimum of 8 weeks later by the PPV23, and patients who have already received PPV23 first should wait 1 year before receiving PCV13.(29)

CONCLUSIONS

SPN infections remain an important source of morbidity and mortality in adults, especially among older adults (≥ 65 years) and those at risk (e.g., COPD).Vaccination is the only public health measure likely to reduce the burden of pneumococcal diseases.

For adults aged ≤ 64 years at risk (e.g., cigarette smokers, asthma, COPD), is recommend PPV23 vaccination alone (Grade 1B). It must not be forgotten that the PPV23 provides incomplete protection, it does not elicit long-lasting immunity, and no anamnestic effect occurs at revaccination.

For all adults ≥ 65 years of age, is recommend sequential vaccination (PCV13 followed by PPV23) (Grade 1B). PPV23 revaccination (5 years after prime dose) is recommended for those persons who received PPV23 before 65 years of age. PCV13 is not recommended for immunocompetents adults ≤ 64 years and for revaccination.

Among general population, most meta-analyses demonstrate that PPV23 strongly protects healthy persons and older patients against IPD, with a global value of 52%. RCTs have consistently failed to demonstrate a significant benefit of PPV in patients with COPD, in preventing COPD exacerbations, pneumonia, hospitalizations or all-cause mortality. Recommendations for vaccinating COPD patients are based on PPV effectiveness against IPD among the general population (it is possible that PPV may provide some protection in persons with COPD).Vaccination of younger patients with COPD appears best supported, while the evidence of a benefit in older patients is weaker.

More effective vaccination strategies are needed. CAPiTA trial demonstrated an increase efficacy of PCV13 against IPDs and vaccine-type pneumonia, among adults aged ≥ 65 years.In the next few years, the results of ongoing trials evaluating the efficacy of the PCVs in adults will be critical in determining the position of the anti-pneumococcal vaccines in the prevention of PDs in patients with COPD.

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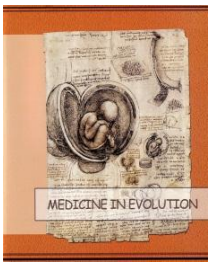
VULVAR SARCOMA – LITERATURE REVIEW



NICOLAE BACALBAŞA[1], BEATRICE LINTOIU[2], IRINA BALESCU[3]

[1]"Carol Davila"University of Medicine and Pharmacy, Bucharest, Romania
[2]"Profesor Dr. Agripa Ionescu"Clinical Emergency Hospital, Bucharest, Romania
[3]"Ponderas"Hospital, Bucharest, Romania

ABSTRACT

Vulvar soft tissue sarcomas are rare malignancies accounting for almost 2% of all vulvar malignancies characterized by a high capacity of developing local recurrence, lymphatic metastases into the inguinal and pelvic lymph nodes or hematogenous distant metastases. This is a literature review of the most important studies regarding the therapeutical options and outcomes of the most frequently seen histopathological subtypes of vulvar sarcomas

Key words: *vulvar sarcomas, surgery, recurrence*

Correspondence to:

Nicolae Bacalbaşa
Address: Dimitrie Racoviță Street, no. 2, Bucharest, Romania
Phone: +40723540426
E-mail address: nicolaebacalbasa@gmail.com

INTRODUCTION

Vulvar cancer is an uncommon disease, marked by typical long delays in diagnosis due to lack of awareness by doctors and patients. It is the fourth most common gynecologic cancer (following cancer of the uterine corpus, ovary and cervix) and comprises 5% of malignancies of the female genital tract and less than 1% of all malignancies in women. Within the United States, almost 5000 cases of vulvar cancer are diagnosed each year, with over 1000 of those women expected to succomb to their disease (1).

RISK FACTORS FOR VULVAR CANCER

Risk factors for vulvar cancer include: vulvar dystrophy (e.g. lichen sclerosus), human papillomavirus (HPV) infection, immunodeficiency syndromes, past history or family history of cancer and cigarette smoking (2). The cause of vulvar malignancies remains unknown, although currently there are considered two independent pathways of vulvar carcinogenesis. The first one is related to mucosal HPV (high risk type 16,18,33) infection and affects a younger population group (less than 45 years of age). The second pathway is related to chronic inflammatory or autoimmune processes and affects older women (2,3).

HISTOPATHOLOGICAL SUBTYPES OF VULVAR CANCER

Approximately 90% of vulvar cancers are are squamous cell cacinoma. Less common vulvar malignancies include malignant melanoma, adenocarcinoma, basal cell carcinoma, sarcoma and Paget's disease (1,3).

Soft tissue sarcomas make up less than 2 % of vulvar malignancies. In general, they have been characterized by local recurrence and death from distant hematogenous metastases. Lesions can show a broad range of differentiation including many categories: leiomyosarcomas, malignant fibrohistiocytomas, rhabdomyosarcomas, epithelioid sarcomas, angiosarcomas, neurofibrosarcomas, dermatofibrosarcomas and many others exceptionally rare types (4). The average age at diagnosis is 50 years. The most common presenting symptom is local discomfort with a vulvar mass, chronic vulvar pruritus, pain with voiding or intercourse. Lesions are frequently misdiagnosed as Bartholin's cysts or abscesses (5).

Anatomically, the vulva is composed of the mons pubis, labia majora and minora, clitoris, vaginal vestibule and perineum. Vulvar cancer spreads by direct extension to adjacent structures (vagina, urethra and anus) and by lymphatic embolization. Lesions may be single or multifocal, unilateral or bilateral. Hematogenous metastases occurs in the liver, lungs and bones.. The dermal lymphatics drain into the superficial inguinal nodes, then to the deep femoral nodes and then to the external iliac nodal chain. Most vulvar cancers occur in the labia majora and drain to ipsilateral lymph nodes in the groin. Bilateral drainage occurs with midline lesions. Direct spread to deep pelvic nodes (bypassing inguinal nodes) rarely occurs. Spread to the pelvic lymph nodes almost always occurs in the presence of groin node metastases. If the groin nodes are positive then the risk of pelvic nodes being positive is 20 %. Patients with no palpable nodes have a high as 25% occult metastatases. The incidence of lymph node

metastases is related to the depth of stromal invasion, histologic grade of the lesion, presence of lymphovascular space involvement, clitoral or perineal location, clinical node assessment and age of the patient (6,7).

No gross or microscopic features are diagnostic of vulvar carcinoma. Diagnosis is based on histologic examination of biopsy material or excision specimen. A high index of suspicion and a large number of biopsies are necessary in evaluating suspicious vulvar lesions.

PREOPERATIVE EVALUATION

Routine staging evaluation includes complete physical examination, biopsy, and imaging studies, such as computerized tomography scan or magnetic resonance imaging to show the extent of tumor, which is often underestimated by physical examination. Magnetic resonance is a very important imaging method since it has good soft tissue contrast resolution, can demonstrate the relation to adjacent anatomic structures, extension and depth of the tumor and show the possible lymph node involvement (8).

The revised FIGO (9) staging system for gynecologic malignancies-2009 established a sarcoma staging system based on the criteria used in other soft tissue sarcomas. Cystoscopy, proctoscopy, X-ray examination of the lungs, and intravenous urography (as needed) are used for staging purposes. Suspected bladder or rectal involvement must be confirmed by biopsy (9).

Between 20% and 40% of patients with carcinoma in situ of the vulva have prior, concurrent, or subsequent neoplasias elsewhere within the anogenital tract. Colposcopic and cytologic review of these areas is necessary (10).

An awereness of the possibility of invasive vulvar carcinoma, even in a relatively young patient, should lead to prompt and through histologic evaluation of any vulvar lesion. Immunohistochemistry is used to rule out nonmesenchymal tumors and to define the mesenchymal cell lineage. A useful reference detailing how immunohistochemistry assists the pathologist to make the correct diagnosis is provided by Heim-Hall and Yohe (11).

HISTOPATHOLOGICAL CHARACTERISTICS AND THERAPEUTICAL PROTOCOLS

Leiomyosarcoma, the most common type of vulvar sarcoma is usually diagnosed in women older than 40 years of age, commonly presenting as painless masses and misdiagnosed as Bartholin cysts. They are thought to originate from smooth muscle within the erectile tissue or blood vessels walls, the round ligament and the dartos muscle. These tumors often rise difficulty in distinguishing between their benign and malignant forms (12). In the largest series of smooth muscle tumors of the vulva, published by Nielsen et al.(13), the most important pathologic findings for a correct diagnosis were: tumor diameter >5 cm, an infiltrative margin, a mitotic count of 5 or more per 10 high-power fields and grade 2 or 3 nuclear atypia. If three of these four criteria were fulfilled, the lesion was diagnosed as leiomyosarcoma. Also,they stain positive for desmin, smooth muscle actin, muscle specific actin, estrogen/progesteron receptor, vimentin, S100 and citokeratin and negative for CD34, CD117, and DOG 1 (11,12).

Epithelioid sarcoma, first described by Enzinger in 1970 is a rare soft-tissue sarcoma typically presenting as a subcutaneous mass originating in the extremities of adolescents and young adults (distal type). There is also a proximal type (occuring in the trunk, pelvis and pubic region) which appears to be more aggressive even if it is very rare. Epithelioid sarcoma of the vulva was first defined by Piver et al. in 1972 (14). It often occurs in the labia majora of young women and frequently is misdiagnosed as benign lesion such as infectious granuloma, Bartholin's cysts, fibroma, lipoma, dermoid cysts, fibrous histiocytoma, viral warts. Slow growth of the tumor, paucity of symptoms, benign appearance in early stage and indistinctive pathologic findings in some cases makes the diagnosis challenging. Slow growing of the tumor with a high rate of recurrence and metastases despite negative surgical margins justify the poor prognosis. Unlike most other sarcomas, epithelioid sarcoma, has a tendency for lymph node metastases (15). Genetic testing shows promise in differentiating different types of sarcomas, including epithelioid sarcoma. INI1, a subunit of chromosome 22 acts as a tumor suppresor gene. Recently, the loss of INI1 gene has been shown in more than 80% of patients with epithelioid sarcoma, so INI 1 can be used for the diagnosis (16). Also, immunohistochemistry of epithelioid sarcoma reveals cytoplasmic immunoreactivity for cytokeratin and vimentin, negative for S100 and CD 34 stainings (17). Wide surgical resection, with a safety margin of at least 2 cm and bilateral inguinofemoral lymphadenectomy remains the most recommended treatment modality. It has recurrence rates up to 77 % in some studies (15). The role of adjuvant therapy, chemotherapy and radiation remains unclear. 5 year survival and 10 year survival rate for patients with ES are 50-70 % and 42-55 % respectively (18).

Malignant fibrous histiocytoma (MFH), described by O'Brien and Stout in 1964, represent less than 1% of vulvar sarcomas and the second most frequent sarcoma of this region (19). In 2005, Vural et al.(20) gathered eight cases of MFH from the English literature. It usually occurs in elderly postmenopausal women, with a peak incidence in the seventh decade. The primary treatment modality ranges from wide local excision to radical vulvectomy with groin dissection. Metastasis of MFH correlates well with the depth of invasion of the original tumor (21). Oda et al. reported that a deep tumor location and high AJCC stage (i.e. stages III and IV) were independent factors indicating a poor prognosis (22). Immunohistochemistry displays vimentin reactivity and negativity for keratins and leukocytic common antigen. The 5 year survival rate of tumors less than 5 cm is 82%, for tumors 5-10 cm size is 68% and for tumors larger than 10 cm is 51% (23).

Dermatofibrosarcoma protuberans (DFSP) is a rare low-grade soft tissue sarcoma that occurs in dermis and usually invades the subcutaneous tissue and muscles. It is composed of spindled cells in a storiform pattern, stains with CD34 and vimentin on immunohistochemical studies (24) and is characterized by local invasion and recurrence. It accounts for 5-6% of all soft tissue sarcomas. Involvement of the vulva is extremely rare, with only 28 cases reported. Its characteristic clinical appearance is an irregular flesh-coloured, reddish-brown to bluish nodule or violaceous plaque. DFSP could dedifferentiate to the high grade sarcomas with an increased risk of local recurrence and metastasis. Advanced age, high mitotic index and increased cellularity are associated with poor outcome. American Musculoskeletal Tumor Society set a staging system considering the tumor

grade and compartment. Ultrasonography (US) or computed tomography (CT) can demonstrate DFSP as a heterogenous subcutaneous solid mass with spiculated or lobulated margins. A wide surgical resection with a margin of 2-3 cm of normal tissue is recommended as the optimal treatment for both primary and recurrent DFSP. Mohs micrographic surgery has presented as an alternative approach as well (24,25).

Liposarcoma of vulva is an extremely rare malignant tumor, with only sixteen reported cases up to date. There are five subtypes by histologic features: well-differentiated liposarcoma (the most common), dedifferentiated liposarcoma, myxoid liposarcoma, round cell liposarcoma and pleomorphic liposarcoma. The last two subtypes are more aggressive and tend to metastasize so histologic subtype is the main prognostic factor regarding clinical outcome. The treatment of choice is complete surgical resection. For high-grade lesions or positive resection margin, adjuvant radiotherapy may provide benefit to control recurrence (26,27).

Synovial sarcoma, contrary to its name, does not arise from the synovial membrane, but from multipotent stem cells. It comprises 10 % of all soft tissue sarcomas and is classified in biphasic, monophasic and poorly differentiated. Although the fourth most commonly sarcoma, it rarely arises in female genital tract (fewer than 15 cases reported, with less than 10 in the vulva). The diagnosis is difficult. RT-PCR for specific chromosomal translocations appears to be regarde as a standard method for diagnosis. The presence of metastases, tumor size >5 cm, invasiveness, high histological grade, positive surgical margins and poor histological differentiation are associated with adverse prognostic outcome. Radiotherapy seems to improve local control of the disease,but the role of adjuvant CHT remains controversial (28,29).

Roth et al.(2004) and Murakami et al.(2013) described low-grade myofibroblastic sarcoma (LGMS) of the vulva. Myofibroblast are mesenchymal spindle cells sharing ultrastructural features of borh fibroblast and smooth-muscle cells, which occasionally undergo tumorigenic transformation, becoming malignant. Immunohistochemically, tumor cells stain positive for at least one of the myogenic markers (α-SMA, desmin and muscle-specific actin). The clinical course of LGMS is indolent, but the recurrence rate in large series was reported as 40 %. The therapeutic effect of adjuvant CHT or RT is unclear. Aartsen et al. (1994) proposed a 2 cm adequate resection margin. Studies have reported that vulvar sarcoma mainly metastasized via the hematogenous route; rarely LGMS shows lymphogenous metastases so the benefit of therapeutic inguinal lymphadenectomy is minimal (4,5,31).

Type 1 neurofibromatosis (NF1) is a dominantly inherited neurologic disorder affecting primarily the skin, bones and peripheral nervous system. Clinical manifestations including cafe-au-lait spots, skinfold freckling, Lisch nodules and visceral neurofibromas. There is an increased risk for benign and malignant tumors (neurosarcoma). Tumors in peripheral nerves are neurofibrosarcomas, malignant schwannomas or malignant peripheral nerve sheath tumors. NF1 rarely affects the genital tract and extremely rare has an isolated vulvar localization. Few cases of vulvar sarcoma in patients with NF1 have been described (32,33).

Ewing sarcoma and peripheral primitive neuroectodermal tumor (pNET) are two morphological ends of a spectrum of neoplasms characterized by a translocation involving the EWS gene on chromosome 22 and reffered as Ewing family of tumours (EFTs) they are extremely rare in vulva and vagina (fewer than 20 cases in the literature). The majority of these neoplasms are composed of solid

sheets of primitive undifferentiated"small round blue cells". In recent years, CD99 and FLI-1 antibodies have been demonstrated to be useful in diagnosis, as being positive in majority of cases. Also, many of them stain with broad-spectrum cytokeratins. Demonstration of one of the characteristic translocation may be regarded as the gold standard for diagnosis. Neoplasm in the EFTS are aggresive with a poor prognosis even with aggresive treatment, patients with metastases exhibit an ~20 % chance of long term survival (34,35).

Angiosarcoma, neoplasms of endothelial origin, comprise less than 2 % of all sarcomas, with appoximately 50 cases of primary angiosarcoma of the female genital tract described in the literature. They are commonly aggresive and disseminate widely. Extensive local or radical excision is the preferred treatment associated with adjuvant RT. Sanz et al.(2005) described a groin angiosarcoma following radiotherapy for vulvar cancer (36,37,38).

Surgery is the primary treatment modality for vulvar malignancies. Until the 1980s, the standard therapeutic approach for invasive locoregional vulvar carcinomas was radical surgery, including complete en bloc resection of the vulva and bilateral inguinal lymph nodes. The en bloc resection butterfly incision had a significant wound separation rate of at least 50%. Because of the high complication rates (wound healing problems, lymphedema, functional deficits) the trend since then has been toward more limited surgery, often combined with radiation therapy (39).

A number of significant advances have occured including elimination of routine pelvic lymphadenectomy, avoidance of groin dissection of patients with microinvasive vulvar carcinoma, elimination of the contralateral groin dissection of patients with less than 2 cm lesions and negative ipsilateral nodes, use of separate incision for groin dissection, use of preoperative radiation therapy to reduce the need for exenteration in advanced disease, the use of postoperative radiation to lower the incidence of recurrence in patients with two or more positive nodes (40).

An approach using three separate incision, one for radical vulvectomy and one for each groin dissection, ensure better primary healing.Few intraoperative deaths occur, but a postoperative mortality rate of up to 5% is observed because of the geriatric patient group (41).

In stage I lesions the therapeutic options include wide (margin of at least 1 cm) excision or radical local excision and unilateral lymphadenectomy. The lymphadenectomy is unnecessary if the tumor is unifocal, has less than 1 mm of invasion, shows no evidence of lymphovascular space involvement, and is well differentiated. Less invasive procedures (e.g. laser therapy) for ablation of the intraepithelial lesions of the vulva can be used in selected cases (young patients with vulvar intraepithelial neoplasia), but careful attention should be paid to adequate ablation, clear margins and histologic studies of suspicious, thick lesions (39,41).

Stage II and III are treated by radical local excision or radical vulvectomy and bilateral groin nodes dissection with or without radiation therapy. Patients with two or more positive nodes should receive external therapy to the groin and pelvis (39,40,42)

In advanced disease, early attempts to remove all gross disease by radical vulvectomy were associated with a high local failure rate, thus radical vulvectomy combined with pelvic exenteration and bilateral groin dissection has become more popular. Such radical therapy carries a high postoperative morbidity (wound seromas, infection and breakdown,

urinary tract infection, deep venous thrombosis, pulmonary embolization, osteitis pubis, chronic leg edema, stress incontinence, introital stenosis and femoral hernias) and a high psychological morbidity (41). Pelvic exenteration offers the last chance for some women with advanced or recurrent vulvar malignancies. Survival time ranges from 6-74 months (43), but the presence of metastatic lymph nodes markedly decreases survival. In stage IVB there is no standard treatment approach. Local therapy must be individualized depending on the extent of local and metastatic disease. There is no standard chemotherapy for metastatic disease (39,44,45).

Radical radiation therapy has been used in patients unable to tolerate surgery. In selected cases radiation therapy may be given before surgery to reduce disease and define surgical margins. Extended field therapy for the vulva and groin nodes has resulted in the control of the disease for some patients but is accompanied by significant morbidity such as persistent vulvar ulcerations, atrophy, contraction and pain (40,41).

Another strategy to minimize the morbidity incurred by groin-node dissection in patients with early clinical-stage disease is sentinel node dissection, reserving groin dissection for those with metastases to the sentinel node (46).

Authors like Holloway et al. (47) have described a multimodality treatment plan (preoperative radiotherapy, conservative surgery, interstitial brachytherapy boost and postoperative systemic chemotherapy) with good cosmetic and functional outcome in a sexually active female.

Pelvic radiation has been compared to pelvic node dissection in the setting of documented groin node-positive disease; after a median follow-up of 74 months, the 6-year overall survival rate was 51% in the radiation group versus 41% in the pelvic node dissection group; late chronic lymphedema was similar in both groups, but more large studies are needed (48).

There is no standard chemotherapy for vulvar cancer. Given the advances age and comorbidity of many patients with advanced or recurrent vulvar cancer, patient tolerance is a major consideration in the use of these agents (39,49).

Radical vulvectomy is often complicated by problems associated with insufficient closure of large skin defects, contributing to postoperative necrosis of the suture line. The reconstructive strategies includes rectus abdominis myocutaneous flap, gluteal fold flaps, gluteal rotation flaps, gracilis V-Y or advancement flaps and latissimus dorsi flaps. Smaller defects are best treated by local flaps, whereas the rectus abdominis flap remains the standard option for larger defects that additionally requires closure of dead spaces (50).

In the follow-up, patients should be seen every 3 months for 2 years and then every 6 months for the next 5 years. Thereafter, visits should be made annually. A pelvic exam and pap smear of the cervix and/or vagina should be performed at each visit. Recurrence and prognosis depend on three main characteristics: lesion size, tumour involvement of adjacent tissue and mitotic activity (51). Lesions greater than 5 cm in diameter, with infiltrating margins, extensive necrosis, more than 5 mitotic figures per 10 high-power fields are more likely to recur after surgical resection (51). Tumor-free surgical margin less than 8 mm, increased depth of invasion, positive vascular space invasion, extracapsular groin node spread, all corellate with a higher incidence of recurrent disease (52).

Treatment and outcome depend on the site and extent of recurrence. Radical excision of localized recurrence may be considered if technically feasible. Palliative radiation therapy is

used for some patients. Radiation therapy with or without chemotherapy may be associated with substantial disease-free periods in some patients with a small local recurrence. When local recurrence occurs more than 2 years after primary treatment, a combination of radiation therapy and surgery may result in a 5-year survival rate of greater than 50% (50,53).

CONCLUSIONS

The rarity of the disease and the variety of histologic types (more than 70) accounts for the difficulty in aquiring sufficient data to get significant conclusions.

Improved survival will depend on earlier and more accurate diagnosis and treatment.

Adequate knowledge of these malignancies is essential for the diagnosis, adequate surgical treatment, adjuvant therapy and efficient treatment in relapse.

A multidisciplinary approach is crucial in this pathology, including pathologists, radiologists, surgeons, gynecologists, radiotherapists.

Treatment induced anatomic and physiologic abnormalities may significantly impair quality of life, sexual function and sexual satisfaction, but the objectives should be first to cure and, second, to preserve good long-term quality of life.

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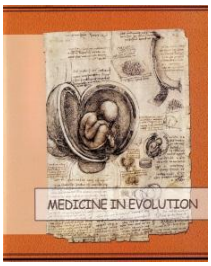

THE MANAGEMENT OF TREATMENT AND DIAGNOSES AT PATIENTS WITH AORTIC COARCTATION

ANA LASCU[1], IOANA MIHAELA CÎTU[2]

[1] Department of Phisiopatology, Cardiovascular Surgery, Institut of Cardiovascular Disease, University of Medicine and Pharmacy"Victor Babes"Timisoara
[2] Department V Internal Medicine I Medical Semiology I, University of Medicine and Pharmacy"Victor Babes"Timisoara

ABSTRACT

The aim of this study was to find the best management of treatment and diagnoses for patients with aorctic coarctation (AoCo). For this we made a retrospective study from 1998 to 2008 from a hospital from Timisoara, Romania.

Methods: We analyzed medical records of 70 patients, obtaining an assessment of clinical, laboratory, and imaging and their treatment results. We followed clinical and preclinical signs for the patients with AoCo.

Results: Best represented ages were 12 to 13 years (5 cases each). Mean age of patients was 14.79 ± evaluated 10.65 years, so patients belong to the young, but with variations in a fairly wide range of minimum 2 months, maximum 44, which shows a great variability within the group. Age groups showed 6 patients of 0-1 years, 11 patients of 2-5 years, 9 patients of 6-10 years 16 patients of the 11-15 age group, 11 patients of 16-20, 5 patients to 21-25, 5 patients from 26 to 30 years, 3 patients to 31-35 years, and 4 patients aged 36-45 years. The Heart rate had an average value of 92.54 ± 21.12 bpm (minimum 46 bpm, 150 bpm maximum) with high variability depending on age groups. Drug treatment for those 70 patients consisted of prescribing AoCo complex therapy depending on the specific case (given the heterogeneity associated diagnoses) in 3 major therapeutic classes: beta - blockers, ACE inhibitors and angiotensin converting diuretics, along with other therapeutic options (calcium channel blockers, cardiac glycosides etc). In total there were practiced 51 istmoplastia, 7 bypasses and 10 resection with end-to-end.

Conclusions: No specific laboratory tests are necessary for AoCo, but for the best diagnosis is to follow the clinical signs and chest radiography, echocardiography or MRI. The medical treatment of less severe AoCo beyond the neonatal period include: administration of digoxin and diuretics for chronically increased afterload and signs of CHF and postponement of intervention (surgery or balloon dilatation) until the patient is hemodynamically stable.

Key words: *aortic coarctation, diagnosis, istmoplastia, staturo-ponderal deficit, treatment*

Correspondence to:

Ioana Mihaela Cîtu
Address: Departamentul V Medicina Interna I Semiologie Medicala I, Universitatea de Medicina si Farmacie „Victor Babes"Timisoara
Phone: +40723280623
E-mail address: ioanamihaela2010@gmail.com

INTRODUCTION

Of all heart malformations, Aortic Coarctation (AoCo) has an incidence of 6-8%, which untreated and undiagnosed has bad consequences, such as late of the development, hypertension and heart failure [1].

AoCo is a narrowing of the aorta usually positioned between the origin of the left subclavian artery, proximal and the junction of the aorta and arterios distal ductus. The AoCo was first described in 1760 by Morgan after an autopsy, and then in 1791 first described the clinical manifestations [2]. In 1835, Legrand presents the first clinical diagnosis of thoracic aortic stenosis, while in 1892 Potain implies the existence of hypertension in the upper portion of the body. Aortic coarctation for adult is classification like a postductal and the infantile type is preductal, described in 1903 by Bonnet [3]. AoCo repair premiere was performed in 1944 in Stockholm, Sweden. Also, the success of surgical coarctation of the aorta in was reported in 1951 by Lynxwiller, then the Mayo Clinic in 1952, the anastomosis technique used end-to- end; Following the Vosschulte to achieve aortoplastia with patch [4].

The Aortic Coarctation was clasiffied by Jönsson and Broden in 1951 in four types [5]:

- Type I: when prestenotic portion of the aortic arc is long and wide
- Type II when prestenotic portion of the aortic arc is long and narrow;
- Type III: when prestenotic portion of the aortic arc is short;
- Type IV: when prestenotic portion of the aortic arc is atresia.

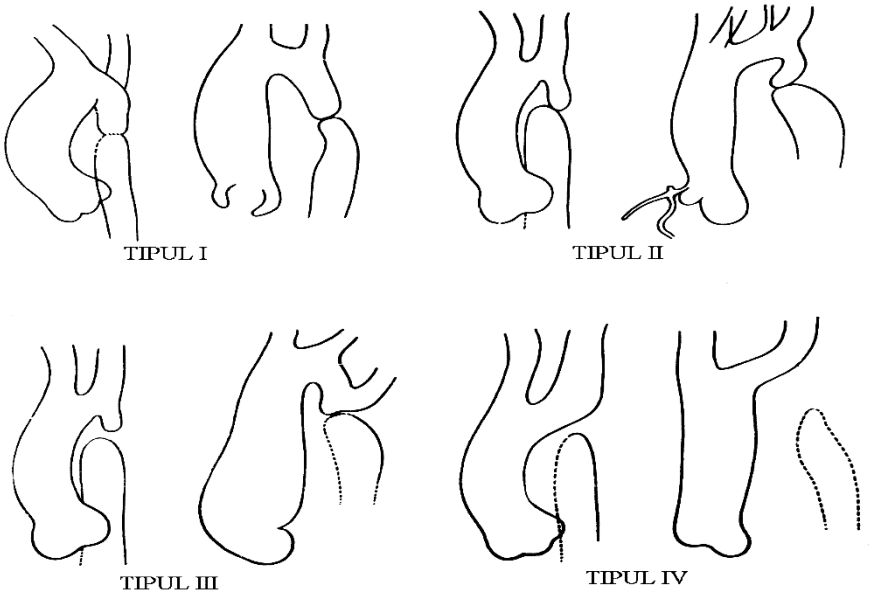

Figure 1. Aortic Coarctation Classification by Jönsson and Broden [6]

The vascular malformation responsible for coarctation is a defect in the vessel media, giving rise to a prominent posterior in folding, which may extend around the entire circumference of the aorta. Aortic coarctation is a narrowing of the aorta most commonly found just distal to the origin of the left subclavian artery [7]. Coarctation of the aorta is a heterogeneous disease process with multiple associated complications both with and without treatment. Covered stents have evolved to provide greater support to the aortic wall and a varied approach with choice of stent tailored to the anatomy of the patient is required. Covered stent implantation

for treatment of aortic coarctation is safe and highly effective in selected patients. Self-expanding stent grafts may be preferable to balloon expandable stents when there is aneurysm formation in the setting of aortopathy [8].

Medical treatment is necessary to reduce high blood pressure in the upper limbs and control of heart failure. At newborns and infants with heart failure the treatment is diuretic therapy and digoxin. In case of severe heart failure, may be used dopamine for improvement of kidney function. In order to maintain the channel open arterial prostaglandin E1 will be administered by continuous infusion at a dose of 0.05-015 microgram / kg / min. At older children and adults the treatment of hypertension will be preoperative beta-blockers; postoperative beta-blockers, and vasodilators like sodium nitroprusside [9].

The treatment of aortic coarctation can be managed in different ways, and depends upon the age of presentation, the anatomical details of the aortic coarctation, the anatomy of aortic arch, and whether the coarctation is native or a recurrence following surgery. For infants, surgery is the preferred treatment of choice, but for the older children and adults the percutaneous procedures, such as balloon angioplasty or stent implantation, are the most popular methods for treating coarctation. In neonates, balloon angioplasty provides effective palliation only. [10] The treatment of aortic coarctation can be made with stent implantation for aortic recoarctation and native coarctation gives good immediate results. Careful follow up is necessary to evaluate complications and the long term effect on blood pressure. [11,12,13]. Various types of stents have been used and this paper specifically addresses the use of covered or graft Jomed stents in a small group of patients with coarctation [14]. Stent implantation represents a therapeutic option that can safely and effectively reduce gradien in challenging patients with mild postoperative coarctation [15]. Furthermore, our data suggest that aortic obstruction often coexists with ventricular diastolic dysfunction in these patients and that relief of obstruction may play a role in improvement of function. [16]

Balloon angioplasty is safe and effective in the treatment of native aortic coarctation; significant incidence of recoarctation is seen in neonates and infants [17,18]; repeat balloon angioplasty for recoarctation is feasible and effective; and the time has come to consider balloon angioplasty as a therapeutic procedure of choice for the treatment of native aortic coarctation. [19]

METHODS

The purpose of this paper is to highlighting the complex issues surrounding the place this condition in congenital heart diseases, therapeutic methods available, from the"classical"and ending with the most modern (angio CT, RMN) for the best management of diagnosis and treatment of AoCo.

For this we performed a retrospective study of clinical cases treated statistically and clinically diagnosed (TimCenter case studies) over 11 years (1998- 2008). Patients were selected on the basis of specific pathologies, CoAo who made a surgical intervention). Our sample analyzed medical records of 70 patients, obtaining an assessment of clinical, laboratory, and imaging and their treatment results. We followed the next parameters: age, sex, height and weight of patients heart rate, systolic murmur parasternal left intercostals artery pulsation, pulse absent, marked diminished or delayed

femoral arteries, hypertension in the upper limbs, headache, chest pain, epistaxis, lower limb lameness cold sometimes.

Laboratory examinations were: electrocardiography (heart rate, electric axis, signs of hypertrophy), chest radiography (coastal erosions), echocardiography - left atrial diameter (AS), the ventricles (LV, RV), left ventricular ejection fraction (LVEF), the diameter of the scending aorta (AoAsc), the maximum speed of blood flow in the aorta (Vmax), the maximum pressure (Pmax), angiographic examination, magnetic resonance imaging (MRI) and angioCT. For diagnostic accuracy in patients lot was very useful and a number of functional imaging examinations: electrocardiography, chest radiography, ultrasound, angiography, magnetic resonance imaging (MRI), angioCT.

RESULTS AND DISCUSSIONS

During the study was performed a total of 7027 interventions of which 1400 were MCC, and of these 70 were AoCo. AoCo had an incidence of 5.29 % of MCC (1323 cases) compared to 19.33 % of MCC dark heart (362 cases). From the 70 patients with a diagnosis of CoAo ratio male / female is 1.3-2.1 in most series in the literature. It shows a predominance of males in the retrospective study, the ratio M / F was 1.8: 1, which is consistent with data from literatură.33

Best represented ages were 12 to 13 years (5 cases each). Mean age of patients was 14.79 ± evaluated 10.65 years, so patients belong to the young, but with variations in a fairly wide range of minimum 2 months, maximum 44, which shows a great variability within the group. Age groups showed 6 patients of 0-1 years, 11 patients of 2-5 years, 9 patients of 6-10 years 16 patients of the 11-15 age group, 11 patients of 16-20, 5 patients to 21-25, 5 patients from 26 to 30 years, 3 patients to 31-35 years, and 4 patients aged 36-45 years. Age groups showed 6 patients of 0-1 years, 11 patients of 2-5 years, 9 patients of 6-10 years 16 patients of the 11-15 age group, 11 patients of 16-20, 5 patients to 21-25, 5 patients from 26 to 30 years, 3 patients to 31-35 years, and 4 patients aged 36-45 years. Somatic parameters evaluated showed a shift to the left of the frequency distribution (8 and 9 cases percentile 0-5) with a relative flattening the midpoint.

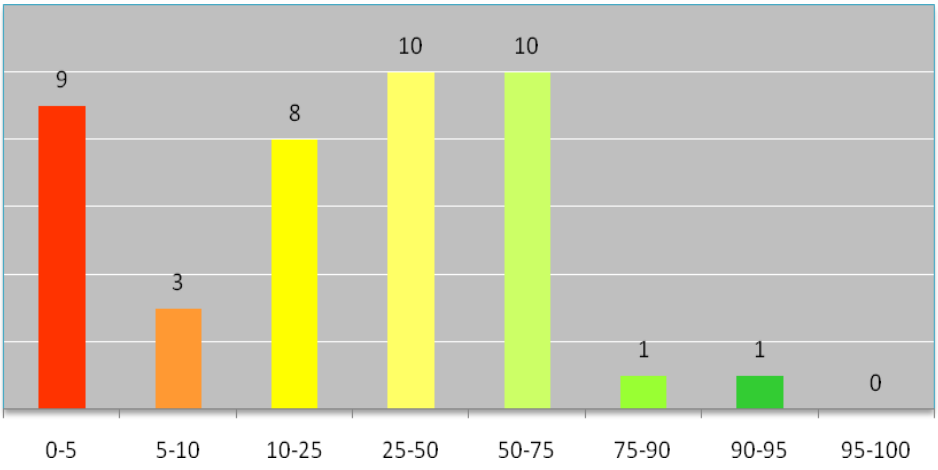

Figure 2. Frequency histogram percentiles for weight gain in patients aged 0-16 years

Somatic parameters evaluated showed a shift to the left of the frequency distribution (8 and 9 cases percentile 0-5) with a relative flattening the midpoint (whole underdeveloped stature - weight). Distribution of height percentiles for boys and girls aged 0-16 years (28 boys) shows the same effect (7 boys height or weight percentile 0-5), representing a marked delay in the stature-weighted development; and 14 girls show a similar phenomenon, representing a growth retarding weight. Statistical data shows that the height of male patients is significantly higher than those of females ($p = 0.006336$) in patients between the age of 2-5 years. In the age group 6-12 years ($p = 0.7348$) were not significant differences between male and female.

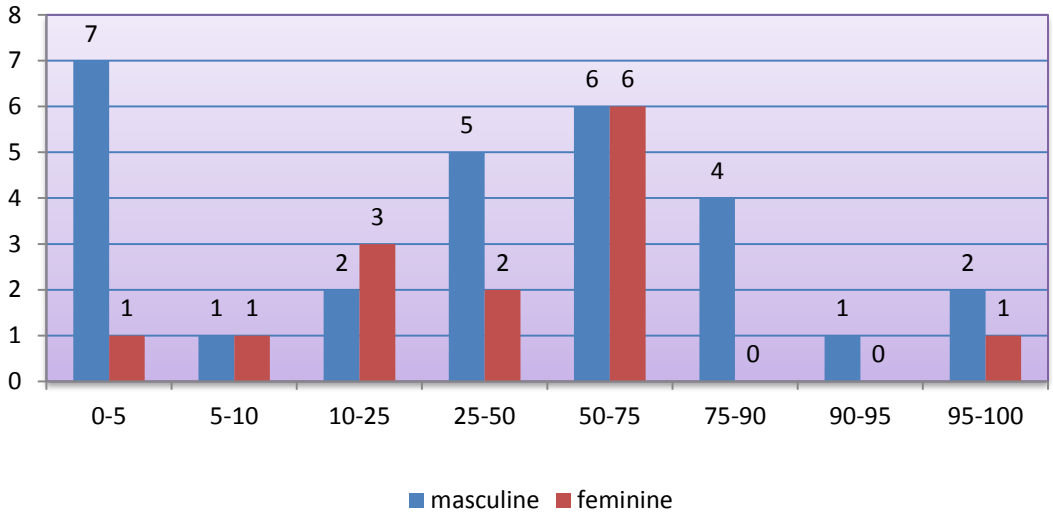

Figure 3. Frequency histogram height percentiles for boys and girls aged 0-16 years

For diagnosis of the patients were evaluated existing clinical signs in patients with coarctation of the aorta: the presence (and intensity) of a left parasternal systolic murmur, intercostal artery pulsation, and pulse in the femoral artery. The Heart rate had an average value of 92.54 ± 21.12 bpm (minimum 46 bpm, 150 bpm maximum) with high variability depending on age groups.

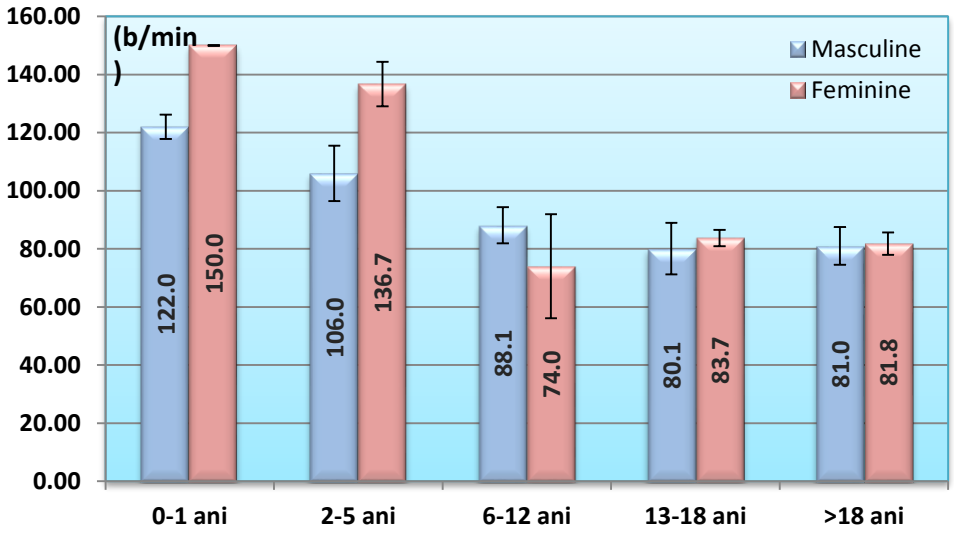

Figure 4. Heart rate by age and sex at patients with AoCo

Left parasternal systolic murmur was undetectable in 8 patients, other patients with varying degrees breath of 19 patients blast grade II / VI, 32 patients grade III / VI, 10 patients grade IV / VI and one patient blast grade V / VI. A blast to the chest mezosistolic anterior, posterior spinous processes and may become continuous if stenosed lumen is sufficient so as to cause a high flow through the lesion throughout the cardiac cycle. Additional systolic murmurs and continuous lateral chest wall may reflect increased flow through collateral vessels dilated and tortuous. Collateral circulation is also an important sign and can develop in the newborn period, but is observed especially in adolescent and adult. When relevant, can cause coastal erosion pulses palpable and visible intercostals chest radiography. The femoral artery pulse was absent in 44 cases, weak detectable in 21 cases and well beaten (near normal) only in 5 cases. The sign marked characteristic is to reduce the delay or loss of femoral pulse, contrasting with large artery pulsations upper limb. The difference can be objectified by measuring blood pressure at the upper and lower limbs. Were found TASDI significant differences in all age groups studied in males (p = 0.00160765, FS).

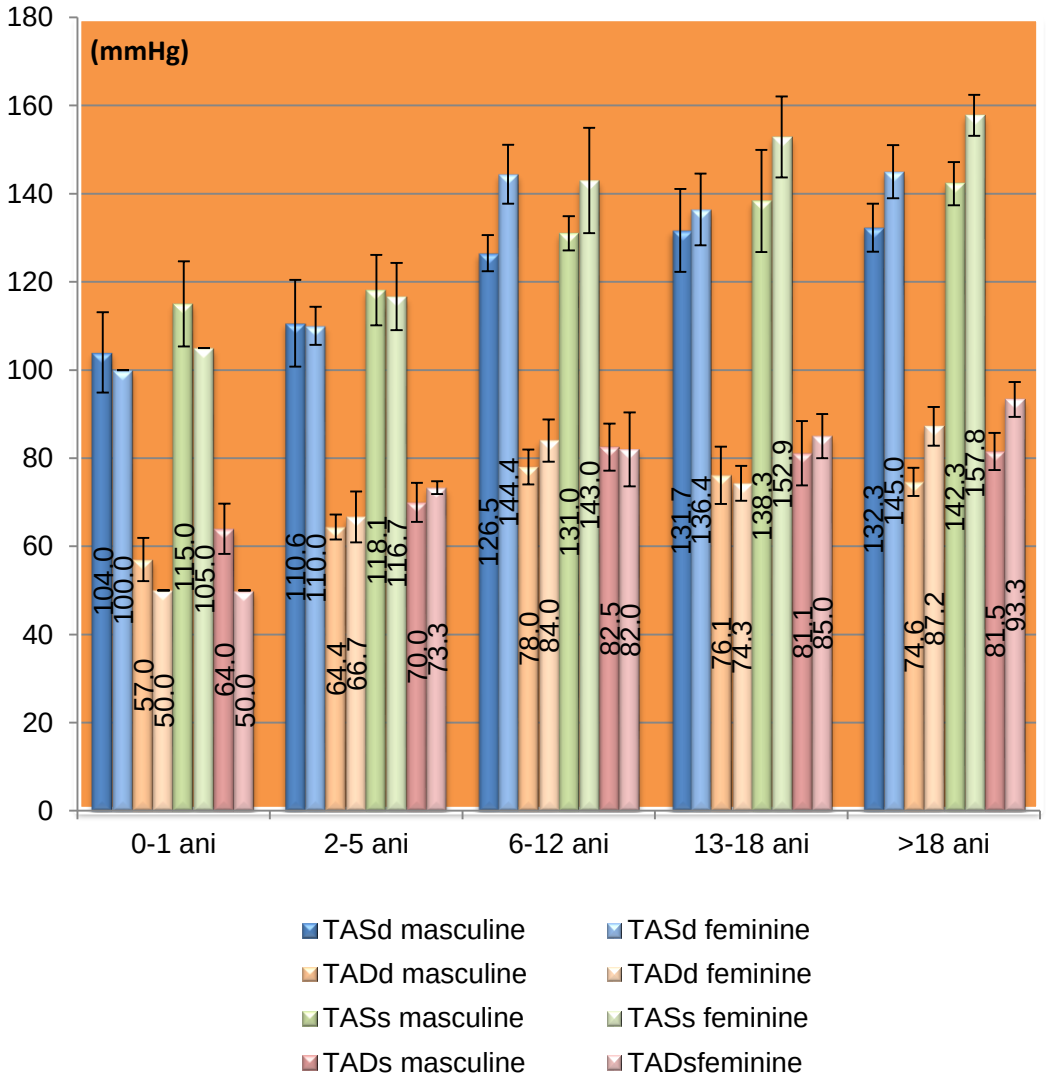

Figure 5. Mean arterial pressure by age and sex in patients with AoCo

Exploring ECG rhythm were highlighted flutter, atrial fibrillation - FIA) and changes in QRS like right bundle branch block highlighting - BRD or left ventricular hypertrophy. The most common change was left ventricular hypertrophy associated with sinus rhythm in 29 cases, 4 cases with RS and right bundle branch block and one patient with atrial fibrillation. Almost half of the patients (48.57 %) was determined electrocardiographic LVH. Other aspects ECG were identified RS + BRD, 7 patients, RS + HVD in 1 patient, RS + + HVD also BRD in 1 patient or flutter isolated in 1 patient. Have a relatively high weight and 26 patients with electrocardiographic changes (RS).

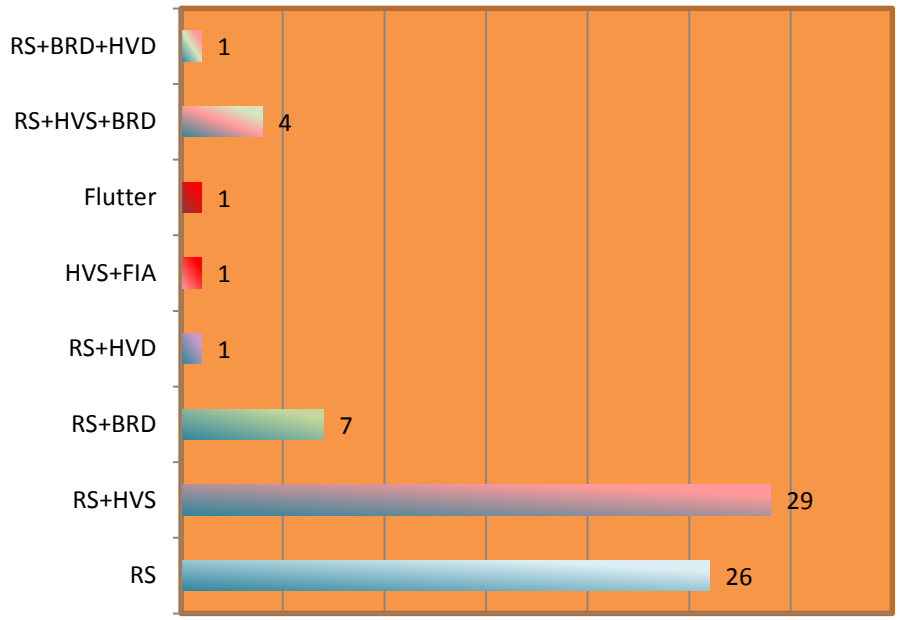

Figure 6. Electrocardiographic changes in patients with AoCo

Coastal erosion is important for observing AoCo place in relation to the origin of the subclavian artery. Fingerprints appear on the lower rib ribs (3-8), usually after the age of 12 years. Unilateral and bilateral coastal erosion can be observed in many other conditions and usually related to changes in the arteries, veins, nerves intercostals. The meeting provides the clinician and surgeon coastal erosion data traffic degree colaterale. Were determined echocardiography left atrial diameter (AS), the ventricles (LV, RV), left ventricular ejection fraction (LVEF), the diameter of the ascending aorta (AoAsc, the maximum speed of blood flow in the aorta (Vmax), the maximum pressure (Pmax). Two-dimensional echocardiography by para- or suprasternal section identifies the location and length CoAo, while studies Doppler pressure gradient recorded and quantified. Left atrial diameter was 3.17 ± 0.88 cm (1.80 inches minimum, maximum 5.80 cm), the average variability, depending on the age, sex and the degree of expansion / left atrial hypertrophy LV ejection fraction was 66.06 % on average, at least 30 %, maximum 91 %, with average variability parameter depending on the change of cardiac contractile function. 98, 75% of patients were determined to have a normal LVEF. AoCo associated Diagnostics From the lot of 70 patients, a total of 62 were diagnosed with primary AoCo and 8 have associated diagnosis like atrial septal defect (DSA)- 3 patients, aortic stenosis -2 patients, heart failure- 2 patients, lung grafts window, the transposition of great arteries – 1

patient, 1 patient with DSA and DSV and 2 with aortic stenosis AoCo.

Drug treatment for those 70 patients consisted of prescribing AoCo complex therapy depending on the specific case (given the heterogeneity associated diagnoses) in 3 major therapeutic classes: beta - blockers, ACE inhibitors and angiotensin converting diuretics, along with other therapeutic options (calcium channel blockers, cardiac glycosides etc).

The most common treatment option, either alone or in combination with other groups was the class of beta (used alone in 21 cases - 30% dual therapy with ACE inhibitors in 17 cases - 24.29 % with diuretics 5 cases - 7.14 %, beta blocker therapy Triple + ACEI + diuretic in 2 cases - 2.86 %). On the second place as frequency of use were angiotensin-converting enzyme inhibitors: alone in 5 cases - 7.14 %, double or triple therapy in 21 cases - 30% and diuretics were used only in 10 cases - 14.29 %: the alone in one case - 1.43 %, in combination with other medications in 9 cases - 12.86 %

The surgery treatment most commonly practiced was istmoplastia with synthetic patch in 27 cases or associated with diaphragm resection in 8 cases. Another option was istmoplastia with pericardial patch in 6 cases (8.57 %) or associated with diaphragm resection at 10 cases. Coarctation area resection with end-to-end suture 10 patients. Bypass technique with Dacron tubular prosthesis was used in 7 patients. A special case was solved by bypass with tubular prosthesis required extracorporeal circulation - 119 minutes.

In total there were practiced 51 istmoplastia, 7 bypasses and 10 resection with end-to-end.

CONCLUSIONS

No specific laboratory tests are necessary for AoCo, but for the best diagnosis is to follow the clinical signs and chest radiography, echocardiography or MRI. The diagnosis of AoCo generally can be made on physical examination, were relieved the differential blood pressure and pulse delay are path gnomonic. Tratamentul si prognosticul coarctației de aortă sunt bune în situația în care diagnosticul este pus precoce si se actioneaza terapeutic imediat. Medical treatment of neonates with severe AoCo include: intubation, infusion of prostaglandin E1, acidosis correction and inotropic support for improvement of symptoms of congestive heart failure (CHF). The medical treatment of less severe AoCo beyond the neonatal period include: administration of digoxin and diuretics for chronically increased afterload and signs of CHF and postponement of intervention (surgery or balloon dilatation) until the patient is hemodynamically stable.

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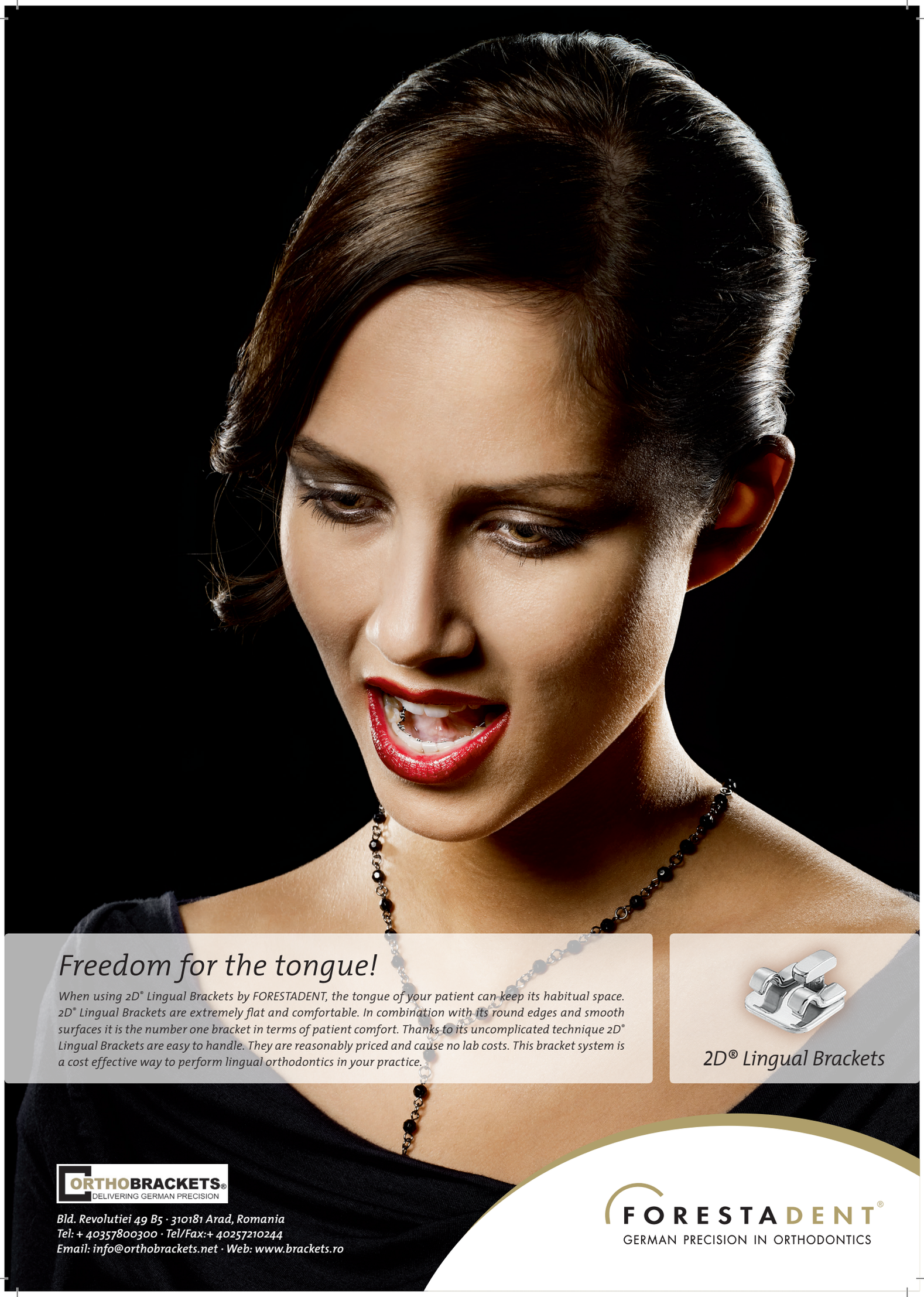
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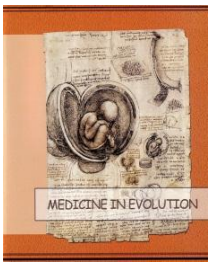

THE EXPECTATIONS OF SENIOR STUDENTS FROM THE FACULTY OF DENTAL MEDICINE REGARDING THEIR FUTURE PROFESSIONAL CAREER

MARINEL CHIRCA[1], CORNELIA BICLEŞANU[2]

[1]C.M.I. Dr. Chirca Marinel
[2]Faculty of Dental Medicine, Titu Maiorescu University

ABSTRACT

The study aims to present the expectations of students from the faculties of dental medicine regarding their future medical career, depending on the social-economic level, the theoretical and practical abilities obtained after graduation, professional targets etc.

Materials and method The study lot is formed by 358 senior students from years V and VI of the faculties of dental medicine from Bucharest and Iaşi, aged between 23 and 27 years. For the research it has been used the query method which consists of 12 questions. The collected data from the subjects have been centralised in a database for further interpretation.

Results: 152 (42,5%) respondents were informed before the admission about the educational system, style and training structure and 52,8% appreciate that the activities undergone in the syllabus well strucutured, flexible and with practical importance. 180 (50,3%) respondents are determined to pursue the path of being a dentist after graduation. Crosstabular analysis revealed that the majority of students who opt for the same career are from Iaşi, 95 (65,5%) compared to the 85 (39,9%) from Bucharest. Instead, 39,4% from the Bucharest students consider themselves unsure compared to 16,6%, the procent of those from Iaşi. The huge differences observed have a statistical significance, p=0,001. Learning from professionals represents the option for more than a half of the respondents. 47,5% respondents want to begin their activity in their own cabinet, only 39,7% will take part in the residency exam.

Conclusions: It is necessary to found centres for counseling and career guidance in every faculty to support the students in taking professional decisions.

Key words: *query, professional success, career expectations, professional satisfaction*

Correspondence to:

Dr. Marinel Chirca
Address: Bulevardul Republicii, Nr.43, Pitesti
Phone: +40745819487
E-mail address: marinelchirca@yahoo.com

INTRODUCTION

One chooses his career path as a consequence of a complex system of motivations of intrinsical and extrinsical nature which define his interest and emerge from his normal tendency of self-fulfillment [6]. The road to fulfilling the destiny of a medical career takes continuous effort (there is no clinical or intellectual rest), continuous learning (obsolete practices are not accepted), as long as professional and human perfectioning with no pause (compentence mixes with character and forms the profile of the appreciated doctor, praised by apprentices and pacients) [6].

MATERIAL AND METHODS

The study was taken on lot formed by 358 senior students from years V and VI of the faculties of dental medicine from Bucharest and Iaşi, aged between 23 and 27 years, of which 158 (44,1%) were males and 200 (55,9%) females. 191 (53,4%) were students from year V and 167 (46,6%) from year VI. From Bucharest it has been interviewed a number of 213 (59,5%) and from Iasi 145 (40,5%) senior students. The study was undergone by creating a query which consists of 12 questions divised in 3 sections: educational system and academic curriculum (first 5 questions), options regarding professional future (questions 6,7,8,9), choosing the work place (last 3 questions).

For the realisation of the statistical analysis it has been used SPSS18 (for Windows) with the help of which the following were calculated: the elements of descriptive statistics – the frequency of answers to the questions and crosstabular analysis. Also it has been tested the statistical significance of differences between answers with the aid of Chi-square test. In the situation of which over 20% of cells have the expected frequency less than 5, we called on correction with the Fisher test. The ethical aspect of the study concerned data confidentiality and the impossible identification of participants of the study in case of publishing the results.

RESULTS

The answers from section I revealed that 152 (42,5%) were informed before admission about the educational system, style and training structure and 52,8% appreciate that the activities undergone in the syllabus are well structured, flexible and with practical importance. The number of practical classes during the clinical internships is appreciated as being reduced by the majority of students, 153 (42,7%). The answers prove that the group division by the year of study is appreciated by most of the students, 192 (53,6%). A low number of students, 92 (25,7%) could not manage to identify their objectives for the future profession.

Section 2 of the query showed that most of the students, 149 (41,6%) hope to get an annual salary between 10.000-20.000 euro, in their own cabinet, 170 (47,5%). The majority, 180 (50,3%) are determined to continue becoming a dentist, and 70 (19,6%) of the total of 358 students wish to change their career. The number of unsure people is also remarkable, 108 (30,2%) fig. 1.

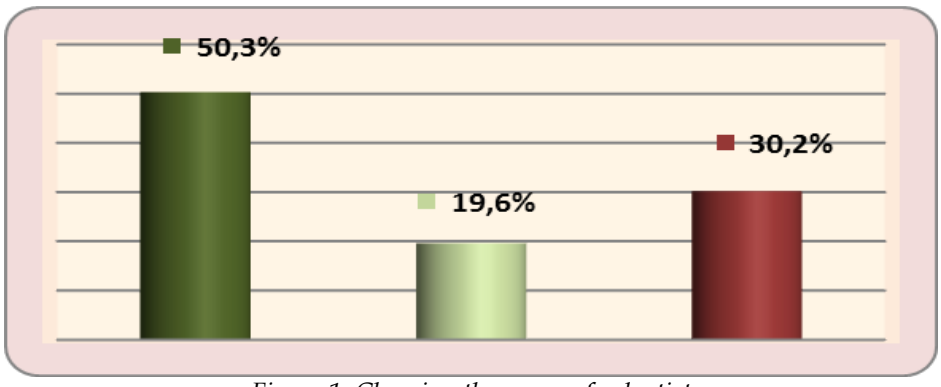

Figure 1. Choosing the career of a dentist

Continuing the crosstabular analysis for this question depending on the variable *faculty*, I observed that most of the students who opt for the same career are from Iasi, 95 (65,5%) compared to 85 (39,9%) from Bucharest. Still, 39,4% from the students of Bucharest are considering themselves undecided, compared to the 16,6% of those from Iasi. The observed differences have a statistical meaning, p=0,001.

Choosing the first workplace varies from the possibility of learning from the professionals, where it has been observed the biggest proportion of positive answers, 52,8%, until the obtaining of undetermined period work contract, where only 7,8% considered it an important element for their future career; for 29,6% of the students, on the first place is the obtained salary (fig. 2).

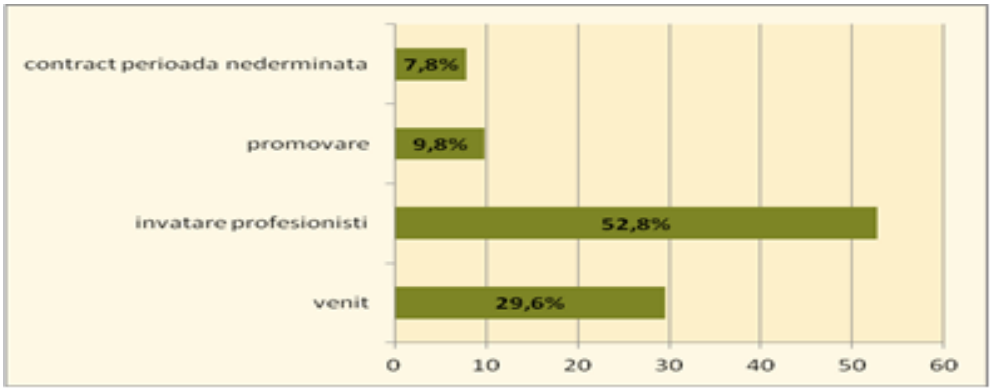

Figure 2. Important elements at the workplace

134 (37,4%) of the respondents consider that the most important aspect from the beginning of their career is professional perfecting, while 126 (35,2%) appreciate financial security as being the safest. Just 71 (19,8%) appreciate workplace stability, while 7,5% accord the highest importance to social insurances. (Fig. 3)

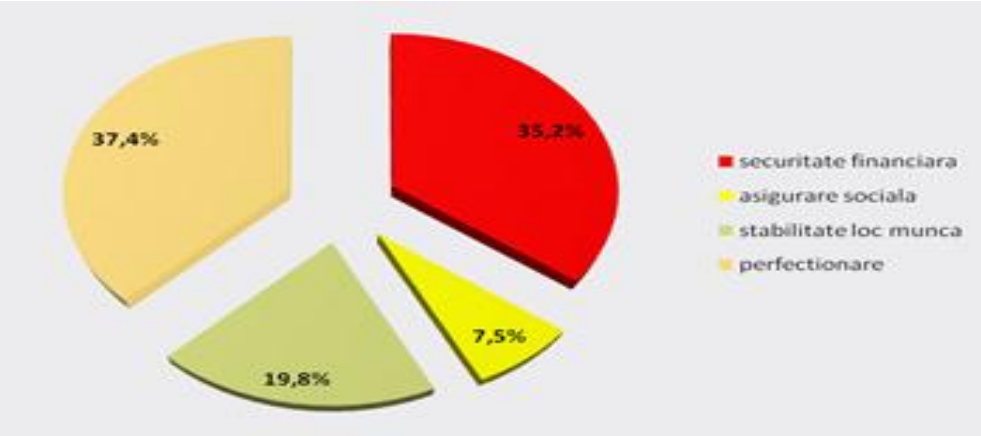

Figure 3. The most important aspects at the beginning of the career

The last question of the query accentuates professional development and the time provided for it. Sustained work consists in the basis of professional development of the majority of students, 161 (45%), the rest being divided in the following way: 84 (23,5%) consider that continuous medical education is the most important element for the professional development, 66 (18,4%) put on the first place personal needs and 47 (13,1%) agree that family represents the most important aspect for which it needs to be allocated the majority of time (fig. 4).

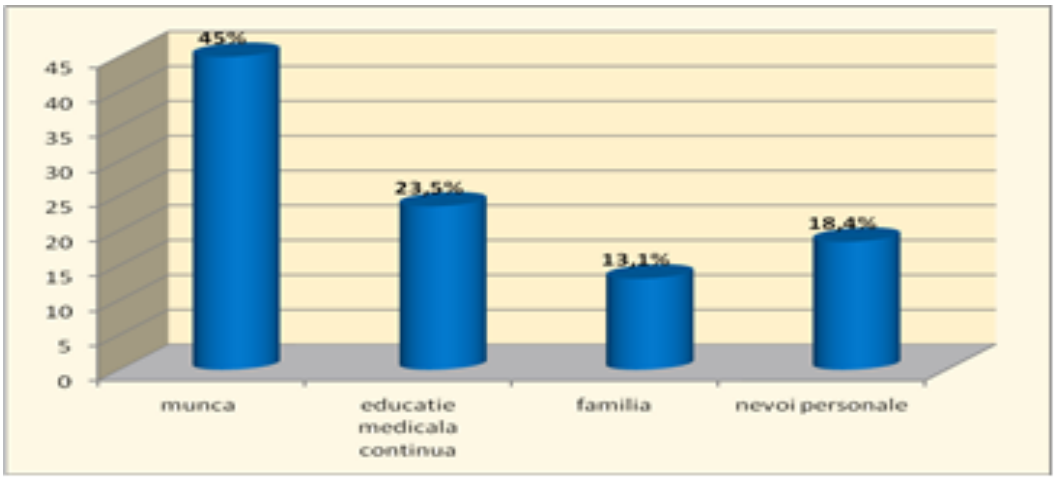

Figure 4. Important elements for career development

DISCUSSIONS

Choosing a career is done by taking into account the values, motivations, abilities and personal skills [4]. This thing presumes a previously rigorous documentation about what it means to work in the medical field. Information about profession and its environment is very important. Other information consists of possibilities of obtaining the diploma, how tough is the admission competition, how much do the studies last and how much do they cost. Most of the times objective or subjective barriers intervene in choosing the medical career: these can be due to family's influence or pressure, financial terms or even to the admission process [8, 9]. In this situation, information received by the high school graduate can come not only from the teachers and family, but from mass-media or acquaintances' group, so it's necessary for each faculty to promote its own

universitary curriculum, way of running the exams, practical internships or even professional fulfilment opportunities.

Contrary, failure or insuccess can overcome, due to the fact that motivations and expectations do not match up to the satisfaction and practical achievable possibilities after graduation. This could be a possible explanation of the fact that 70 of the respondents would not choose being a dentist for a second time. There are universities which offer students counselling and career guidance centres which have the role of supporting students in taking professional decisions according to their personality profile and in inserting them in the labor market [2]. For this reason, I consider appropriate, giving the context of the obtained results from the study, to found these counselling and career guidance centres at the medicine universities throughout the country which today do not dispose of these programmes for students. Their role would consist in informing students regarding to potential employers, options that they could have at the end of their studies, support offered regarding career planning, or taking a realistic career decision. In other speciality studies, like the one from India realised by Aggarval and his contributors on a lot of 369 dental medicine students, the proportion of subjects content with the academic process is 64,5% [1]. In another study from Canada, authors observed that students wish for the educational process to have more clinical activities, starting even with the first year of study, another wish being that of compressing preclinical activities [5]. In Canada the SYLLABUS is conceived as that the students who have not received high grades in the first years of studies cannot access a superior educational level, which implies their exclusion from professional training in certain specialties, like ortodentistry and dento-alveolar surgery [10]. In Romania, medical school adapted rapidly to the European standards of superior medical education with reference to the 5.500 hours of training for doctors and 4.600 hours of training for nurses and medical assistants, regarding practical education, curriculum component, the necessary readjustments being minimal and, in a very short time, managing to update to present what was necessary for their diplomas to be recognised in every country of the EU.

Foreign emigration is seen as an opportunity for 22,1% of the Romanian students; 24,1% being the percent of male students, compared to 20% of female students in choosing this option. In the actual situation of work force in Europe, when in the richer countries is observed a fall in the number of general dentists, taking into account the fact of curriculum uniformisation and the possibility of recognising the diploma from Romania, this option is not neglecting at all. Other studies quote the same tendency: for example, in India, the percent of those who wish to work abroad is of 30,1%, and in Thailand this reaches even 44,2% [1, 7]. In France, a dentist earns to about 15.000 euros a month, while in other countries from the EU a salary reaches a value of 40.000 euros an year. Nowadays, according to international statistics, the medium salary of dentists from the EU countries is about 3.500 euros, meaning almost 10 times bigger than the one from Romania [3].

CONCLUSIONS

Information received before admission aid in choosing correctly the medical career. To support students in taking professional decisions according to their personality profile and to insert them in the labor market, it would be

useful to found counselling and career guidance centres in each faculty.

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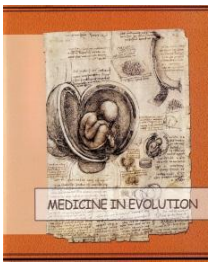
LATERAL CEPHALOMETRIC ANALYSIS – A REVIEW



CRISTINA DUMITROIU[1], ILINCA POPOACĂ[1], ANCA TEMELCEA[1], RADU STANCIU[1], DRAGOŞ STANCIU[1]

[1]"Carol Davila"University of Medicine and Pharmacy, Bucharest, Romania

ABSTRACT

Cephalometric analysis helps not only by diagnosing dento-maxillary anomalies but also by identifying the complications. It is an important tool for monitoring the treatment and follow-up and, also the screening of different skeletal maloclussions.

Key words: *dento-maxillary anomalies, cephalometric analysis, orthodontic diagnosis*

Correspondence to:

Anca Temelcea
Address:"Carol Davila"University of Medicine and Pharmacy, 37 Dionisie Lupu, Bucharest
Phone: +40213180720
E-mail address: office@leodent.ro

INTRODUCTION

Dento-maxillary anomalies represent a common pathology, often with varying degrees of diagnosis difficulty because of its various clinical types. Proper diagnosis and treatment can significantly reduce the morbidity associated with this condition.

If orthodontists confronts patients with suspected or established skeletal maloclussion, it is likely to recommend an imaging investigation. Frequently, ortopantomograph (OPG) is performed as initial test, but in modern orthodontics, lateral cephalometric radiograph is the imaging modality of reference for the diagnosis of dento-maxillary anomalies (1).

CEPHALOMETRIC ANALYSIS IN ORTHODONTICS AND DENTOFACIAL ORTHOPEDICS

Cephalometric analysis serves many goals. It is initially used to diagnose or exclude a skeletal malocclusion. During this process, cephalometric radiograph is frequently only one of many treatment stages and is combined with information provided by the clinical examination and patient history in order to establish the right diagnosis. After the diagnosis, cephalometric radiograph is useful to assess the pacient response to treatment. This is a method to evaluate if the response to treatment is adequate, or to see if a change of therapy is required in cases of inadequate response (7).

Cephalometric analysis can also be used for screening in order to detect anomalies that are already present but are poor in symptoms (6). This approach alows a premature dento-maxillary anomalies detection in early stages of evolution, so that specialists begin an appropriate treatment.

For this reason, it is often the most important diagnostic tool for suggesting the correct diagnosis and monitoring the outcome of orthodontic treatment (Fig.1).

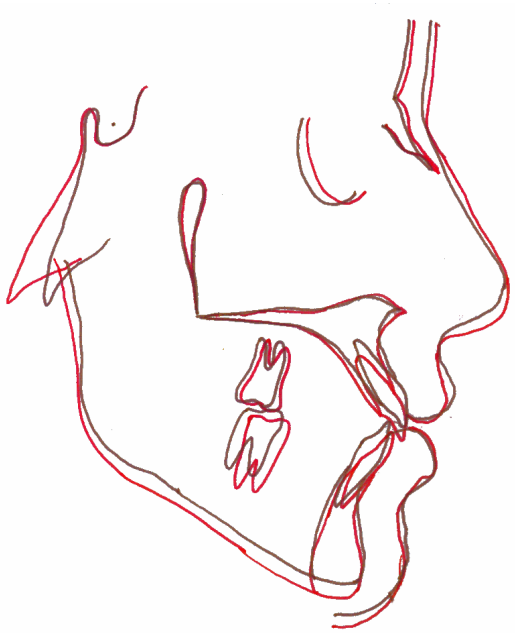


Figure 1. Manual cephalometric tracing pre (black)- and post (red)- orthodontic treatment; the superimposition has been made using N-S line, în S point

Profile cephalometric radiograph is challenging, since the investigated area contains a large volume of air with no signal and a number of artefact sources, that could lead to distortions in images (5). This issue has been revised by recent technologies. Modern devices produce images with great contrast and they are well suited to orthodontic diagnosis. An important aspect of the interpretation process is knowledge of the clinical setting behind the radiological findings. This means that it is important to be provided with accurate clinical details.

Undergoing cephalometric radiograph involves no discomfort. In comparison with OPG, profile cephalometeric radiograph involve relatively large doses of ionising radiation, which are potentially harmful to human tissue. However, nowdays this procedure uses ionising radiation in very low doses; a single examination or even several examinations do not produce harmful dose levels for patients. Over the last decades, techniques have been developed to reduce drastically the dose of radiation involved in an examination and to allow for the dose to be adapted. This ensures that only the amount of radiation absolutely necessary will be used as a diagnostic indicator.

Cephalometric analysis plays an important role in the assessment of treatment response and in the follow-up of patients surgically treated for severe dento-maxillary anomalies (4).

Sometimes, even when a proper treatment is conducted, dento-maxillary anomalies relapse. Most recurrences occur within the first two years following completion of the orthodontic treatment. Therefore, it is important to schedule a tighter follow-up for the patient during that period. Profile cephalometric radiograph is essential for investigating recurrences (3).

Recent advance in radiology include the transition from analogue to digital techniques (Fig.2). The advantages of digital lateral cephalometric radiograph include a more consistent and optimised image quality (2). Image data can be transferred and computerised analysis of the data is available.

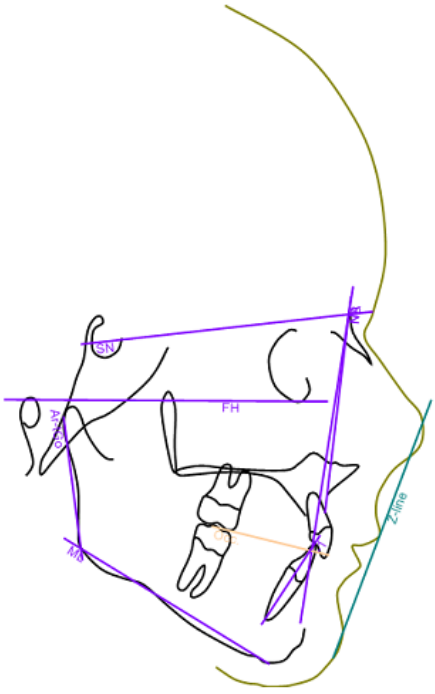


Figure 2. Computerized cephalometric analysis

In the future, cephalometric radiographs will provide more detailed images, quicker and with less radiation. Cephalometric analysis is an essential tool in orthodontics and will certainly continue to play a key role in the future.

CONCLUSIONS

It can be stated that cephalometric analysis is still in everyday use and represent a prognostic indicator for treatment success. The technique has been constantly improved over the last few years and it serves as a baseline examination for dento-maxillary anomalies of great value..

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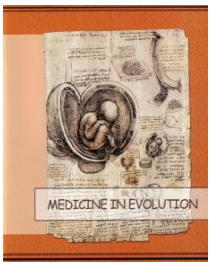
IMMUNOHISTOCHEMICAL STAINING ANALYSIS OF PLATELET CONCENTRATE



IRIMIA CRISTIAN[1], DINCĂ OCTAVIAN[1], BUCUR MIHAI-BOGDAN[1], VLĂDAN CRISTIAN[1], BUCUR ALEXANDRU[1]

[1]"Carol Davila"University of Medicine and Pharmacy, Bucharest, Romania

ABSTRACT

Platelets play an important role in haemostasis, but recently it was demonstrated that platelets contribute to wound healing. Activated platelets initiate endothelial cell activation and produce a number of pro-inflammatory mediators and growing factors. The aim of this study was to investigate the expression of platelet membrane glycoproteins CD31 (vascular marker) and CD61 (platelet marker) in PRP.

Key words: *platelets, plasma concentrate, immunocytochemistry*

Correspondence to:

Cristian Vlădan
Address: Clinica de Chirurgie Oro-Maxilo-Facială a UMF „Carol Davila"Calea Plevnei 19 010221 Bucureşti, Romania
E-mail address: crystalvladan@gmail.com

INTRODUCTION

Autologous platelet-rich plasma (PRP) therapies have seen a dramatic increase in breadth and frequency of use for dental conditions in the past years (5,6). Rich in many growth factors that have important implications in healing, PRP can potentially regenerate tissue via multiple mechanisms (3). However, there is limited reliable clinical evidence to guide the use of PRP.

A high amount of platelet into platelet concentrates (PRP) might reasonably be expected and may contribute to wound healing (1). To investigate this possibility, we studied the pattern of PRP, using immunocytochemical methods with two anti-bodies. One of these detects platelet membranes (CD61) and the other labels vascular endothelium (CD31).

MATERIAL AND METHODS

Blood was obtained from a healthy adult patient and collected into tubes containing acid-citrate-dextrose solution formula A (1:4 vol/vol) anticoagulant. PRP was prepared using a double-spin method; the citrated blood was centrifuged in a standard laboratory centrifuge for 3 min at 1,800 rpm to remove red blood cells. Subsequently, a second round of centrifugation was performed for 6 min at 1,900 rpm, to obtain the platelet pellet. A 1:1 mixture of 0.5 M calcium chloride and thrombin was prepared in advance as an activator. A 10:1 mixture of PRP and activator was incubated at room temperature.

Several smears were obtained from PRP material. The smears were double immunostaining stained with a monoclonal anti-CD-31 antibody (Clone: 1A10; Novocastra-Leica Biosystems, Newcastle Upon Tyne, U.K; dilution 1:100) and anti-CD-61 antibody (Clone: 2f2; Novocastra-Leica Biosystems, Newcastle Upon Tyne, U.K; dilution 1:100). Subsequently, diaminobenzidine (DAB) based staining system was used to visualize antibody binding. Sections were counterstained with hematoxylin.

RESULTS

In the present study we investigated the immunophenotypic profiles of platelets from PRP samples. CD31 staining was diffusely positive (Fig.1).

CD61 staining was similar to that for CD31 (Fig.2).

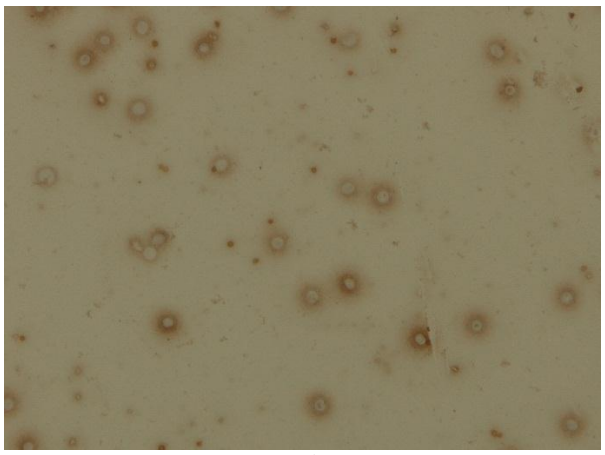


Figure 1. Positive staining for CD31 in PRP. CD31 immunostaining,×400.
Image courtesy of Prof.dr.Alexandru Bucur

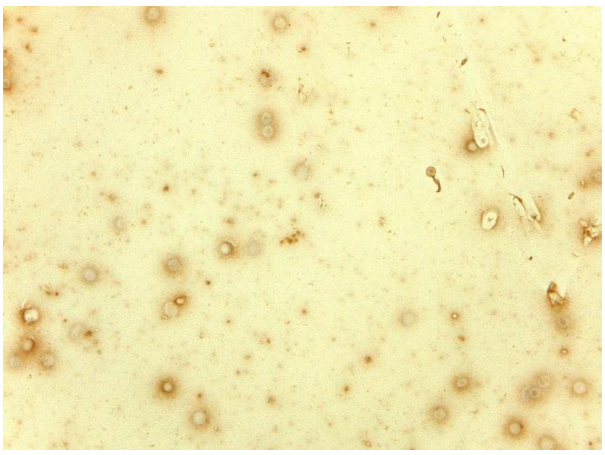


Figure 2. CD61 expression in PRP. CD31 immunostaining, ×400.
Image courtesy of Prof.dr.Alexandru Bucur

DISCUSSIONS

During wound healing, platelets are activated by contact with collagen, and release their α-granule content after aggregation. This secretion is intense in the first hours and platelets continue synthesizing more cytokines and growth factors for a couple of days (7).

The aim of the topical application of PRP is to provide tissues concentrated growth factors to induce or to accelerate the wound healing process. The relationship among platelet concentration, growth factor concentration and clinical effectiveness is far from being clear, since most of the studies claiming clinical efficacy of PRP were not controlled trials and/or reported small group of patients (10); majority of reported clinical studies do not have sufficient statistical power to give conclusive results (2).

In view of multiple potential PRP applications in dentistry, comparative analyses of different clinical situations would be useful. These comparisons are not easy because PRP is prepared using different protocols. Another issue is whether there is a correlation between the platelet concentration or the PRP volume applied (8). Recent studies have demonstrated that low platelet concentration is inefficient and that high concentrations have an inhibitory effect on cell growth, but results were still unclear (4, 9).

CD31 and C61 were found in large quantities on the platelets surface in PRP. This observation is of particular importance, as the exact mechanisms of therapeutic effect of PRP are still not fully understood and the effects of activated platelets have not been reported yet. We believe that the results of this study will allow a better understanding of the therapeutic effects of PRP, with an important implication for improving the effectiveness of platelet concentrate and reducing its side effects during treatment. However, further study of more cases would be needed to confirm this.

CONCLUSIONS

Although it remains unclear whether the effect of platelets plays a primary or secondary role in wound healing therapy when compared to other cell types such as white blood cells, these findings may help refine the therapeutic algorithm in dentistry, if

their effectiveness is confirmed in larger series of patients.

Authors Contribution

All authors designed the study, contributed in the acquisition of the material and interpretation of the data, reviewed the paper and approved the paper for publication.

Acknowledgments

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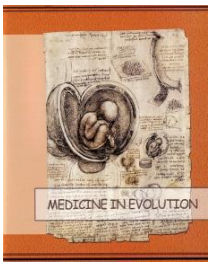
BISPHOSPHONATE-RELATED OSTEONECROSIS OF THE UPPER JAW



CRISTINA PĂDURARIU[1], BUCUR MIHAI-BOGDAN[1], DINCĂ OCTAVIAN[1], VLĂDAN CRISTIAN[1], BUCUR ALEXANDRU[1]

[1]"Carol Davila"University of Medicine and Pharmacy, Bucharest, Romania

ABSTRACT

Bisphosphonates are a class of agents mainly used in the treatment of osteoporosis. Osteonecrosis of the jaws has emerged in the last decade as a severe complication for patients receiving this medication. Our case presentation focuses on a 58 years old woman with a 5 years history of weekly use of alendronic acid, resulting in bisphosphonates-related osteoradionecrosis of the jaws (BRONJ). This entity remains a challenge for the diagnosis set, as well as for the administered treatment. The goal of our work is to provide a fair method of treatment of early stages of BRONJ.

Key words: *osteonecrosis of the jaw, bisphosphonates*

Correspondence to:

Cristian Vlădan
Address: Clinica de Chirurgie Oro-Maxilo-Facială a UMF „Carol Davila"Calea Plevnei 19 010221 Bucureşti, Romania
E-mail address: crystalvladan@gmail.com

INTRODUCTION

In patients treated with oral bisphosphonates, BRONJ has been reported as a relatively rare, but potentially severe complication. Clinically, it is described as an area of exposed bone in the jaws that has failed to heal within a period of eight weeks in a patient currently or previously exposed to bisphosphonates and who has not undergone radiation therapy in head and neck region (4). BRONJ progression is three-staged, identified by clinical signs and symptoms (6). Patients receiving oral bisphosphonates have a lower risk to develop BRONJ when compared to cancer patients receiving intravenous medication (6). Despite the low rate, the risk of developing BRONJ increases when oral bisphosphonates use is longer than four years (7). In most cases, bisphosphonate-associated osteonecrosis occurs in the mandible (5). There are several studies on the occurrence of the disease in the maxilla, but no subgroup for examining the symptoms was defined (2).

A case of BRONJ of the upper jaw without involvement of the maxillary sinus is reported. Surgical intervention comprising sequestrectomy is described.

CASE REPORT

A 58 years old female patient was reported to Oro-Maxillo- Facial Surgery Clinic of Bucharest with complaint of swelling over left upper jaw for 6 months. She accused pain with foul smelling discharge asoociated with an extraction wound for the last 4 months. Empiric antibiotic treatment was administered with no success.

Her medical history was significant; she was diagnosed with osteoporosis 6 years ago and she underwent treatment with weekly doses of alendronic acid. She was also receiving properly medication for high blood pressure and a type 2 diabetes.

Dental history revealed that she underwent removal of 28 tooth eight months earlier.

The oral examination revealed she was partially edentulous with dehiscence in the alveolar mucosa in left upper jaw and exposure of bone with an yellowish-white appearance in the posterior region of the maxilla (Figure 1). There was no purulent discharge from the oral wound.

Figure 1. Initial oral examination showed exposure of bone in the area of teeth 27, 28

Orthopantogram showed radiopaque sequester with irregular peripheral radiolucency. Correlating history, clinical evaluation and radiologic examination, diagnosis of BRONJ was established.

Sequestrectomy with limited debridement under local anaesthesia was performed (Fig.2).

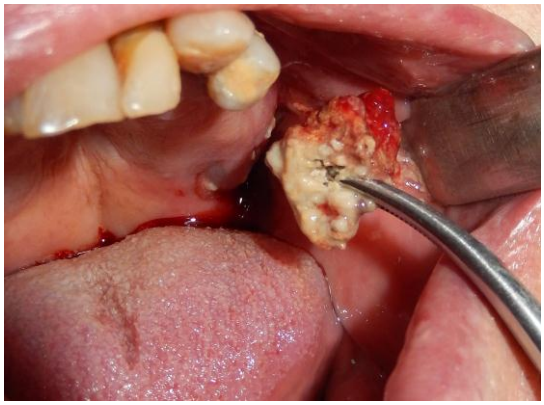


Figure 2. Bone sequester

Bone fragments were sent for histopathology analysis; the histologic report revealed lamellar bony trabeculae and inflammatory cells infiltration comprising of lymphocytes, other plasma cells and histiocytes, cells specific for an inflamatory process.

Four months follow-up showed no recurrence. Patient is currently under regular follow-up and properly monitorised.

DISCUSSIONS

BRONJ is a severe complication after bisphosphonate therapy for osteoporosis. Treatment of this complication is a major challenge for oro-maxillo-facial surgeons (1).

Principles of conservative treatment involves administration of an antimicrobial therapy and superficial curettage of the necrotic bone, therapeutic conduct appropriate in most of the cases of BRONJ (3).

The described case shows that the involvement of the upper jaw requires sequestrectomy as there was no other way to create conditions for the healing process in the surrounded soft tissues.

However, further observations to specify the indications for sequestrectomy in a larger population of patients with BRONJ are necessary.

CONCLUSIONS

Clinical management of BRONJ presents several problems. Compared with other therapeutic alternatives, the described protocol has introduced the use of sequestrectomy as therapeutic support.

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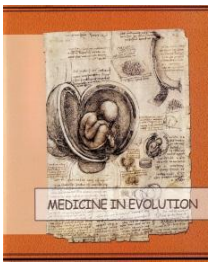
PLATELET-DERIVED ENDOTHELIAL CELL GROWTH FACTOR (PDEGF) EXPRESSION IN FIBRIN-RICH PLASMA



PARAYIALIS ANDREAS[1], DINCĂ OCTAVIAN[1], BUCUR MIHAI-BOGDAN[1], VLĂDAN CRISTIAN[1], BUCUR ALEXANDRU[1]

[1]"Carol Davila"University of Medicine and Pharmacy, Bucharest, Romania

ABSTRACT

Platelet concentrates are potentially useful in wound healing applications because they function as a tissue sealant and a host of growth factors too. It is wellknown that each platelet-rich plasma preparation method may differ in regard to growth factor profiles. Despite their expanding clinical availability and the large use, effectiveness of Choukroun's Platelet Rich Fibrin (PRF) is scientifically unclear, as sufficient characterization of PRF has not been thoroughly performed. The purpose of our work was to determine the presence of two major growth factors released from the platelets from PRF.

Key words: *growth factors, PRF, wound-healing*

Correspondence to:

Cristian Vlădan
Address: Clinica de Chirurgie Oro-Maxilo-Facială a UMF „Carol Davila"Calea Plevnei 19 010221 Bucureşti, Romania
E-mail address: crystalvladan@gmail.com

INTRODUCTION

Many experimental and clinical studies have demonstrated effects of growth factors on the healing process. Wound healing is a complex process that involves several cell types including platelets (2). Growth factors released from activated platelets initiate and modulate wound healing in both soft and hard tissues (4).

Autologous fibrin-rich plasma (PRF) has gained popularity as a treatment in a variety soft- and hard-tissue applications in oral and maxillo-facial surgery (3,6).

Platelet-derived endothelial cell growth factor (PDEGF) and transforming growth factor-beta (TGF-beta) markedly potentiate tissue repair *in vivo* (12). The purpose of this study was to identify PDEGF and TGF-beta released from a fibrin-rich plasma.

MATERIAL AND METHODS

Eight study participants were selected from patients who required alveoloplastic tooth extraction, ranging in age from 43 to 56 years (six women and two men), with no relevant medical history.

The blood collection was performed quickly, and the tubes were immediately centrifuged at 3,000 rpm for 14 minutes with a specific centrifuge (7). After centrifugation, the PRF clot was removed from the tube and separated from the red blood cells fraction.

Histological sections were stained with hematoxylin-eosin or processed for immunocytochemistry. Immunohistochemical staining was performed on the paraffin tissue sections using the monoclonal antibodies anti-TGFβ (Novocastra-Leica Biosystems, Newcastle Upon Tyne, U.K., 1:40, clone TGFB17) and anti-PDGEF (Novocastra-Leica Biosystems, Newcastle Upon Tyne, U.K.,1:120, clone P-GF.44C). As detection system, we used Novolink Polymer (Leica/Novocastra). Immunoreactive proteins were visualized by a polymer-based peroxidase detection system. Slides were counter-stained with Mayer's Hematoxylin, rehydrated and mounted with glycerol gelatin. mmunohistochemical stains were analyzed using a microscope Nikon 80i. Cells showing positive reaction were counted in five random fields.

RESULTS

Positive results were obtained in all the specimens examined. Pletelets in the PRF clot were reactive for both monoclonal antibodies used. Intense diffuse positivity for TGFβ was observed mostly in aggregated platelets in PRF (Fig.1). Also, there was intense diffuse positivity for PDEGF highlighting both aggregated and isolated platelets (Fig.2).

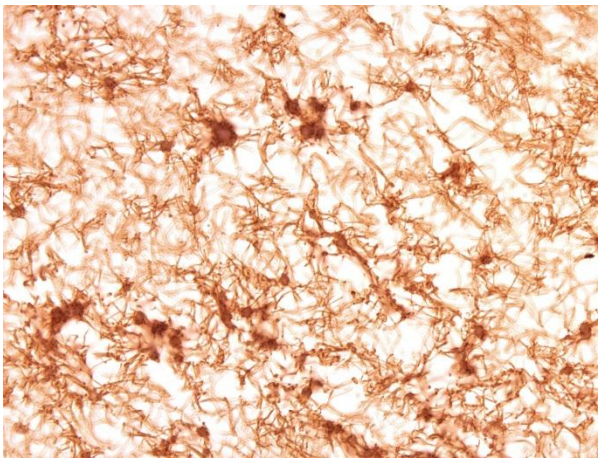


Figure 1. Intense diffuse positivity for TGFβ mostly in aggregated platelets in PRF. TGFβ immunostaining, ×1000. Image courtesy of Prof.dr.Alexandru Bucur

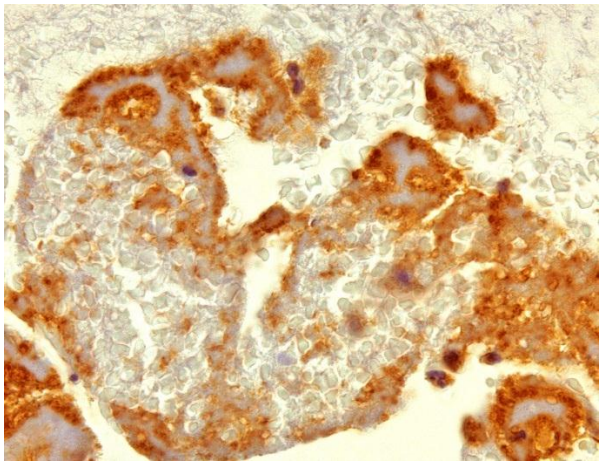


Figure 2. PDEGF positivity in PRF. PDEGF immunostaining, ×1000. Image courtesy of Prof.dr.Alexandru Bucur

DISCUSSIONS

The mechanisms responsible for wound healing are not yet fully understood. With the progress in molecular biology there is increasing evidence suggesting that the wound healing is regulated by cytokines and several growth factors (8,9).

These growth factors are released from the platelets and recruits leukocytes and mesenchimal cells to injury site. Tissue repair is aided by cell proliferation and differentiation, chemotaxis, angiogenesis induced by growth factors produced by the patelets, including platelet derived growth factors (PDGF), vascular endothelial factor (VEGF), transforming growth factor (TGF), platelet derived endothelial growth factor (PDEGF) (1).

In the present study we used TGFβ and PDEGF, which are potent mitogens for cells of mesenchymal origin. PDEGF drives cellular responses, including deposition of extracellular matrix and tissue remodeling factors (10). Both TGFβ and PDEGF stimulate collagen production improving wound strength (5).

Because PRF contain an increased number of platelets, our results showed a beneficial effect of PRF in wound healing. However, the growth factor concentration is dependent upon the amount of whole blood used and the platelet content of the final PRF product. Studies are showing that it is impossible to replicate a consistent level of platelet concentration (11).

CONCLUSIONS

Our study shows a increase in the expressions of growth factors TGFβ and PDEGF in the PRF clot. However, the variability of concentrations of platelets and growth factors should be considered in treatment of PRF.

Author contribution

All authors have equally contributed to this study.

Acknowledgement

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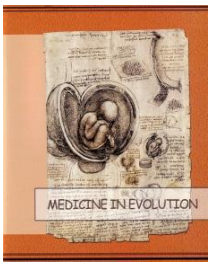
AN ANALYSIS OF TWO MIXED-DENTITION ANALYSIS



ILINCA POPOACĂ[1], CRISTINA DUMITROIU[1], RADU STANCIU[1], ANCA TEMELCEA[1], DRAGOŞ STANCIU[1]

[1]"Carol Davila"University of Medicine and Pharmacy, Bucharest, Romania

ABSTRACT

Mixed dentition arch analysis system is an major criteria in determining the type of orthodontic treatment plan. Different mixed dentition arch analysis system are available and among them the estimation of 2-6 distance by radiographic measurement. In this work we compared the accuracy of space prediction by digital ortopantomography measurement vs estimation by study models.

Key words: *mixed dentition analysis, 2-6 distance, digital orthopantomography*

Correspondence to:

Anca Temelcea
Address:"Carol Davila"University of Medicine and Pharmacy, 37 Dionisie Lupu, Bucharest
Phone: +40213180720
E-mail address: office@leodent.ro

INTRODUCTION

It is essential to analyze the relationship between tooth size and dental arch size. This isespecially important during the transition from the deciduous arch to the permanent arch. Mixed dentition arch analysis is important in determining whether the orthodontic treatment plan is going to involve extraction, guidance of eruption or space maintenance. Thus, the determination of tooth size - arch length discrepancy in the mixed dentition requires an accurate prediction of the mesiodistal width of the unerupted permanent teeth. The sum of the mesio-distal widths of the primary cuspid and first and second primary molars is larger than that of the permanent cuspid and premolars by 1 mm per quadrant in the maxilla and 2 mm per quadrant in the mandible (3).

There have been reports in the literature on methods for predicting the mesiodistal dimension of unerupted canines and premolars (1,6). The mixed dentition analysis developed by Moyers utilized the sum of the mandibular permanent incisors as the independent variable (2). Determination of the space existing for the unerupted permanent cuspid and premolar is accomplished by measuring from the mesial surface of the permanent first molar to the distal aspect of the permanent lateral incisor (the 2-6 distance) (4). Generally, estimation of the 2-6 distance rely on observation of study models and and digital orthopantomographs (5). It seems that both methods could have potential errors, which can occur during measurement. Based on this, it might be argued that digital radiographs analysis would be no more likely to give accurate results than measurement of study models. Therefore, we have investigated and compared the accuracy of the digital orthopantomography evaluation of the 2-6 distance with estimation of study models of pediatric patients in the mixed dentition.

MATERIAL AND METHODS

Digital orthopantomographs and study models of maxillary and mandibular arches of ten consecutive children (five females, five males; age range 7 to 10 years) were selected. The measurements were fee of caries, malformations, restorations, fractures and lack of any previous orthodontic treatment. All measurements for the 2-6 distance technique were made by a single specialist. The values were calculated by using Moyer's probability chart at 75% level as suggested by Moyers, for given sum of mandibular incisors.All the recorded data was entered into Excel for Windows and statistically analyzed to determine whether significant differences were present in the values obtained by both methods.

RESULTS

Analysis indicated that differences between gendres were statistically not significant for both methods (Fig.1).

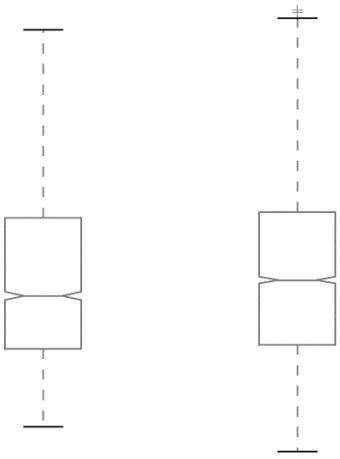


Figure 1. Comparison of the 2-6 distance measurement with gendre

No significant differences were present in teeth in right and left side, where P value was not under 0.5, showing greater amount of similarity in size of both sides.

A moderate correlation (Pearson's correlation P< 0.05) between the measurements produced by the two techniques. Differences were independent of the arch examined (upper vs lower).

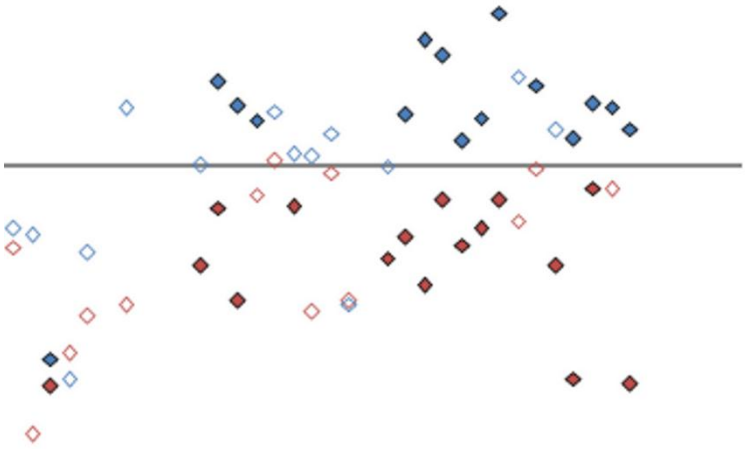


Figure 2. Comparison of the 2-6 distance measurement on digital orthopantomography vs study models

One-way ANOVA for 2-6 distance between groups failed to reach any level of significance for study models.

DISCUSSIONS

The range of variability on study models is higher when compared to the variability of digital OPG method. The radiographs examination was more reproducible than the study model technique. The simple observation of the study model is an entirely subjective and the results show that measurements may vary in the prediction of space discrepancy by this method.

The purpose of measurment of the 2-6 distance is to evaluate the amount of space available in the arch

for the succeeding permanent teeth. Although many methods have been suggested, none of them are as precise as one might like. Digital orthopantomography have been largerly used to estimate the 2-6 distance (2,4). However, distortions, enlargements, and rotations of the tooth germs make this method less effective.

The digital orthopantomography analysis represents a good method because it has minimal systemic error, it can be perfomed by the less experienced doctors, does not require special tools and it may be used for both dental arches. The limited number of records used in the present study precludes any definite conclusions regarding improvement of space evaluation methods.

CONCLUSIONS

The 2-6 distance measurement on digital OPG technique produces more consistent data when compared to study model observation. Further study has to be performed on a larger population and for a longer duration of time to get a more accurate result.

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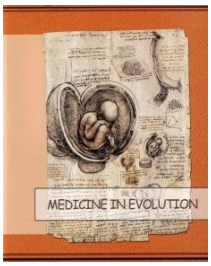

CORRELATION OF SUBGINGIVAL BACTERIAL PLAQUE AND ATHEROSCLEROTIC LESIONS IN PATIENTS SUFFERING FROM PERIODONTITIS

IOANA MIHAELA CÎTU[1], RUXANDRA SAVA ROŞIANU[2], ANGELA CODRUȚA PODARIU[2], DANIELA JUMANACA[2], ATENA GĂLUŞCAN[2], MELINDA ONEȚ[3], ȚICĂ PAUL[3], RAMONA AMINA POPOVICI[2], ANA LASCU[1]

[1]Faculty of General Medicine, University of Medicine and Pharmacy „Victor Babes"Timisoara
[2]Faculty of Dentistry, University of Medicine and Pharmacy „Victor Babes"Timisoara
[3]Faculty of Dentistry, University of West „Vasile Goldiş"Arad

ABSTRACT

Background: Chronic bacterial infections have been associated with an increased risk for atherosclerosis and coronary artery disease. The ability of oral pathogens to colonize in coronary atheromatous plaque is well known. The aim of our study was to detect the presence of four common periodontal pathogens in coronary plaques. We detected the presence of Treponema denticola, Eikenella Corrodens, Porphyromonas gingivalis and Campylobacter rectus in subgingival and atherosclerotic plaques of CABG surgery. Methods: 51 patients in the age group of 40 to 80 years with chronic periodontitis were recruited for the study. These patients were suffering from Coronary Artery Disease (CAD) and underwent Coronary Artery Bypass Grafting (CABG). Results: T.denticola, E.corrodens, C.rectus and P.gingivalis were detected in 49.01 %, 27.45 %, 21.51% and 45.10% of atherosclerotic plaque samples. In both subgingival and coronary plaque samples, T. denticola was detected in 39.21% of the cases, E.corrodens in 19.60%, C.rectus in 11.76% and P.gingivalis in 39.22% of the cases respectively. Conclusion: Our study revealed the presence of significant bacteria of oral pathogens in coronary plaques. This suggests possible relationship between periodontal infection and atherosclerosis and can help devise preventive treatment strategies.

Key words: *periodontal disease, coronary artery disease, periodontal pathogens, atherosclerotic plaques*

Correspondence to:

Ramona Amina Popovici
Address: University of Medicine and Pharmacy „Victor Babes"Timisoara,, Faculty of Dentistry, address: Splaiul T. Vladimirescu, nr. 14
Phone: +40762006828
E-mail address: ramona.popovici@umft.ro

INTRODUCTION

Cardiovascular diseases are a leading cause of morbidity and mortality in developed countries. The disease process that underlies the majority of cardiovascular events is atherosclerosis, an inflammatory disease of the blood vessel wall. The earliest physical evidence of atherosclerosis is fatty streaks, which are typically present in childhood. In the presence of arterial endothelial dysfunction, which is involved in the initiation and progression of atherosclerosis, these early lesions progress through to complex atheromatous lesions in adulthood, finally resulting in occlusion, plaque rupture and ischemic events [1].

Periodontal disease is inflammation of the tissues surrounding teeth and results from a complex interplay between bacteria and host risk factors such as long-term smoking, poor oral hygiene, poorly controlled diabetes, stress and genetic predisposition [2]. Not only have periodontal organisms adapted to survive within an environment that is constantly besieged by host defenses, but they flourish in the presence of inflammation, enabling their capacity to invade host tissues and gain direct access to the circulation [3]. Repeated bacteremias and endotoxemias are characteristic of periodontal infection, and periodontal organisms have been found to co-localize within atheromatous plaques [4]. The constant exposure of the vasculature to these pathogens provides an opportunity for endothelial inflammatory activation and functional impairment. Clinically, periodontal disease manifests as deepening of the epithelial attachment around teeth, loss of periodontal attachment and, ultimately, tooth loosening.

Periodontal disease has been associated with atherosclerosis [5], cardiovascular disease [6], diabetes [7], pre-term low birth weight [8], stroke [9], and premature death [10]. Accordingly, periodontal disease may account for a portion of the risk for cardiovascular disease via a shared pathogenic underlying inflammatory response [3].

Coronary Artery Disease (CAD) and periodontal diseases are common inflammatory conditions in human population [11]. Chronic infections have been implicated as an increased risk factor for coronary atherosclerosis [11]. CAD is one of the leading causes of premature death in the adults. Coronary atherosclerosis is the most frequent cause of CAD and plaque disruption with superimposed thrombus is the main mechanism of myocardial infarction. A key role for inflammation has been established, suggesting that inflammatory processes underlie all phases of coronary atherosclerosis, from the initial formation of plaques to their progression and rupture, which lead to clinical events such as unstable angina, acute myocardial infarction, and sudden death [2].

Oral surgical procedures may lead to bacteremia which may produce bacterial endocarditis and other systemic complications. Anaerobic bacteria have been detected in human blood under a variety of conditions including bacteremia after oral surgery [12]. Similarly, the periodontal infection is a chronic infectious disease characterized by inflammatory changes in periodontal tissues. The disease occurs as a result of infection associated with a small number of predominantly *gram-negative* micro organisms and spirochetes [12]. Oral *gram-positive* and gram-negative bacteria have frequently been identified in bacteremia and may play a role in vascular diseases. Recent epidemiological studies suggest that periodontal disease may be an important risk factor for CAD. Various case control studies have shown a

significant association after correction for conventional risk factors like smoking, hypertension, obesity and dyslipidemia [13]. Possible mechanism could be endothelial injury by oral microbial toxins and systemic inflammation triggered by oral infections. In addition phagocytes in periodontal lesions may engulf various bacterial cells and their antigens. The bacterial cells and phagocytes may then penetrate the gingival tissues and get transported through circulation to the heart, and adhere to the coronary artery endothelium. These deposited bacteria can then stimulate the release of inflammatory cytokines and initiate the formation of the characteristic foam cells associated with atherosclerosis [13].

The aim of our study was to detect the presence of four common periodontal pathogens in atherosclerotic plaques and subgingival plaques. We detected the presence of *Treponema denticola, Eikenella Corrodens, Porphyromonas gingivalis* and *Campylobacter rectus* in subgingival plaque and atherosclerotic plaques of patients undergoing Coronary Artery Bypass Grafting (CABG).

MATERIAL AND METHODS

51 patients (11 females and 40 males) in the age group of 40–80 years from 2013-2014 with chronic periodontitis were recruited consecutively from the Institut of Cardiovascular disease, Timisoara, Romania. These patients were suffering from CAD and were scheduled to undergo CABG. Exclusion criteria included major systemic illness, advanced malignancy, antibiotic intake and periodontal treatment in the previous 6 months.

The medical and dental history of each subject was obtained by an interview. Patients fulfilling the inclusion criteria were selected for the study and an informed consent was obtained from them. The ethics committee approved the protocol of this study.

The samples were taken one day before the patients underwent CABG. A periodontal examination was performed by the dental surgeon. Clinical evaluation included plaque index, probing depth, periodontal index, total number of teeth present and clinical attachment loss. The deepest periodontal sites with periodontal depth >5 mm were selected for the microbial sampling. The teeth were gently dried with sterile cotton swab. After the removal of supragingival plaque, the subgingival plaque samples were obtained with the help of curette from the two deepest periodontitis sites and were pooled for analysis. A biopsy was obtained from the coronary atherosclerotic plaque during the CABG.

The surgeon excised one or two small bits of plaque (0.5 to 1 mm) from the edge of the coronary arteriotomy performed for anastomosing the graft. To eliminate blood contamination, the samples were placed in sterilized phosphate buffered saline and mixed gently and tissue samples were transferred to fresh vials containing the transport media and placed on culture media to detect the four pathogens.

To explore the association between the presence of Treponema denticola, Eikenella corrodens, Campylobacter rectus and Porphyromonas gingivalis in both the plaque samples with the periodontal parameters, correlation coefficient was calculated and are shown in Table 1 and 2 respectively.

RESULTS

Treponema denticola, Eikenella corrodens, Campylobacter rectus and *Porphyromonas gingivalis* were detected in 66.66%, 47.06%, 29.41% and 64.71% of subgingival plaque samples and in 49.01%, 27.45%, 21.51% and 45.10% of atherosclerotic plaque samples. In both subgingival plaque and coronary atherosclerotic plaque samples *Treponema denticola* was detected in 39.21%, *Eikenella corrodens* in 19.60%, *Campylobacter rectus* in 11.76% and *Porphyromonas gingivalis* in 39.22% respectively. 9.80% of *Treponema denticola*, 7.85% of *Eikenella corrodens*, 9.75% of *Campylobacter rectus* and 5.88% of *Porphyromonas gingivalis* was found only in coronary atherosclerotic plaque without the presence of these microorganisms in subgingival plaque.

Results revealed that there was a significant correlation between the plaque index and presence of *Treponema denticola* in atherosclerotic plaque ($P<0.05$). There was also significant correlation between the *Treponema denticola* in atherosclerotic plaque with clinical attachment level and probing depth ($P<0.05$) (Table 1). There was statistically significant correlation between E.Corrodens in atherosclerotic plaque with plaque index, periodontal index, clinical attachment level and probing depth ($P<0.05$). There was also a highly significant association ($pd<0.01$) of the microorganism in the subgingival plaque with plaque index, clinical attachment level and probing depth and significant association ($p<0.05$) with the periodontal index (Table 1). There was a statistically significant association between the C. rectus in atherosclerotic plaque and subgingival plaque with the total number of teeth and plaque index respectively (Table 2). There was a highly significant association between plaque index and the periodontal index for the presence of P. gingivalis in subgingival and atherosclerotic plaque. ($P<0.01$). There was a statistically significant association between probing depth and P. gingivalis in both the plaque samples ($P<0.05$) (Table 2). The above observations reveal that the amount of periodontal destruction directly correlates with the presence of the four periodontal microorganisms in coronary atherosclerotic plaque samples.

Chi-square analysis was carried out to find out the significant difference between the four microorganisms in subgingival plaque and coronary atherosclerotic plaque samples. It was found that the value of chi-square was significant and hence variables were dependent.

Table 1. Correlation coefficient analysis of Treponema denticola and Eikenella corrodens

	Treponema denticola		**Eikenella corrodens**	
Periodontal parameters	**Subgingival plaque**	**Coronary atherosclerothic plaque**	**Subgingival plaque**	**Coronary atherosclerothic plaque**
No. of teeth	-0.0073 ($p>0.05$)	-0.0580 ($p>0.05$)	-0.0638 ($p>0.05$)	0.012 ($p>0.05$)
Plaque index	-0.0051 ($p>0.05$)	0.336 ($p<0.05$)	0.3697 ($p<0.01$)	0.2845 ($p<0.05$)
Gingival index	-0.0096 ($p>0.05$)	0.0197 ($p>0.05$)	0.0415 ($p>0.05$)	0.2499 ($p>0.05$)
Clinical attachment	0.0352 ($p>0.05$)	0.3382	0.5599($p<0.01$)	0.2846 ($p<0.05$)
Probing depth	0.0183 ($p>0.05$)	0.3212	0.4972 ($p<0.01$)	0.2820 ($p<0.05$)

Table 2. Correlation coefficient analysis of campylobacter rectus and porphyromonas gingivalis

	Campylobacter rectus		Porphyromonas gingivalis	
Periodontal parameters	**Subgingival plaque**	**Coronary atherosclerothic plaque**	**Subgingival plaque**	**Coronary atherosclerothic plaque**
No. of teeth	0.0931 (p>0.05)	0.2764 (p<0.05)	0.2147(p>0.05)	0.0792(p>0.05)
Plaque index	0.2847 (p<0.05)	0.2118(p>0.05)	0.5455 (p<0.01)	0.3809 (p<0.01)
Gingival index	-0.0841 (p>0.05)	-0.0671(p>0.05)	0.1123(p>0.05)	-0.1234(p>0.05)
Clinical attachment	0.1613 (p>0.05)	0.1331(p>0.05)	0.2570(p>0.05)	0.2252(p>0.05)
Probing depth	0.2076 (p>0.05)	0.1059(p>0.05)	0.3207 (p<0.05)	0.2841 (p<0.05)

DISCUSSIONS

A number of epidemiological studies have shown a statistical association between periodontitis and CAD. Presence of oral pathogens in coronary atherosclerotic plaques has been reported by Haraszthy et al. [14]. Periodontitis and atherosclerosis have many pathogenic mechanisms in common. Both the diseases have complex causation, genetic and gender predisposition and might share many risk factors, such as age, education, smoking, social status, and stress [15].

Our study was done with the aim to investigate and compare the presence of four common oral pathogens namely *Treponema denticola, Eikenella corrodens, Campylobacter rectus* and *Porphyromonas gingivalis* in coronary atheromas recovered from patients undergoing CABG. Coronary atheromas and subgingival plaque samples were collected in 51 patients and analyzed for universal bacterial primers followed by the specific primers for these microorganisms. The prevalence of *Treponema denticola, Eikenella corrodens, Campylobacter rectus* and *Porphyromonas gingivalis* was detected in 66.66%, 47.06%, 29.41% and 64.71% of subgingival plaque samples and in 49.01 %,27.45 %, 21,51% and 45.10% of coronary atherosclerotic plaque samples respectively. In both subgingival plaque and coronary atherosclerotic plaque samples *Treponema denticola* was detected in 39.21%, *Eikenella corrodens* in 19.60%, *Campylobacter rectus* in 11.76% and *Porphyromonas gingivalis* in 39.22% respectively. 9.80 % of *Treponema denticola,* 7.85 % of *Eikenella corrodens,* 9.75% of *Campylobacter rectus* and 5.88% of *Porphyromonas gingivalis* was found only in coronary atherosclerotic plaque without the presence of these microorganisms in subgingival plaque. *Treponema denticola* is a gram-negative bacterium from the Spirochetes family that is motile, slender, helically shaped and flexible. The organism consists of periplasmic flagella, which allows for mobility by using a proton motive force to cause thrusting through rotation. It is commonly found in the human oral cavity, specifically in subgingival dental plaque, and it is often associated with periodontal disease. The organism causes disease by aggregating in subgingival plaque with Porphyromonas gingivalis and it uses several mechanisms in order to survive harsh conditions, such as oral biofilms [15]. Eikenella corrodens is a facultative gram-negative bacillus, which is a common inhabitant of the oral cavity, intestinal and genital tracts. It is often present in the supra and subgingival plaque of periodontal healthy subjects. It appears to be somewhat more prevalent in subgingival plaque samples of patients with periodontitis than healthy individuals [16]. Porphyromonas gingivalis is a black pigmented gram negative anaerobic coccobacillus which

is one of the major pathogen implicated in chronic periodontaldisease. It is known to express various pathogenic factors such as fimbriae, adhesins, lipopolysaccharides, enzymes like collagenase and protease [12]. Campylobacter rectus, a gram-negative, microaerophilic, and motile bacterium, has been proposed to play a pathogenic role in human periodontitis. Surface components, such as the flagellum, surface layer (S-layer), and cytotoxin, have been reported as possible virulence factors of the microorganism. Our results have shown that oral pathogens detected in the periodontal sites are also detected with a higher prevalence in the diseased coronary artery. This is in accordance with the study by Aimetti et al. [17] which showed the prevalence of *T. denticola* in subgingival plaques and atherosclerotic lesions as 54.5%. The chronic cyclic nature of periodontal disease provides multiple opportunities for repeated dissemination of pathogens in the blood. This can also explain the absence of organisms in the subgingival plaque when it is detected in the coronary plaque. Gingival ulceration and vascular changes in the periodontal tissues increase the incidence and severity of transient bacteremia. The bacteremia could affect the vascular endothelial integrity, metabolism of plasma lipoproteins, blood coagulation and platelet function [18].

In conclusion, our study revealed the presence of significant bacteria of oral pathogens in coronary plaques. This suggests possible relationship between periodontal infection and atherosclerosis and can help devise preventive treatment strategies.

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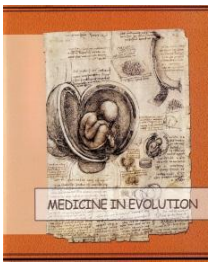
ORTODONTICHS AND PERIODONTOLOGY



MELINDA ONEȚ[1], SZEKERES KATALENA[1], LILE IOANA[1], HOSSZU TIBERIU[1], ANGELA CODRUȚA PODARIU[2], RUXANDRA SAVA ROSIANAU[2], ATENA GALUSCAN[2], DANIELA JUMANCA[2], ALEXANDRA PODARIU[2], RAMONA AMINA POPOVICI[2]

[1]Faculty of Dentistry, University of West „Vasile Goldiş"Arad,
[2] Faculty of Dentistry, University of Medicine and Pharmacy „Victor Babes"Timisoara

ABSTRACT

During the last years, the number of adult patients who benefit from orthodontic treatment is increasing. Teeth can be malpositioned from the beginning or can migrate into malpositions over the years [1]. The most important factor that causes tooth migration is periodontal disease which has as symptoms inflammation and alterations of the gingiva at different stages [2,3]. Periodontitis is a multifactorial disease, bacterial plaque being the initializing factor of the condition[4]. The evolution of disease is genetic determined, factors like social status and environment representing other risk factors. Regular check-ups are very important for the early recognition of the disease and application of treatment. In adults, orthodontic treatment can be used when periodontal disease is more advanced if no inflammatory processes are seen during treatment. Interdisciplinary collaboration between the orthodont and the periodontologist is essential. [5]

Key words: *adult orthodontic treatment, periodontal disease, periodontitis*

Correspondence to:

Ramona Amina Popovici
Address: University of Medicine and Pharmacy „Victor Babeş"Timişoara, adress: Splaiul T. Vladimirescu nr. 14 A
Phone: +40762006828
E-mail address: ramona.popovici@umft.ro

INTRODUCTION

Adult orthodontic treatment can be very complex in comparison to child orthodontic treatment because the adult patient can have: prosthetic treatments, endodontic treatments, implants, dysfunctions of the temporo-mandibular joint. Collaboration between the orthodontist and the periodontologist is very important in order to achieve a proper diagnosis and create a precise treatment plan. [5]

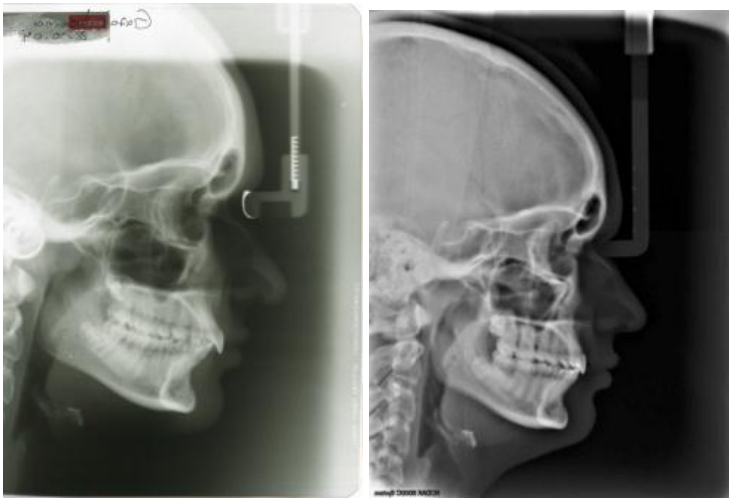
MATERIAL AND METHODS

Subgingival micro organisms are responsible for the start of periodontal disease. The high number of periodontal patients is given by the presence of many unspecific gram-negative bacterial types. Epidemiological studies have shown that improper hygiene and the presence of bacterial plaque show a higher prevalence in the appearance of periodontal disease. [4]

Orthodontic treatment can start only if inflammatory processes are completely arrested[5]. Systematic treatment in case of periodontis tries to remove periodontal infection and maintain the tooth integrity. [4]

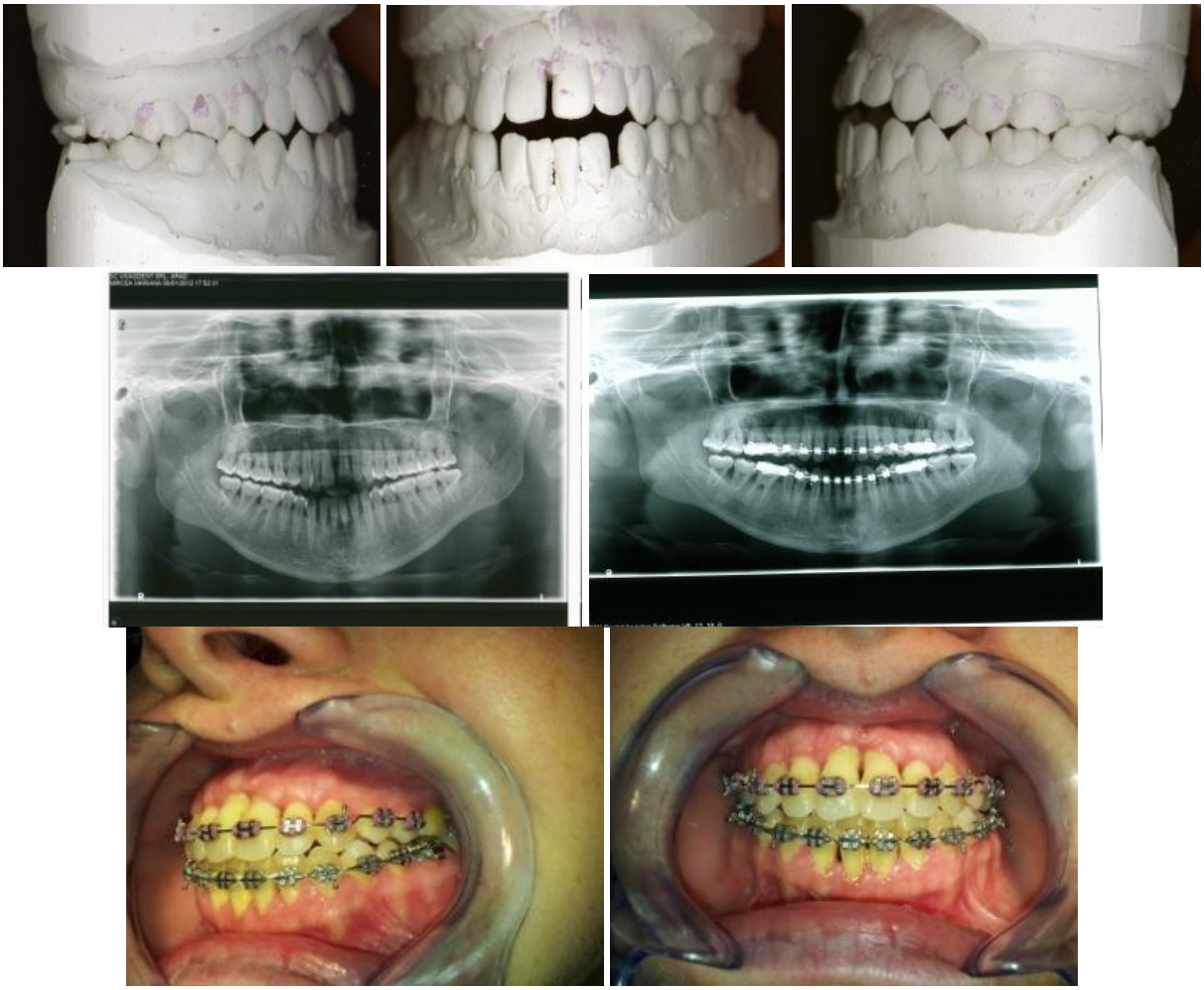
1. Anti-infectious therapy supposes: professional hygiene by scalling and rootplaning, educating the patient in order to obtain control of bacterial plaque. Scaling and rootplaning can be done on closed field. At this stage, mouth disinfection using clorhexidine is recommended.
2. Corectional perio-surgical therapy: 2-4 months after the anti-infectious therapy, the patient is evaluated to see the results. If the processes haven't disappeared, some surgical procedures can be done for teeth with pocket depths over 5.5 mm. The results of correctional therapy are clinically and radiologic evaluated after 6-12 months.
3. Dispensarization aims to maintain the obtained results and to recognize eventual new signs.

16 years old patient comes to the practice for orthodontic treatment. First of all, periodontal treatment by scalling and rootplaning and mouth washes with clorhexidine has been done. Results haven't been satisfying, so the patient was scheduled for surgical treatment associated with antibiotic therapy, 4 month after. Satisfying results has been achieved and orthodontic treatment could be started. It lasted for one and a half years. The following images show the status before and after orthodontic treatment.



68 years old patient, following periodontal treatment, without inflammatory processes, benefited from orthodontic treatment in the upper jaw, in order to close the existing gaps.

45 years old patient, having an open bite due to the interposition of the tongue, frontal teeth in the upper and lower jaw showing IInd degree mobility, gingival retraction vertical and horizontal bone atrophy. Following periodontal and orthodontic treatment, considerable bone regeneration has been obtained with tooth stability and closing of the open bite. The patient is still under treatment.



DISCUSSIONS

Patients with periodontal treatment who choose to have also periodontal treatment, have to be evaluated three months after intervention. This way, an actual treatment is obtained, which indicates treatment solutions that can be applied.

In patients with advanced periodontitis we can take micro biological tests and can apply combined mechanical and antibiotic medication.

A major importance has the collaboration with the patient to individually control dental plaque.

Over the following of the patient, we have to base our decision on the collaboration and understanding of the patient regarding the importance of respecting the scheduled appointments. Orthodontic treatment starts then, when we know for sure (after a couple of month of following up) that inflammatory processes are not present and the result is stabile.

In patients with vertical bone defects who have been treated using regenerative therapy, orthodontic treatment can be discussed as soon as 6 weeks after surgical intervention, in order to stimulate the activity of fibroblasts and osteoblasts, necessary to heal the defect. Following regenerative intervention, early starting of the orthodontic treatment can negatively influence the healing process.

Unlike young patients, who are orthodontic treated and esthetic and occlusal problems are ideally resolved, periodontal patients can aspire to more realistic objectives what occlusal,

periodontal and prosthetic restoration is concerned and according to the material possibilities of every patient.

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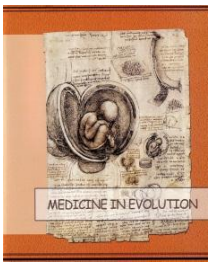
CLINICAL AND RADIOLOGICAL CONSIDERATIONS IN TRANSPOSITIONS



ANCA PORUMB[1], LIGIA VAIDA[1], IOANA IGNAT-ROMANUL[1], ANDA MUNTEAN[2], BOGDAN ŞINK[3], SERGIU TOFAN[3], SILVIU BRAD[3], OVIDIU MOTOC[1], RAMONA AMINA POPOVICI[3]

[1] Faculty of Dentistry, University from Oradea
[2]University of Medicine and Pharmacy „ Iuliu Hatieganu"Cluj-Napoca
[3] Faculty of Dentistry, University of Medicine and Pharmacy „Victor Babes"Timisoara

ABSTRACT

Transposition is being characterised by changing the place on the arch of 2 neighbouring teeth, due to disturbing the rythm of eruption of temporary teeth and delays of eruption of permanent teeth.

Transpositins can be partials or complete, on one side or both sides.

The anomaly attracts attention through physiognomics and mandibular dynamic disturbances; it is mandatory artificial remodelling of teeth or prosthetic treatements for the elimination of the occlusal disturbances. [1,2,3]

Key words: *transposition, dental X- ray*

Correspondence to:

Ramona Amina Popovici
Address: University of Medicine and Pharmacy „Victor Babes"Timisoara, adress: Splaiul T Vladimirescu nr. 14A
Phone: +40762006828
E-mail address: ramona.popovici@umft.ro

INTRODUCTION

Transposition is a position anomaly which belongs to I Class Angle. It is characterised by changing the place on the arch of 2 neighbouring teeth, usually canine with lateral incisive or canine with first premolar. [4]

MATERIAL AND METHODS

Matherial and method: on each case we have used: a detailed clinical exam, examination models, photos and dental X- rays (OPT and retroalveolar dental X- rays).

RESULTS AND DISCUSSIONS

We will present a few clinical cases:

CASE No 1:

B.A., 14 years, female:

Exooral, the patient does not exhibit any changings, neither at the front examinations or at the side one.

Static exam of oclussion:

Figure 1.

Incisive: S: circumscribe; T: deviation of interincisive lower line towards right with 3 mm; V: cover 1/3.

Canine: static exam of oclussion at canine it is impossible to be done because of the lack of canine from their places on the arch; They changed the places with first premolar, complete on the right and partial on the left.

Molars: 1.6 and 2.6. can not be recovered.

As a result of clinical observation, examination models and photos we stablished the presumptive diagnosis of complete transposition of canin with first premolar on right and partial transposition of canine with first premolar on left.

This dignosis was confirmed by X-ray examinations: OPT and retroalveolar dental X- ray in canine-first premolar area.

Figure 2.

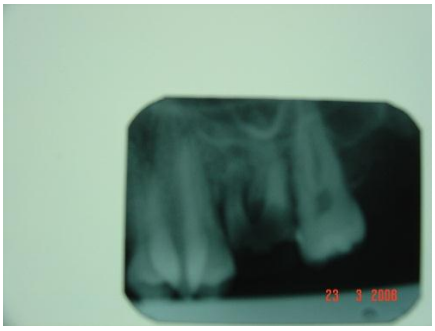


Figure 3.

CASE No 2:

C.E., 14 years, female:

Exooral, the patient does not exhibit any changings, neither at the front examinations, neither at the side one.

Static exam of oclussion:

Figure 4.

Incisive: S: circumscribe; ; T: deviation of interincisor lower line towards right with 1 mm; V: cover 1/3.

Canine: static exam of oclussion at canine it is impossible to be done because of the lack of canine from their places on the arch; They changed the places with first premolar.

Molar: left:

S: distal of ½ cuspid

T: circumscribe

V: cuspid-cuspid rapport

right:

S: distal of ½ cuspid

T: circumscribe

V: cuspid-cuspid report

We can see 1.4 partial included, with decrease space.

Figure 5.

As a result of clinical observation, examination models and photos, we stabilished the presumptive diagnosis of complete transposition of canine with first premolar, both left and right, with 2.4 included.

This diagnosis was confirmed by X- ray exam: OPT and retroalveolar dental X- ray.

We can see 1.4 partial included with decrease space and 2.4 included, towards palate.

Figure 6.

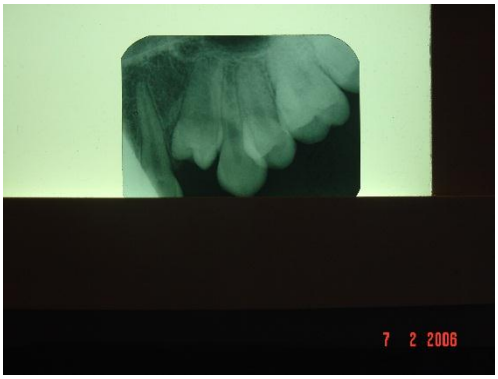


Figure 7.

Treatment:

First of all, we started with surgical treatment: odontectomy of 1.4 and 2.4.

We continued with a fixed orthodontic treatment, with the purpose of the alignment of 1.3 and 2.3 near to 1.2 and 2.2 and alignment of upper arch.

Figure 8.

Figure 9.

Case no 3:

M.M., 19 years, male.

Exooral, the patient does not exhibit any changings neither at the front examination, neither at the side one.

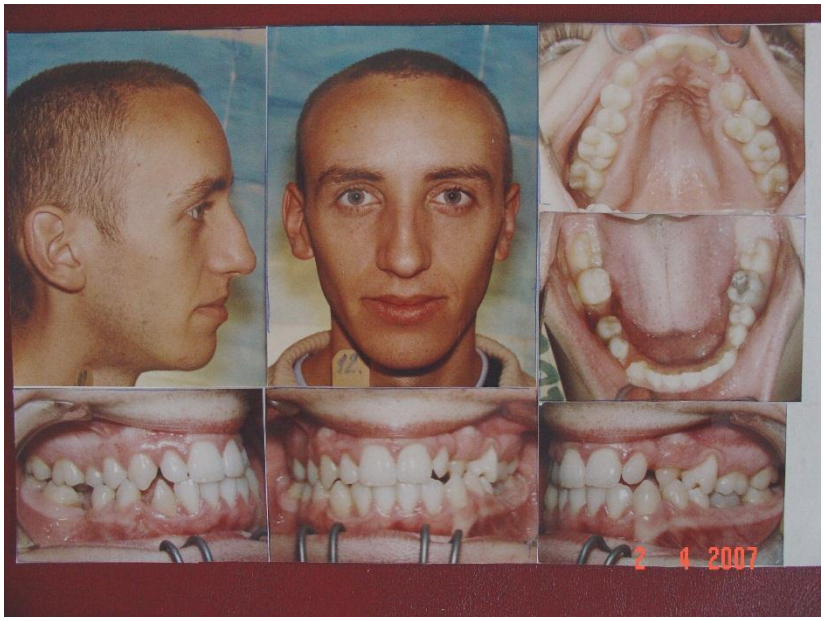


Figure 10.

As a result of clinical observation, examination models and photos, we stabilished the diagnosis of partial transposition of first and second upper premolars.

This diagnosis was confirmed by X –ray exam: OPT.

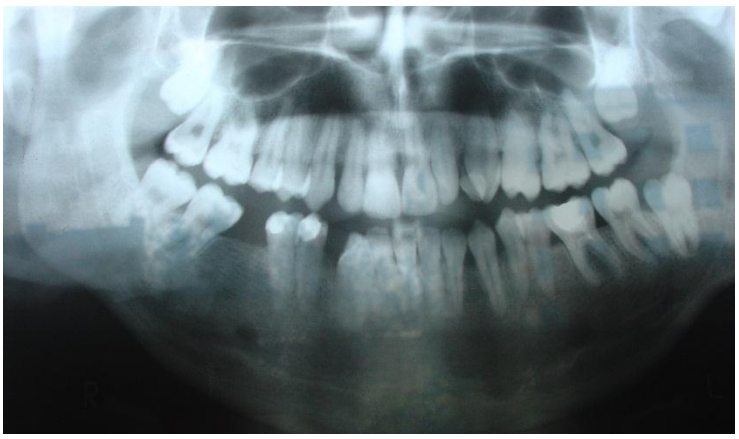


Figure 11.

We apply a fixed orthodontic device for alignment of teeth from upper arch.

Figure 12.

To be followed with the lifting of occlusion for the achievement of articular jump, at the upper left canin.

CONCLUSIONS

In complete transpositions, do not intervene orthodontically. It is being done only modelling of canine cuspid, to improve physiognomical appearance, or polishing from palatinal cuspid of the first premolar in order to eliminate occlusal disturbances.

In incomplete transpositions, we will try propper adjustement of the teeth on the arch by moving those teeth which need the smaller movement of the apex.

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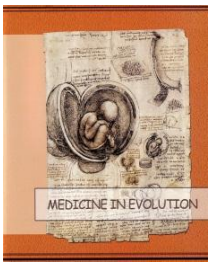
THE ALTERATIONS OF SALIVARY GLANDS SECRETION IN DIABETES MELLITUS



OVIDIU MOTOC[1], ANGELA CODRUȚA PODARIU[2], ATENA GĂLUŞCAN[2], DANIELA JUMANACA[2], RUXANDRA SAVA ROŞIANU[2], ANCA PORUMB[1], FLORINA ANDRICA[3], RAMONA AMINA POPOVICI[2]

[1] Faculty of Dentistry, University from Oradea
[2] Faculty of Dentistry, University of Medicine and Pharmacy „Victor Babes"Timisoara
[3] Faculty of Pharmacy, University of Medicine and Pharmacy „Victor Babes"Timisoara

ABSTRACT

Saliva is an organic secretion of the exocrine salivary glands that plays an important role in oral health due to its numerous functions and compounds. The salivary dysfunction is frequently associated with dental caries and also with oral pain. Recently, salivary secretion is also used for detecting biomarkers in patients with systemic diseases for example cancer, diabetes mellitus (DM) and viral infections including HIV. For example, experimental and clinical trials indicated that DM can cause various physiological and morphological complications of the salivary glands including the reducing of salivary flow rate and the increase in total protein content, altering the saliva composition. Further prospective and multicenter randomized clinical trials are needed to elucidate the complex connection between chronic diseases especially DM and oral diseases and to prove the efficiency of salivary biomarkers detection in various systemic pathologies.

CPK = creatine phosphokinase
DM = diabetes mellitus
MI = myocardial infarction
T2DM = type 2 diabetes mellitus
Key words: *saliva, biomarkers, diabetes mellitus*

Correspondence to:

Florina Maria Andrica
Address: University of Medicine and Pharmacy „Victor Babes"Timisoara, Faculty of Pharmacy, Pharmaceutical Chemistry, adress: str. Cetatii nr.38-39, Timisoara
Phone: +40724875021
E-mail address: florina.andrica@yahoo.com

INTRODUCTION

Saliva is an organic secretion of the exocrine salivary glands including sublingual, submandibular, parotid and the minor glands and plays an important role in oral health due to its numerous functions and compounds.

The salivary secretion contains water (99%), electrolytes, proteins especially mucin, enzymes, trace metals and other biochemicals and minerals including cystatins, proline-rich peptides, sodium, chloride, calcium, potassium, magnesium, phosphate and bicarbonate, all of these being also found in blood [1, 2].

The fundamental roles of saliva consist of the food tastes perception, digestion of food, physical protection against bacteria and wound healing. Another important function of the saliva is to lubricate the oral mucosa, the mucin being responsible of it [3].

Saliva is also considered one of the most important constituents of the antioxidant defense systems, protecting against the harmful effects of reactive oxygen species for example gingivitis, periodontal disease, caries, but also diabetes and cardiovascular diseases [4].

Saliva may be also a route for transmitting infections, although the possibility of HIV transmission through saliva is possible only in immunocompromised patients [5, 6].

In normal conditions the unstimulated salivary flow rate is 0.1-2 mL/min [7], the salivary secretion being controlled by both sympathetic and parasympathetic nervous system [8]. The saliva secretion is directly influenced on one hand by the age of patients and on the other hand by the number and type of administered drugs [9].

Xerostomia is a common side effect of different prescription and nonprescription drugs especially antimuscarinic drugs and chemoradiotherapy for head and neck carcinomas. The salivary dysfunction is frequently associated with dental caries and also with oral pain [10]. Unfortunately, the decreasing of saliva secretion can cause difficulty in speaking, tongue pain, a burning sensation in the mouth and bad breathe [9, 11, 12].

Hyposalivation is a serious health problem treated with oral lubricants and saliva substitutes. Nevertheless, the properties of these health products are not very similar with that of natural saliva [13].

Recently, a clinical study on 134 patients with T2DM proved that immunologically active saliva substitutes can be used for reducing the plaque and gingivitis [14].

This mini review analyzes the most common salivary biomarkers that are used for detection of various systemic diseases.

SEARCH STRATEGY

All available data provided in English language by a www.PubMed.com search until February 2015 was used in the survey. The following words were selected for our research: saliva, chronic disease, diabetes mellitus and salivary biomarkers.

SALIVARY BIOMARKERS FOR CLINICAL APPLICATIONS

Recently, salivary secretion is also used for detecting biomarkers in patients with systemic diseases for example cancer, diabetes mellitus (DM) and viral infections including HIV [15]. According to clinical evidences, the

saliva analysis is an advantageous alternative in the chronic diseases management, because the saliva is abundant and its procedures of collection are noninvasive and painless [16]. Furthermore, the salivary medium is safe, simple and inexpensive, specialized personnel being not required [17].

The close correspondence between saliva and plasma concentrations may be explained by the diffusion process between these two fluids [18]. For example, all steroids of routine clinical endocrinology may be detected in saliva. Moreover, the concentrations of antibodies, hormones and also of some kind of drugs may be determined in saliva [19]. Moreover, a large number of metabolites related to the oxidative stress, inflammation and bacterial metabolism may be detected and measured using saliva collection methods [20]. In this regard, a clinical study on type 2 diabetes mellitus (T2DM) patients showed that lipid peroxidation and antioxidant parameters measured from saliva may be very important not only in detecting, but also in monitoring the disease activity and severity [21].

SALIVARY BIOMARKERS IN DM

Experimental and clinical trials indicated that DM can cause various physiological and morphological complications of the salivary glands including the reducing of salivary flow rate and the increase in total protein content, altering the saliva composition. For example, an experimental study on streptozotocin-induced diabetic mice showed a significant decrease in salivary flow [22]. According to a clinical study the heat shock proteins like heat shock protein 60 from human saliva proved to play a fundamental role in the DM pathogenesis [23].

Furthermore, the frequency of oral complications such as gingivitis, dental caries, periodontitis, xerostomia and fungal infections proved to be higher in diabetic patients [24]. Periodontal disease and DM are related to the oral inflammatory burden. In this regard, the increasing of the salivary myeloperoxidase and IgA may worse periodontal diseases in the diabetic subjects [25-27]. Clinical evidence suggested that the increasing of salivary parameters may lead to caries also in diabetic children [28]. Another clinical study in diabetic and non-diabetic patients also indicated significant differences regarding the levels of potassium and total proteins between both groups [16, 29].

The most important salivary biomarkers in DM subjects proved to be glucose, ghrelin hormone, alpha-amylase, immunoglobulins, glycated end products and other markers of oxidative status, including myeloperoxidase, salivary peroxidase, and other oxidants [13-18]. Most of these biomarkers were monitored using infrared (IR) spectroscopy [30].

It is known that oxidative stress is considered to be one of the major risk factors for DM. Thus, the salivary total antioxidant capacity may be considered a useful marker of periodontitis, a serious condition found in DM [31, 32]. The oxidative modification of salivary DNA and proteins were also observed in DM patients [33, 34].

Saliva can be also used as an alternative noninvasive technique for glucose determination in patients with DM. According to a clinical study the high salivary glucose levels might be involved in increasing of the oral candida carriage rate in these patients [35]. Moreover, it was demonstrated that there is a correlation between salivary acetone concentrations and the blood ketone bodies concentrations at the same time. In this regard, the

salivary acetone may be considered a useful biomarker in the follow-up of DM subjects [36]. The levels of mono- and dicarboxylic acids were higher in the saliva of DM patients than in that of the healthy subjects. For example, clinical trials showed an increasing of D-lactic acid concentration in the first group [37].

Another noninvasive metabolic marker in identifying T2DM patients is 1,5-anhydroglucitol and it may be included in national screening programs [38].

OTHER APPLICATIONS OF SALIVARY BIOMARKERS

The high salivary levels of different enzymes like glutamate pyruvate transaminase, glutamate oxaloacetate transaminase can be explained by the immunological attack that affects both pancreatic beta cells and salivary glands [39].

Another application of saliva consists in early detecting of patients with acute myocardial infarction (MI) by salivary creatine phosphokinase determination [40]. For example, a clinical trial in patients with acute MI showed that there is a strong correlation between salivary CPK levels and serum CPK level in the first two days after acute MI [17].

The other application of salivary biomarkers refers to the nicotine replacement therapy by detection the salivary cotinine levels [41].

Clinical evidence emphasized that salivary oxycodone concentrations were higher than in plasma ones, but lower than in urine. Consequently, salivary concentrations of oxycodone may be used also for drug monitoring [18].

CONCLUSIONS

Further prospective and multicenter randomized clinical trials are needed to elucidate the complex connection between chronic diseases especially DM and oral diseases and to prove the efficiency of salivary biomarkers detection in various systemic pathologies. Furthermore, the discovery of new oral products for preventing and treating the oral health problems should be also a major target for medical research.

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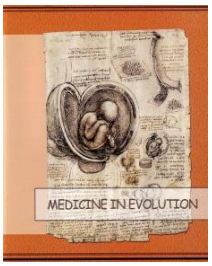
CHANGING THE LIFESTYLE AT CHILDREN'S BETWEEN 6 AND 8 YEARS, FROM RURAL AREA



ȚICĂ PAUL[1], ANGELA CODRUȚA PODARIU[2], DANIELA JUMANACA[2], ATENA GĂLUŞCAN[2], ALEXANDRA PODARIU[2], ANAMARIA BICA[2], RAMONA AMINA POPOVICI[2], MELINDA ONEȚ[1]

[1] Faculty of Dentistry, University of West „Vasile Goldiş"Arad
[2] Faculty of Dentistry, University of Medicine and Pharmacy „Victor Babes"Timisoara

ABSTRACT

Introduction: With almost no home education about the correctly hygiene, healthy food (a lot of parents send to children's a lot of sweets, for replacement their missing) and with psychological problems from be away from their parents the quality life of the children's from rural area Romania is not so good.

Methods: We purpose in this study was to investigate the effects of early childhood caries (ECC) on children's oral health-related quality of life (QOL) before and 4 weeks after its treatment, as assessed by the children themselves as well as by their parents/guardians.

Results: One hundred eight children diagnosed with ECC and sixty one children without caries and their parents/guardians responded to face-to-face administered surveys before a dental treatment was started (baseline assessment). Seventy-nine children with ECC completed dental rehabilitation. Four weeks after the treatment was completed, these seventy-nine children as well as their parents/guardians responded to a second survey (follow-up assessment). The results show that children's from rural area have oral health problems, and at the earlier education can improve their life quality. The children with ECC who received dental treatment had a significantly improved oral health-related life quality at the follow-up assessment when compared with their baseline measurement as measured both with the children's self-ratings of oral health-related life quality and the parents'/guardians' perception of their child's oral health-related life quality.

Conclusions: The lifestyle and life quality of children's from rural area was improved by oral health education and health education and was changed the perception about the dental treatment and dental doctors.

Key words: *changing comportments, dental caries, lifestyle, life quality, rural area*

Correspondence to:

Ramona Amina Popovici
Address: University of Medicine and Pharmacy „Victor Babes"Timisoara Faculty of Dentistry, adress: Splaiul T. Vladimirescu nr. 14 A
Phone: +40762006828
E-mail address: ramona.popovici@umft.ro

INTRODUCTION

People from rural areas are working in many services other than agricultural, which help rural communities to diversify their economic activity, their knowledge's and education. Unfortunately a lot of young people from rural area Romania, goes every year for the spring, summer and autumn to work in agriculture abroad, and leave their children's at home with grandfathers or other relatives, which are not give a lot of importance to healthy style life, hygiene, and healthy food, so in the adolescence a lot of children's have dental anomalies, a lot of caries, or already missing teethes. With almost no home education about the correctly hygiene, healthy food (a lot of parents send to children's a lot of sweets, for replacement their missing) and with psychological problems from be away from their parents the quality life of the children's from rural area Romania is not so good.

In addition, the qualitative deficiencies of the education system and health system in the rural area are significant worst than that from the urban area. The passive educational methods, with not very good teachers and lack of educational material to support learning process for both teachers and students, rigidity of calendars and schedules; lack of parental and community involvement in health education is a failure to provide a health quality lifestyle. A good accessibility to health providers has a good influence of improvement of the life quality.

For the past few years, the term 'lifestyle migration' has been used to refer to an increasing number of people who take the decision to migrate based on their belief and comportment to other which is more healthier. [1] Lifestyle migration is thus a growing, disparate phenomenon, with important but little understood implications for both societies and individuals.

In last decades low-income countries, like Romania experienced an increasing trend in dental caries among children that's affect children's life.[2] The challenge for health authorities will be continuously to keep the disease level low. In spite of the low level of dental caries the dentists in rural area are not sufficient in number to deal with the treatment need of the population leaving little time for preventive measures.

Oral diseases are major public health problems owing to their high prevalence and incidence in all regions of the world, and as for all diseases. The severe impact is from pain and suffering, impairment of function and effect on quality of life must also be considered. Traditional treatment of oral diseases is extremely costly in several industrialized countries, and not feasible in most low-income and middle-income countries. [3]

Despite its relatively recent emergence over the past few decades, oral health-related quality of life (OHRQoL) has important implications for the clinical practice of dentistry and dental research. OHRQoL is a multidimensional construct that includes a subjective evaluation of the individual's oral health, functional well-being, emotional well-being, expectations and satisfaction with care, and sense of self. It has wide-reaching applications in survey and clinical research. OHRQoL is an integral part of general health and well-being. In fact, it is recognized by the World Health Organization (WHO) as an important segment of the Global Oral Health Program (2003). [4]

The prevalence of oral pain was high among these children, and had a considerable impact on both the children and their parents. [7]

Furthermore, dental health services in general have a strong curative focus and suffer from limited capacity to deliver population-based

essential health care. The health authorities should therefore focus on planning and implementation of population-directed oral health promotion programmes through schools, in active collaboration with the education authorities. Schools play an important role by providing a health promoting environment and healthy lifestyles. For the prevention of dental caries access to food items, drinks and snacks rich in sugars should be discouraged and healthy choices have to be supported. Within schools, oral health education also should be oriented towards oral health self-care instrumental to prevention of dental caries and the poor gingival health of children. While the health personnel should provide technical input to such programme, the challenge of the educational system is to apply skills based learning and relate the rather theoretical oral health curriculum directly to the daily life of the children [2].

The effect of a relatively common chronic disease, severe dental caries, affects young childrens' growth and well-being. Children with severe caries weighed less than controls, and after treatment of decayed teeth there was more rapid weight gain and improvements in their quality of life. This may be due to dietary intake improving because pain affected the quantity and variety of food eaten, and second, chronic inflammation from caries related pulpitis and abscesses is known to suppress growth through a metabolic pathway and to reduce haemoglobin as a result of depressed erythrocyte production. [5]

Toothache in children is a sizeable problem in rural area but not only and had substantial consequences for children and their guardians. Freedom from disabling dental pain/discomfort is an outcome indicator of oral health and could be used as an explicit goal by dental systems. It is important to note however, that the present study did not assess the extent to which the dental pain was associated with avoidable dental problems as opposed to normal physiological processes. It is important that future work try and separate the prevalence of dental pain caused by physiological from avoidable pathological factors. In addition, future work is needed to assess how effectively and efficiently dental services are responding to people suffering with dental pain.[6]

Oral rehabilitation results in the immediate improvement of children's oral health and physical, emotional and social quality of life. It also has a positive impact on the family. [9]

MATERIAL AND METHODS

The purpose of this study was to investigate the effects of early childhood caries (ECC) on children's oral health-related quality of life (QOL) before and 4 weeks after its treatment, as assessed by the children themselves as well as by their parents/guardians. We measured life satisfaction oriented on measuring the psychological functioning in evaluating the outcome of interventions and ameliorating the oral cavity hygiene, disabling physical problems and community-wide social problems.

The paper presents a progress report on developing and applying a research approach to improve the quality of life. Steps in the plan include:

- Surveys which showing needs of the children's analyzing 15 factors defining quality of life;
- Illustrations showing the advantages of using in-depth studies of individuals to identify the determiners of quality of life;

This study had a longitudinal intervention design which analyzes the changing comportments of lifestyle

quality at 169 children's after oral health education lessons and after oral treatments.

RESULTS

One hundred eight children diagnosed with ECC and sixty one children without caries. and their parents/guardians responded to face-to-face administered surveys before a dental treatment was started (baseline assessment). Seventy-nine children with ECC completed dental rehabilitation. Four weeks after the treatment was completed, these seventy-nine children as well as their parents/guardians responded to a second survey (follow-up assessment).

Table 1. The characteristics of the study lots

	mean age	**low**	**Maxim**	**Range**	**masculine**	**feminine**
children's with ECC n=108	7 years and 2 weeks	6 year and 1 week	8 years and 7 months	6 year and 8 months	49	59
children's without caries n=61	7 years and 3 months	6 year and 4 months	8 years and 3 months	6 year and 11 months	27	34

The results of the surveys showed that the children's life was improved by the lowest pain, absence of the masticators problems, which are illustrated in table 2.

Table 2. Indicators of quality life before and after the oral health education lesson

	before	**After**	**statistics**
dental caries	108	79	p= 0,067
dental pain	81	32	p= 0,035
dental anomalies	49	41- but 20 went to the orthodontic doctor	p=0,92
feeding problems	59	21	p=0,03
finger sucking	32	12	p=0,05
eating daily sweets	153	82	p=0,05
eating daily fruits	62	89	p=0,03
eating daily vegetables	78	91	p=0,05
eating daily meet	124	167	p=0, 76
drinking daily juice	92	43	p=0,05
staying every day outside minimum one hour	121	154	p=0, 67
staying every day minimum one hour in front of TV or PC	145	121	p=0,84
sleeping minimum 9 hours per night	133	145	p=0,123
doing sports minim 30 minutes every day	32	56	p=0,81
anxiety about dental treatment and dental doctors	122	53	p=0,03
good body hygiene	66	82	p=0,34

The results show that children's from rural area have oral health problems, and at the earlier education can improve their life quality. The children with ECC who received dental treatment had a significantly improved

oral health-related life quality at the follow-up assessment when compared with their baseline measurement as measured both with the children's self-ratings of oral health-related life quality and the parents'/guardians' perception of their child's oral health-related life quality. The anxiety about the dental treatment and dental doctors was shown in 90% of children's with ECC and in 45% of children's without ECC, but this is straight correlate with parents education also.

Totals of 44.8% from children's without ECC and 83% of the children's with ECC had fair to poor oral hygiene (OHIS > 1) whereas 73.5%, 69% and 38% had at least one tooth with plaque, calculus and bleeding, respectively. The mean OHIS score was 1.5, SD 0.9 range (0-4, good-bad) corresponding to a clinical level of fairly good oral hygiene. Totals of 46.2% (in the main sample) and 503.8% (clinical sub sample) reported at least one oral impact on daily performances (OIDP > 0).

The most frequently reported impacts were eating problems (52.8%) and problems tooth cleaning (37,3%), whereas the least frequently reported impacts were problems speaking (14.5%) and problems school work (9.9%). Totals of 45.4% versus 58.0% of students having well (OHIS ≤ 1) and poor (OHIS > 1) oral hygiene reported any oral impact on daily performances. Table 2 depicts the overall differences in frequency of children's having poor oral hygiene (OHIS > 1) and any oral impact (OIDP > 0) by socio-demographic and behavioral characteristics. As shown, the frequency of oral impacts were higher at children's with ECC and parents with lower income, higher in subjects having father and mother with low education, higher in subjects from families and in those having parents that could not afford dental care. The frequency of having any oral impacts also increased significantly in relation to decreased level of tooth brushing decreased intake of sugar sweetened soft drinks increased dental visiting. The frequency of having poor oral hygiene increased significantly in relation to increased age, being a male, having father with lower level of education, being in the most poor category of the household category and having parents that could not afford dental care index and in relation to not performing regular tooth brushing ($p < 0.05$).

DISCUSSIONS

The present results should be interpreted in the light of limitations that include a cross-sectional design, use of self-reported measures and the fact that the estimates presented are not weighted using sample weight. Another weak aspect is the lack of a measure of dental problems in the present study area and might assumingly impact children's OHRQL from rural area, but is not always better neither in urban area from the west of Romania. Structured, self-administered questionnaires as applied in this study have certain limitations with bias due to social desirability, acquiescence and lack of recall being frequently encountered, particularly in younger age groups [10]. In spite of a reportedly optimal tooth brushing frequency that might counteract the deleterious effects of dental plaque and sugary diets, about 80%, 70% and 30% of the study population presented with plaque, calculus and gingival bleeding. This finding do indicate that response inaccuracy due to recall bias and social desirability is a methodological problem that might have confronted the identification of relationships between oral behaviors and oral health outcomes in this study. However, most findings were in accordance with expectations. The sugar frequency

questionnaire applied has been found to be acceptable with respect to classifying adolescents into broad categories of high and low sugar consumption [11,12].

CONCLUSIONS

The lifestyle and life quality of children's from rural area was improved by oral health education and health education and was changed the perception about the dental treatment and dental doctors. Gender differences should be considered in intervention studies, and modifiable behaviors have some relevance in reducing social disparity in oral health.

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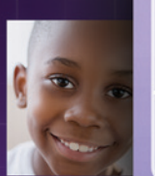





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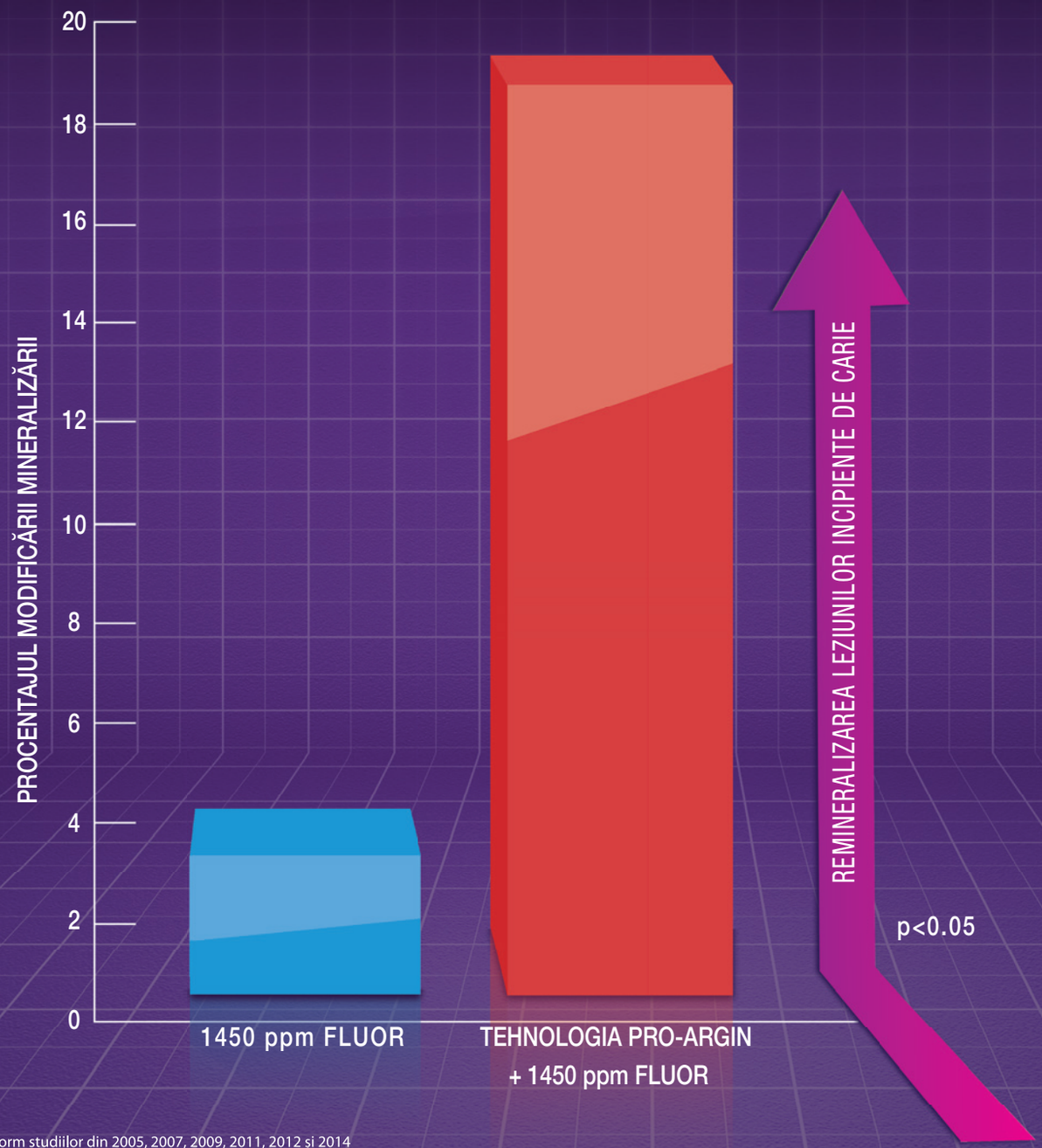

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6. PRESENTING THE MANUSCRIPT

For the journal *„Medicine in evolution"*, the manuscript must be typed double spaced, on white A_4 paper – 210 x 297mm, on one side (2.5cm upper and lower borders, 3cm left and 2cm right border, respectively), in clear characters, no further corrections or addings. It is advisable that articles are presented on CD or other data transfer methods, in Word format, 12 Times New Roman fonts - using Romanian characters – respecting the same page order, accompanied by a printed version. Graphs – black and white or coloured – may be generated in MS Excel or MS Graph, inserted in the body of the paper or presented in a different file.
Infected materials will not be used.

6.1. FIRST PAGE (TITLE PAGE)

Together with the title and names of the authors, the first page must include the affiliation, professional and university degree (if applicable), marked by asterisc for every author; it is advisable to give at least a phone and/or fax number or e-mail address of the first author who may be contacted by the editors for additional recommendations or explanations.

6.2. ABSTARCT OF THE PAPER

6.2.1 Recommendations for original studies

Original studies must include a structured abstarct of maximum 150 words, containing the following titles and informations:

- Aim and objectives;
- Material and methods;
- Results;
- Conclusions;
- Key words: give 3-5 key words;
- The abstract will be translated into an international circulation language.

6.3 CONTENT OF THE PAPER

6.3.1 For original articles

The text will usually be divided into sections:

- Introduction – presentation of general aspects, in the context of the approached theme
- Aim and objectives – Define the aim of the article. Briefly expose the rationale of the presented study or observation. Make strictly pertinent referals and do not exhaustively review the subject. Do not include data or conclusions from the paper.
- Material and methods – Describe the selection of observations or subjects for the experiment (including controls). Identify methods, equipments (with the name and address of the manufacturer in brackets) and give sufficient details on procedures. Give references for the selected methods, including statistical methods; offer details and brief descriptions for

previously published methods which are not well known; describe new or substantially modified methods, justify their use and assess their limitations. Precisely identify all used drugs and chemicals, including generic names, dosage and administration ways. Describe statistical methods with sufficient details for reported results to be verified. Whenever possible, quantify discovered aspects and present them with appropriate measurement indicators for the uncertainty or error of measurement (such as confidence intervals).

- Results – Present results in a logical succession as text, tables and illustrations. Emphasize or briefly describe only important observations.
- Discussions – Underline new, important aspects of the study. Do not repeat in detail data which have been presented in previous sections. Include implications of revealed aspects and their limitations, including implications for future studies. Connect your observations to other relevant studies. Relate the results to the aim proposed for the study.
- Conclusions – organize conclusions which emerge from the study. In the end state: a) contributions to be acknowledged but which do not justify paternity right; b) thanks for technical support; c) thanks for financial or material support.

6.3.2 Indications for case reports

Themes may be selected from all medical fields. Manuscripts which offer a special gain for daily activity will have priority. The title must be clearly, precisely stated. It may be completed by a subtitle. It is advisable to include in the key words of the title the main message, the special element which may be observed from the case evolution. The content of a case report must be divided into three parts:

- Introduction – It must include a maximum of 15 typed rows (half page). Here, the main medical problem is summarized in order to place the case in a specific domain.
- Case report – It contains essential specific information on the case.
- In order to make a logical, chronological and didactical case report the following 5 chapters are needed:

  I. Anamnesis;
  II. Clinical examination data;
  III. Laboratory data;
  IV. Additional paraclinical investigations;
  V. Treatment and evolution.

- Discussions – The reason for the case report must be stated. The report must be patient-centered. Occasional deviations from typical (characteristic) evolutions, nosologically important facts must be presented in such a manner to expose the clinical picture as completely as possible. The case report must not appear as an appendix of a general review. Dimensions of a case report: maximum 6-8 typed pages, 30 rows of 60 characters/page.

6.4. MEASUREMENT UNITS, SYMBOLS, ABREVIATIONS

All measurements must be expressed in International System (IS) units. Abreviations must be fully explained when first used.

6.5. TABLES

Tables are noted with Roman figures and they will have a brief and concise title, concordant with their content.

6.6. ILLUSTRATIONS

Number all illustrations in Arabic figures in a single succession. Apply a label on the back side of every illustration, containing its number and an arrow indicating the upper side. Coloured illustrations may be accepted but it is the choice of the editors, according to particular technical abilities of each journal issue, or it may involve a fee in special cases.

6.7. EXPLANATIONS FOR DRAWINGS AND GRAPHS

Explanation for drawings and graphs must be clear and in readable dimensions, considering the necessary publishing shrinkage.

6.8. PHOTOGRAPHS

Offer glossy, good quality photographs. Any annotation, inscription, etc. must contrast with the ground. Microphotographs must include a scale marker.

6.9. ILLUSTRATION LEGENDS

Include explanations for each used symbol, etc. Identify the printing method for microphotographs.

6.10. REFERENCES

A numbered list of references must be provided at the end of the paper. The list should be arranged in the order of citation in the text of the publication, assignment or essay, not in alphabetical order(according to the Vancouver rules). List only one reference per reference number. It is very important that you use the correct punctuation and that the order of details in the references is also correct.

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In order to accelerate publishing, the main author will send a set of printed sheets presenting the final version of the paper, as it will appear in the journal. It is really helpful that texts to be also sent on electronic support, diacritic characters mandatory.

8. REJECTION OF PAPERS

If a paper does not meet publishing conditions, whatever these may be, the editors will notify the first author on this fact, without the obligation of returning the material. Original photographs or the whole material will be returned only if the author comes to the editor and takes them.

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Journal Medicine in evolution
Department of Preventive, Community Dental Medicine and Oral Health
Splaiul Tudor Vladimirescu no. 14 A
300041, Timişoara
Email: proiectetm@yahoo.com

Dana Brehar-Cioflec, MD, PhD
Institute of Public Health *"Prof. Dr. Leonida Georgescu"* Timişoara
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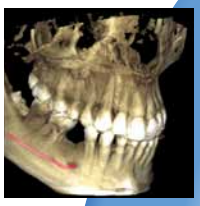

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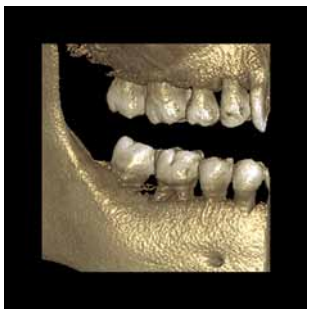

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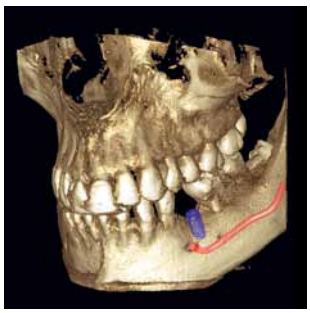

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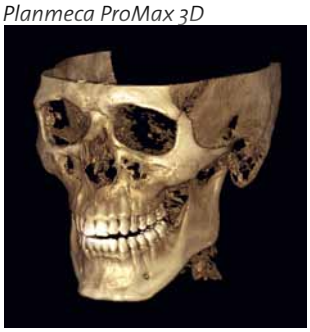

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