### TABLE OF CONTENTS

**INFLUENCE OF ASSISTED REPRODUCTIVE TECHNOLOGIES ON VITAL STATISTICS** ..................................................5

Original article / Originalni naučni rad
C677T POLYMORPHISM OF METHYLENETERAHYDROFOLATE REDUCTASE (MTHFR) GENE IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA AND DIFFUSE LARGE B CELL LYMPHOMA

C677T POLIMORFIZAM GENA METILENTRAHIDROFOLAT REDUKTAZE (MTHFR) KOD PACIJENATA SA HRONIČNOM LIMFOCITNOM LEUKEMIJOM I DIFUZNIMKRUPNOČELIJSKIM B LIMFOMOM ............................................................9

Original article / Originalni naučni rad
APPLICATION OF METHYPREDNISOLONE SUSPENSION BY IONTOPHORESIS IN PATIENTS WITH ARTHROSIS OF THE KNEE

PRIMENA SUSPENZIJE METILPREDNOZOLONA JONTOFOREZOM KOD PACIJENATA SA ARTROZOM KOLENA ...........................................................................13

Literature review / Pregled literature
CLINICAL IMPORTANT OF BIOCHEMICAL MARKERS OF CARDIAC DAMAGE IN HEMODIALYSIS PATIENTS

KLINIČKI ZNAČAJ BIOHEMIJSKIH MARKERA SRČANOG OŠTEĆENJA KOD PACIJENATA NA HEMODIJALIZI............................................................................19

Professional article / Stručni rad
OCULAR MANIFESTATIONS OF CHRONIC SARCOIDOSIS

OFTALMOLOŠKE MANIFESTACIJE HRONIČNE SARKOIDOZE..................................................................................27

Professional article / Stručni rad
THE IMPORTANCE OF Nd: YAG LASER IRIDOTOMY IN THE THERAPY OF THE CLOSED ANGLE GLAUCOMA

ZNAČAJ Nd: YAG LASER IRIDOTOMIJE U TERAPIJI GLAUKOMA ZATVORENOG UGLA ....................................................31

Case report / Prikaz slučaja
INSERTION OF NASAL SEPTAL BUTTON IN THE TREATMENT OF SEPTAL PERFORATION

INSERCIJA SEPTALNOG OPTURATORA U TRETMANU PERFORACIJE NOSNE PREGRADE ..................................................................................35

INSTRUCTION TO AUTHORS FOR MANUSCRIPT PREPARATION.................................................................................39
Dear readers,

New title, new design, new era!
Being published under the title MEDICUS for nine continual years, our journal has got a new, we hope more appropriate, title Serbian Journal of Experimental and Clinical Research. The decision to change the title stemmed from two reasons. The first is a need to clearly define the country of origin as well as the field of medical science the journal covers. By providing these two pieces of information through the journal title itself, we strive to facilitate, both to our readers and potential contributors, the process of grasping and accepting it. The other reason is more practical, or should we say technical in its nature. A certain level of ambiguity had arisen in relation to the now defunct title MEDICUS, the ambiguity we wanted to avoid. Namely, in addition to our MEDICUS, quality and rating of which are indubitable, there are few other journals with the same or similar titles. We may say with confidence that their quality and rating are on the lower end of the bar, yet they were fertile ground for misunderstanding and we felt that this reason more sufficient significance to necessitate the change of title.

In addition to the new title, this volume features revamped design - in other words, the design of Serbian Journal of Experimental and Clinical Research is improved both aesthetically and functionally. Our friend and associate Vidan Papić will take credit for all positive and the blame for all negative aspects of the journal's new layout.

We would also like to inform you that we feel our quality and maturity are of such exceptional level that we engaged in a qualification match for getting indexed in Medline/Index Medicus base, the objective we set for ourselves nine years ago. We certainly hope to receive the positive answer to our application.

You are looking at the first issue, 9th volume, of the journal formerly known as MEDICUS, now entitled Serbian Journal of Experimental and Clinical Research. In the Editorial, our esteemed associate from Slovenia, Professor Veljko Vlaisavljević, outlines his views on the influence assisted reproduction exercises on vital statistics, in a competent and interesting way. Nataša Rakonjac et al. provide insight to the results of a research concerned with gene polymorphism of Methylentetrahydrofolate reductase in patients with B-cell leukemia and lymphoma, whilst Tatjana Jevtić et al. provide the results of their research on methods of iontophoretic corticosteroid application in degenerative/inflammatory knee joint conditions. This issue contains selection of current literature data relevant to the biochemical markers of heart disease and their significance in dialysis patients. The outline is accompanied by commentary and interpretation by Dejan Petrović et al. Further, you will find the research results for ocular manifestations of chronic sarcoidosis written by Svetlana Jovanović et al., whilst contemporary and efficient method of laser iridotomy in glaucoma treatment is presented by Svetlana Paunović et al. Finally, Branislav Belić et al. exhibit a case of successful septal obturator insertion in the treatment of nasal septal perforation.

Enjoy!
Statistical data uncompromisingly show that Europe is dying. In times when the number of people is globally increasing, most European countries are facing negative demographic trend. The birth rate in European countries is lower than 2 children per woman, below the level needed to review the population (2.1 child per woman). Europeans live longer and have fewer children. A negative demographic trend and the aging of population have constantly been present for at least two decades in most reports on demographic statistics. Geographical position, social status and religion obviously have no influence on these figures. Demographic data for Slovenia do not significantly differ (1-9).

Solving the problem of the aging population is one of the priorities of European countries since the “no solution policy” has undoubtedly economic consequences for the population and in most cases also results in poor social connection among different generations. A different attitude to giving birth results from altered economic and social values (the cost of real estate purchase, women prioritizing career instead of having children, high unemployment rate among young people who still live with their parents, inability to get a job with shorter working hours, care for pre-school children, etc.)

It is estimated that such trends will result in every fourth European older than 65 years in 2040. The population of Europe in 2050 will be the oldest on Earth with the average age of 47 (in comparison to the today’s average age of 39). The aging of population in Europe will have negative consequences for its economic and social security. This will influence the Europeans’ standard of living - less people will be employed and a decline in the national economic progress as well as family’s standard of living is expected. The costs of health care system will increase in next decades. The government’s standard of living - less people will be employed and a decline in the national economic progress as well as family’s standard of living is expected. The government’s standard of living - less people will be employed and a decline in the national economic progress as well as family’s standard of living is expected. The government’s standard of living - less people will be employed and a decline in the national economic progress as well as family’s standard of living is expected. The government’s standard of living - less people will be employed and a decline in the national economic progress as well as family’s standard of living is expected. The government’s standard of living - less people will be employed and a decline in the national economic progress as well as family’s standard of living is expected. The government’s standard of living - less people will be employed and a decline in the national economic progress as well as family’s standard of living is expected. The government’s standard of living - less people will be employed and a decline in the national economic progress as well as family’s standard of living is expected.

Unfortunately, access to ART is in many European countries poor and limited with a restrictive health care policy and health care insurance system, which is often an insurmountable obstacle for couples without children. Not only the fact that more and more women decide to have their first child in the later reproductive period (a general characteristic of all European countries) but also poor prevention of pelvic tubal diseases caused by Chlamydia
(more often in poorer environments) decreases the probability of spontaneous pregnancy, which again increases the need for ART. Nevertheless, some countries have adopted the policy of refunding the costs of IVF, solely as a measure to increase the birth rate [Estonia, South Korea] (8, 11, 14).

ART is based on a method which could hardly be called a therapeutic method at the time it was first applied. Its success amounted to only 0.5% taking into account all procedures worldwide to the moment when the first child was born in 1978. However, it has become a successful method for treating infertility and in many cases also the only possibility of treatment. Today it reaches an average success level of 30–40% for embryo transfer and is thus becoming more successful – measured with the number of conceptions per cycle – than spontaneous conception in any reproductive period of life.

Due to high costs of ART treatment and limited material resources intended for health care, many societies are unable to provide enough financial means for treating infertility because they generally lack money to deal with priority issues of health care. In such cases the problem of abortion (desired termination of pregnancy) is often addressed and its prevention to improve the negative demographic trends of population growth. At the same time it is also used as an excuse (not) to solve individual problems of infertile couples. In the last 25 years the percentage of abortions has nevertheless been decreasing (figure 1) (13).

Women play a different role in modern society than decades ago. Intellectual and economic equality has altered women’s priorities in the early reproductive period. Setting new/different objectives and often making an academic career has set the wish to have children into the late reproductive years. Such „consciously chosen” form of infertility occupies an important position among reasons for infertility in the late reproductive period and the only remaining solution for many couples is ART. Commonly, it is the only possibility in cases where there are no clinical signs of sterility and the ART method is required only due to the late reproductive period and the need for fast conception planning (the so-called „urgent IVF”). The realization of such wishes is often accompanied by enormous financial expenses.

The success of ART certainly belongs among the most important reasons for „urgent IVF” in the late reproductive period when nature is less successful due to physiologic changes in egg cells. The reason may also lie in the patients’ demands, who do not wish a (less successful) surgical procedure on Fallopian tubes and the subsequent waiting (with a negative outcome) for a natural conception. (15, 17, 18).

The medical public has a significantly different attitude when surgical methods for treating infertility are employed (e.g. endoscopic corrections of tubal causes of infertility) than in the case of ART.

Undisputedly, the use of IVF techniques for treating tubal causes of infertility or initiating spermatocyes into cytoplasmic egg cells (ICSI – intracytoplasmic sperm injection), which are most of the time used for states of „not having” children, leaves women and men further infertile (closed fallopian tubes or insufficient number of spermatocytes) but they get a child nevertheless.

Due to the exceptional success rate of ART methods, which in all reproductive periods of life exceed the success rate of spontaneous conception, indications for ART methods have also spread on „planning” pregnancy in the late reproductive period when nature is less successful due to physiologic changes in egg cells. The reason may also lie in the patients’ demands, who do not wish a (less successful) surgical procedure on Fallopian tubes and the subsequent waiting (with a negative outcome) for a natural conception. (15, 17, 18).

The success of ART certainly belongs among the most important reasons for „urgent IVF” in the late reproductive period and IVF as a replacement for surgical techniques of tubal infertility therapy in the second half of women’s reproductive period. Such health insurance in Denmark does not acknowledge the costs of surgery on Fallopian tubes after the age of 33 (in favor of choosing ART). It should also be mentioned that treating male forms of infertility with ICSI techniques is now mainly carried out at centers for treatment with ART methods (gynecology) and no longer at urologic (andrologic) out patients clinics.

Negative demographic trends in European countries are less a consequence of religious or economic reasons than the result of changes in lifestyle and priority list of life values. Women’s age during their first pregnancy is constantly increasing and the birth rate in most European countries is

Although a woman’s role in society is no longer bound to her reproductive role, in many environments infertility still brings about stigmatization within family and friends, suffering, unstable marriage and even violence. It is estimated that the frequency of infertility in Europe amounts to approximately 10%.
lower than 1.4 children per woman. These trends are similar in most European countries. There are neither significant differences between wealthier and poorer social classes, nor even among different groups on the same geographical area. Religion has no greater influence on these trends (although the largest decrease in births is noticeable in „traditionally catholic countries”).

Today we register barely 1.2 child per woman in Slovenia and the average age of mothers at first birth has increased in the last fifteen years for 4 years (to 27.8 years in 2005) (figure 2). Consequently, only a short suitable biological period is left for the birth of a second child (16).

To say that treating infertility is merely a means to solve problems of certain individuals is a definition which is not acceptable nowadays, especially if we consider all the above-mentioned. Even the data from the European ART registry show that in certain environments ART conception methods contribute to a considerable number of newborns in comparison to the entire number of newborns.

The argument of „limited resources” in health care fund is often an excuse for not investing in the development of expensive technology for ART and only a limited group of patients potentially benefit from it. On account of that more attention is paid to prevention of infertility as well as providing ART treatment. The opinion that treating infertility does not belong to priority programs of the health care policy is especially based on a false presumption that such a state does not cause indirect material consequences for an individual as well as the society and endanger life.

Progress and successful use of ART methods is certainly the most extensive and maybe even the most significant event in the course of development of gynecology in the last quarter of the previous century. It is difficult to estimate the cost benefit of ART procedure and compare it to other treatment methods. When evaluating the success rate of treating specific diseases as cancer, it is almost impossible to compare this method which creates life in the same manner as „prolonging life” is estimated and valued. It is estimated that each euro invested in ART returns to the society in the next ten years in the amount of 40 euros through activities of a new inhabitant born by means of ART.

To accept the ideal model of a small family with the average of somewhat more than one child and to voluntarily choose a lifestyle with no children, as acceptable for modern and emancipated women, sets the problem of consciously chosen infertility in the center of our interest even in the case of solving the problem of infertile couples. If a couple is delaying the decision to have their first child, help needs to be offered in order to convince them to conceive at the time the couple feels it has fulfilled all the conditions for parenthood. From the moment a couple realizes that their biological clock is no longer running, only a short period of time remains to realize the decision. The most appropriate period (the most reproductive part of life) for having children has passed. In many European countries such a situation leads to radical measures employed for solving the problem of „not having” children and „infertility” in non-conventional forms of family or incomplete families (women without a partner).

Clearly, ART procedures cannot extensively influence the population policy and significantly improve the unfavorable
demographic movement (figure 5); however, the percentage of newborns conceived by means of ART in Slovenia already exceeded 4% in 2005. Without its contribution to the number of births in Slovenia, demographic trends would be even more unfavorable.

Prof. dr Veljko Vlaisavljevic

REFERENCES


INTRODUCTION

Lymphoproliferative diseases include a heterogeneous group of lymphoid neoplasms characterized by typical morphological, immunophenotypical, genotypic and clinical features (1). In that group, beside characteristic neoplasms which are general for WHO classification, one can distinguish low progressive (fOLLICULAR lymphomas, chronic lymphocytic leukemia, etc.) and high progressive lymphoid neoplasms (AcUTE leukemia, diffuse large B-cell lymphomas, etc.).

Etiology of most cases of lymphoproliferative neoplasms is unknown, although some factors such as immunodeficiency, viral infections, exposure to environmental and chemical factors and genetic factors have been defined (2–4). Certain genetic events during cell differentiation, such as chromosomal translocations, mutations in various genes, genetic polymorphisms and many other chromosomal aberrations play an important role in genesis of lymphoid malignancies. Also, methylation status of various oncogenes or tumor suppressor genes may induce selective growth of cells or its inhibition (5).

Folate is an important nutrient required for DNA synthesis, repair or methylation; it donates a methyl group to uracil and converts it to thymine. Low folate could increase risk of malignancy by following mechanisms: 1) DNA hypomethylation and inappropriate activation of oncogenes or 2) uracil misincorporation during DNA repair and synthesis, leading to DNA strand breaks, chromosom damage and eventually malignant transformation (6–8).

Folate metabolism requires the optimal activity of multiple enzymes including 5, 10-methylenetetrahydrofolate reductase (MTHFR) which catalyses the irreversible conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (5). This reaction catalysed by MTHFR plays a significant role in folate metabolism, contributing to DNA synthesis, methylation and eventually to cancer susceptibility and it has been implicated in cancer risk. In the present study we investigated the association of the common MTHFR C677T polymorphism with B cell chronic lymphocytic leukemia and diffuse B cell large non Hodgkin’s lymphoma. Patients were compared with age and sex matched control subjects.

Our results indicate significantly lower distribution of variant allele 677TT in patients with chronic lymphocytic leukemia compared with control group (frequency of variant allele 677TT 24% vs. 33% respectively). The difference in allelic distribution of MTHFR gene among those two groups was statistically significant (p=0.05). Results were the same when we compared CLL with DLBCL (frequency of variant allele 677TT 24% vs. 34.5%, p=0.05). This was accompanied by a significantly higher frequency of homozygote normal genotype (677CC) among the patients with CLL. The difference in allelic distribution between DLBCL and control group did not reach statistical significance (p=0.065).

Our results suggest that the distribution of polymorphism of MTHFR gene may vary among the different group of lymphoproliferative diseases and that 677CC genotype may represent risk factor for developing of CLL.

Key words: methylenetetrahydrofolate reductase, polymorphism, genetic, leukemia, lymphocytic, chronic, B cell, lymphoma, large B-cell, diffuse

C677T POLYMORPHISM OF METHYLENETERAHYDROFOLATE REDUCTASE (MTHFR) GENE IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA AND DIFFUSE LARGE B CELL LYMPHOMA

Nataša Rakonjac1, Vesna Ilić1, Gordana Supić1, Bojana Cikota1, Olivera Tarabar2, Jovana Jovanović1 and Zvonko Magić1
1Institute of Medical Research, 2Clinic of Hematology, Military Medical Academy, Belgrade, Serbia

ABSTRACT

Methylenetetrahydrofolate reductase (MTHFR) plays an important role in folate metabolism, contributing to DNA synthesis, methylation and eventually to cancer susceptibility and it has been implicated in cancer risk. In the present study we investigated the association of the common MTHFR C677T polymorphism with B cell chronic lymphocytic leukemia and diffuse B cell large non Hodgkin’s lymphoma. Patients were compared with age and sex matched control subjects. Our results indicate significantly lower distribution of variant allele 677TT in patients with chronic lymphocytic leukemia compared with control group (frequency of variant allele 677TT 24% vs. 33% respectively). The difference in allelic distribution of MTHFR gene among those two groups was statistically significant (p=0.05). Results were the same when we compared CLL with DLBCL (frequency of variant allele 677TT 24% vs. 34.5%, p=0.05). This was accompanied by a significantly higher frequency of homozygote normal genotype (677CC) among the patients with CLL. The difference in allelic distribution between DLBCL and control group did not reach statistical significance (p=0.065).

Our results suggest that the distribution of polymorphism of MTHFR gene may vary among the different group of lymphoproliferative diseases and that 677CC genotype may represent risk factor for developing of CLL.

Key words: methylenetetrahydrofolate reductase, polymorphism, genetic, leukemia, lymphocytic, chronic, B cell, lymphoma, large B-cell, diffuse
of 5, 10- méthylénetétrahydrofolate to 5, 10- méthylétrahydrofolate, the methyl donor for the conversion of homocysteine to methionine, which is converted to S-adenosylmethionine (SAM). SAM methylates cytosine residues in DNA (figure 1). The consequence of inappropriate activity of MTHFR is hypomethylation of critical genes and this makes MTHFR a cancer predisposing gene (1).

Several single nucleotide polymorphisms within the MTHFR gene have been described, resulting in variant enzyme activity. Most frequent MTHFR polymorphism is base exchange at nucleotide position 677 (C→T, alanine-valine). This polymorphism leads to the expression of thermo labile form of MTHFR and its reducing enzyme activity (9). Homozygosity for the MTHFR 677 T allele is associated with many diseases such as cardiovascular disease, neural tube defect and with many cancers such as colorectal, ovarian, oropharingeal, breast, endometrial (7, 10–15). Due to role in cancerogenesis of different solid tumors, this polymorphism is of great interest in the pathogenesis of lymphoid malignancies.

Data on the association of MTHFR 677 polymorphism with risk of CLL and DLBCL are controversial. Great number of studies described no association of risk of CLL and this polymorphism (16). Although some authors described that MTHFR 677CT polymorphism is associated with risk of CLL progression (17, 18). In a group of non Hodgkin’s lymphomas, including DLBCL, results are also conflicting. While some of them show decreased risk for DLBCL in patients with 677TT genotype (16, 19), the other authors described controversial results (20), or do not show any connection (21).

Controversial data of influence of MTHFR 677TT genotype in pathogenesis of lymphoproliferative disease could be explained with fact that increased activity of MTHFR enzyme increase availability of méthylénetétrahydrofolate and reduce the frequency of misincorporation of uracil into DNA, reducing the risk of DNA double strand breaks. On the other hand, reduced MTHFR activity might result in hypomethylation of DNA promoter regions, leading to increased expression of protooncogene (22–24).

The aim of the present study was to investigate the allele frequency of MTHFR C677T polymorphism in group of patients with CLL, and in the group of patients with DLBCL. Whereas we considered low progressive disease (CLL) and high progressive disease (DLBCL), we investigated difference of MTHFR 677 polymorphism among these two groups.

PATIENTS AND METHODS

Patients
This study included 26 patients with DLBCL obtained from Oncology and Radiology Institute, Belgrade and 23 patients with CLL obtained from Clinic of Hematology, Military Medical Academy, Belgrade. The patients groups were compared with a control group of healthy individuals (n=35). The control subjects were randomly selected from participants without any sign of a malignant disease.

Methods
Peripheral blood was placed into EDTA containing tubes and lymphocytes were separated by Ficoll gradient centrifugation. Genomic DNA was isolated from peripheral lymphocytes by standard salting out procedure which consist of red cell and mononuclear cell lysis, cell lysis by proteinase K and SDS, salting out by NaCl, DNA precipitation by ethanol and resuspension (25).

Polymerase chain reaction
Genotyping of the MTHFR C677T polymorphism was performed using conventional polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) analysis. A 198 bp region of exon 4 of the MTHFR gene was amplified using the primer and reaction condition (26). The success of amplification was controlled by 2% agarose gel electrophoresis and visualized by ethidium bromide staining.

Digoxin
Ampilified 198 bp PCR product were digested with Hinf I (Fermentas) according to the manufacture’s conditions. Hinf I digest normal 198 bp product into a 175 bp and 23 bp fragments. Polyacrylamide gel electrophoresis (PAGE) Samples of amplificats of MTHFR gene after digestion were electrophoresed in 10% polyacrylamide gel. Gel was run in 0.5X TBE at 150 V and 15 W for 120 min and was silver stained (Serva, Germany). The C allele produced 198 bp band, and the T allele produced 175 and 23 bp fragments. Heterozygote produced bands for each allele.

Statistical analysis
The Fisher exact test was used to determine the difference between the allele and genotype frequencies among the groups. A two sided alpha level of 0.05 was considered statistically significant.

RESULTS

The characteristics of study subject are given in table 1.

<table>
<thead>
<tr>
<th>Number</th>
<th>Gender</th>
<th>Age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>35</td>
<td>Female 10 Male 25</td>
</tr>
<tr>
<td>DLBCL</td>
<td>26</td>
<td>8 18</td>
</tr>
<tr>
<td>CLL</td>
<td>23</td>
<td>7 16</td>
</tr>
</tbody>
</table>

Table 1. Characteristics of study participants.

DLBCL -> Diffuse large B cell lymphoma, CLL -> Chronic lymphocytic leukemia

The characteristic pattern of npolyacrylamide gel electrophoresis for C677T polymorphism of MTHFR gene was shown in figure 2.

The frequency of variant allele was 33% in the control group and 24% in the patients group with CLL, indicating that the variant allele occurred less frequently in patients with CLL compared to control group. That was result of higher frequency of normal homozygote 677CC in patients group with CLL than in control group (56, 5% vs. 45, 7% respectively). The difference of allele distribution among this two groups was statistically significant (p=0.05).
In the group of patients with DLBCL, difference of frequency of variant allele 677T and difference of allele distribution among this group of patients and control group was not statistically significant (34.5% vs. 33%; p=0.065). Our data indicate that there is no considerable difference in the prevalence of the MTHFR C677T polymorphism between control group and DLBCL patients group (table 2).

Table 2. Allele and genotype frequencies in the patients and control groups.

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>677CC n (%)</th>
<th>677CT n (%)</th>
<th>677TT n (%)</th>
<th>T freq. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>35</td>
<td>16 (45.7)</td>
<td>12 (42.8)</td>
<td>4 (11.5)</td>
<td>33</td>
</tr>
<tr>
<td>CLL</td>
<td>23</td>
<td>13 (56.5)</td>
<td>9 (39.2)</td>
<td>1 (4.3)</td>
<td>24</td>
</tr>
<tr>
<td>DLBCL</td>
<td>26</td>
<td>11 (42.3)</td>
<td>12 (46.2)</td>
<td>3 (11.5)</td>
<td>34.5</td>
</tr>
</tbody>
</table>

Given the fact that diffuse large B cell lymphoma and chronic lymphocytic lymphoma have different clinical feature we investigated the difference of allele distribution of MTHFR gene among patients group with DLBCL and patients group with CLL. The frequency of variant allele 677T was significantly lower in patients with CLL (24% vs. 34.5%). The difference of allele distribution among those two patients groups was statistically significant (p=0.05).

**DISCUSSION**

Our study investigated possible role of the common MTHFR gene polymorphism as a risk factor for two groups of lymphoproliferative diseases: chronic lymphocytic leukemia and diffuse large B cell lymphoma. Both groups were compared with sex and age matched control group, and groups of patients were compared to each other. The polymorphism was investigated in patients and controls by PCR-RFLP analysis.

Our findings show that the MTHFR C677T polymorphism occurs less frequently in patients with CLL, compared with the distribution and frequency of variant allele in control group and group of patients with DLBCL. Distribution and frequency of variant allele of MTHFR among control and patients group with DLBCL was not statistically significant. These results suggest that distribution of polymorphic allele 677T may vary among different groups of lymphoproliferative diseases.

Actually, conflicting result has been reported about C677T polymorphism in lymphoproliferative disease. Regarding the potential association of MTHFR genotype with diffuse large B cell lymphoma, only a few studies about non Hodgkin’s lymphoma subentities, including DLBCL, have been published (19–21, 23, 26, 27). While same authors do not find association (21, 27), other describe a protective effect of the MTHFR 677TT genotype (19, 23, 26). One large population based study on 1593 patients found an increased risk of diffuse large cell lymphoma in adult patients being homozygous for the mutated allele (20).

The reports concerning the role of the MTHFR polymorphism in chronic lymphocytic leukemia pathogenesis are also inconsistent (18, 28, 29–31). Most of the results does not show association between C677T polymorphism and risk of CLL (16, 29, 30). Some authors describe significantly more aggressive clinical course in patients with 677CT or TT genotype (18), while some showed association of MTHFR 677CC genotype with high relapse rate in patients with CLL (30, 31).

Association of the normal genotype 677CC with increased cancer risk in patients with CLL, which we have found in our study, may indicate protective effect of MTHFR 677TT genotype.

Protective effect of 677TT genotype in pathogenesis of CLL could be explained with the fact that TT homozygote reduce MTHFR activity and result in the accumulation of 5, 10-methyltetrahydrofolate. This, in turn, reduces the chances for misincorporation of uracil into DNA, which lead to double - strand breaks during uracil excision repair (8,32). Double-strand breaks and deletion in CLL have been reported at specific sites within chromosome 11q where folate sensitive CCG repeats are located (30).

In most cancer types increased cancer risk conferred by MTHFR polymorphism has been associated with homozygote variant of genotype (677TT) (7, 12, 13–15). That could be explained by lower MTHFR enzyme activity, hypomethylation promoter region of oncogenes and their higher expression. Interestingly, these examples show that opposite effects may result from identical causes.

Opposite to our expectation we didn’t find statistical significance in distribution of variant allele among patients in DLBCL group and control group, although, when we consider strictly defined clinical parameters such as progression free interval, survival time, and treatment free interval, DLBCL is more progressive lymphoproliferative disease and have the same B cell origin like CLL. Reason for this is probably small sample size, heterogeneity of DLBCL, and complicated signal pathways which are the base of the different carcinogenesis mechanisms (33).

In conclusion, our study provide evidence that homozygote normal genotype 677CC of MTHFR is observed at higher frequency than heterozygote 677CT or variant homozygote 677TT in CLL, representing risk factor in pathogenesis of CLL. These results need to be confirmed in further studies with larger sample size.
REFERENCES

APPLICATION OF METHYLPREDNISOLONE SUSPENSION BY IONTOPHORESIS IN PATIENTS WITH ARTHROSIS OF THE KNEE

Tatjana Jevtić1, Zeqiri Mejdi2, Jasmina Vukomanović2, Dragan Milovanović2 and Milorad Jevtić3

1Medical Centre, Kosovska Mitrovica, 2Medical Centre, Vranje, 3Medical Centre, Jagodina, “Department of Pharmacology, Medical Faculty University of Kragujevac, Center for Physical Medicine and Rehabilitation, Clinical Centre Kragujevac, Kragujevac, Serbia

PRIMENA SUSPENZIJE METILPREDNOZOLONA JONTOFOREZOM KOD PACIJENATA SA ARTOZOM KOLENA

Tatjana Jevtić, Zeqiri Mejdi, Jasmina Vukomanović, Dragan Milovanović i Milorad Jevtić

1Medicinski centar, Kosovska Mitrovica, 2Medicinski centar, Vranje, 3Medicinski centar, Jagodina, “Institut za farmakologiju Medicinski fakultet Univerziteta u Kragujevcu, 1Centar za fizikalnu medicinu i rehabilitaciju, Klinički centar Kragujevac, Kragujevac, Srbija

INTRODUCTION

The knee arthrosis is a very frequent rheumatic degenerative disease. It primarily represents a damaged joint cartilage, which causes pain and reduction in mobility, inability to walk and associated symptoms. In this clinical syndrome we found synovitis as an attendant symptom of „activated knee arthrosis”. Therapeutic regimen is based on applications of non-specific inhibitors of inflammation, non-steroidal anti-inflammatory drugs, and application of physical therapy. Later on, preparations of hyaluronic acid have been given. Application of corticosteroid by iontophoresis is not so common in clinical practice, instead of intraarticular injection of cortisone preparations (e.g. poorly soluble suspensions of methylprednisolone and betamethasone). In this work we have shown the importance of application of corticosteroids with iontophoresis in patients with arthrosis of the knee joint. The optimal iontophoteric application of methylprednisolone acetate in the cases with knee joint arthrosis was performed by the following protocol: application of the drug with negative electrode, the current of 120 mA/min/cm², with the average time of application (depending on to patient individual sensitivity) of 20 minutes. The improvement of the signs and symptoms and the subjective discomfort in the knee joints were measured by Hubertus test and VAS scale. Our results showed that clinical and subjective improvement was larger and more sustained in the group which was treated with iontophoteric application of methylprednisolone, than in the group treated with placebo (distilled water).

ABSTRACT

The knee arthrosis is a very frequent rheumatic degenerative disease. It primarily represents a damaged joint cartilage, which causes pain and reduction in mobility, inability to walk and associated symptoms. In this clinical syndrome we found synovitis as an attendant symptom of „activated knee arthrosis”. Therapeutic regimen is based on applications of non-specific inhibitors of inflammation, non-steroidal anti-inflammatory drugs, and application of physical therapy. Later on, preparations of hyaluronic acid have been given. Application of corticosteroids by iontophoresis is not so common in clinical practice, instead of intraarticular injection of cortisone preparations (e.g. poorly soluble suspensions of methylprednisolone and betamethasone). In this work we have shown the importance of application of corticosteroids with iontophoresis in patients with arthrosis of the knee joint. The optimal iontophoteric application of methylprednisolone acetate in the cases with knee joint arthrosis was performed by the following protocol: application of the drug with negative electrode, the current of 120 mA/min/cm², with the average time of application (depending on to patient individual sensitivity) of 20 minutes. The improvement of the signs and symptoms and the subjective discomfort in the knee joints were measured by Hubertus test and VAS scale. Our results showed that clinical and subjective improvement was larger and more sustained in the group which was treated with iontophoteric application of methylprednisolone, than in the group treated with placebo (distilled water).

Abbreviations: VAS - visual analogue scale, NSAID - non-steroidal anti-inflammatory drugs, TENS - transcutaneous electrical nerve stimulation

Key words: knee arthrosis, corticosteroids, iontophoresis

INTRODUCTION

The knee arthrosis is the most common degenerative rheumatic disease. Its primary properties are: damaged joint cartilage, pain, motility reduction and inability to walk. In clinical picture, we often find synovitis as a symptom of „activated knee arthrosis” (1). Primary knee arthrosis most often does not have clear etiology, and secondary knee arthrosis is caused by bad position of the genu valgum or genu varum, by inflammatory processes, metabolic joint damage (chondrocalcinosis, gout, diabetes mellitus), by traumatic damage (ligaments damage, chondromalacia), by bleeding in the joint (haemophilia), aseptic necrosis and troubles during growth. It is based on applications of non-specific inhibitors of inflammation nonsteroidal anti-inflammatory drugs and physical therapy. Recently, with the disease at the initial phase, various preparations of hyaluronic acid were given. The use of corticosteroids through iontophoresis has not been much researched so far, but iontophoretic techniques with corticosteroids, some non-steroid antiinflammatory drugs (e.g. sodium diclofenac), and acetic acid were thought to be effective treatment mode for inflammations in several areas of the body (3). Formulated as water soluble salt, the corticosteroid molecule has a negative charge and, during the iontophoresis, such preparations are delivered from the cathode. Dexamethasone sodium phosphate was

Correspondence: Tatjana Jevtić, M.D.
1Medical Centre, Ann Dinana 10; 38220 Kosovska Mitrovica, Serbia, Tel. +381 28 424 006; Mobil +381 64 23 51 642

SAZETAK

Gonartroza je vrlo često degenerativno reumatsko oboljenje. Ona se primarno odlikuje oštećenom zglobovnom hrskavicom, što izaziva bol, redukciju pokretljivosti, nemogućnost hoda i pridružene simptome. U ovom kliničkom sindromu se sreće i sinovitis kao prateći simptom „aktivirane gonarthroze”. Terapijski program se zasniva na primeni inhibitora zapaljenskih nespecifičnih mediatora, nesteroidnih inflamatornih lekova, i primerni fizikalne terapije. Kasnije se daju preparati hijaluronske kiseline. Primena jontoforeze kortikosteroida nije tako česta u kliničkoj praksi za razliku od intraartikularnog davanja kortikosteroidnih preparata (npr. slabo rastvorljive suspenzije metilprednizolona i betametazona). U ovom radu je pokazan značaj primene kortikosteroida putem jontoforeze kod arteroze kolenoškog zgloba. Optimalna primena metilprednizolonacetata putem jontoforeze kod artroze kolena je sprovedena po sledećem protokolu: aplikacija leka sa negativne elektrode, doza od 120 mA*min/cm², a prosečno vreme aplikacije leka (u zavisnosti od individualne osetljivosti pacijenta) je oko 20 minuta.

Poboljšanje simptoma i znakova i subjektivnih tegoba kod arteroze kolenoškog zglobova je mereno Hubertus testom i VAS skalom. Naši rezultati su pokazali da je klinički i subjektivno poboljšanje bilo veće i dugotrajnije kod grupe koja je primala metilprednizolon acetat putem jontoforeze komparativno sa grupom koja je primala placebo.

Skracenice: VAS - vizuelno analogna skala, NSAID - nesteroidni antiinflamatorni lekovi, TENS - transcutan u elektrostimulacija

Ključne reči: arteroze kolena, kortikosteroidi, jontoforeza

Received/Primljen: 22. 01. 2007. Accepted/Prihvaćen: 27. 02. 2008.


13
the most used corticosteroid agent during the iontophoresic procedures (4). It was experimentally proved that the drug penetration into tissue following iontophoresis in primates was considerable (more than 1.5 cm) and included joint capsules (5).

On the other hand, methylprednisolone was occasionally used during iontophoresis probably due to its inability to penetrate the intact skin in significant amount (6). If used, the soluble salt, methylprednisolone succinate, was chosen (7). We were unable to find the study which investigates the iontophoresic penetration of the methylprednisolone or its compounds. However, the esters of methylprednisolone, such as the acetate, sodium succinate, hemisuccinate and the phosphate, were rapidly converted in vivo to parent molecule. In addition, it has been recently reported that in patients receiving the methylprednisolone acetate injection, the drug could be detected in biological fluids with the advanced electrochemical techniques, across the wide range of its concentrations and pH values (8). Electrocytolytic oxidation of methylprednisolone was probably primarily involved in its conversion to electrochemically active compound (9, 10).

Obviously, the use of methylprednisolone during iontophoresis was poorly investigated so far which strongly contrast the fact that in routine clinical practice, intraarticular injection of corticosteroid preparations, among which methylprednisolone was the one (e.g. LemoR depoR) is widely used (11). Taking into account the available evidence about the electrical properties and behaviour of methylprednisolone molecule and its pharmaceutical preparations we hypothesised that the iontophoresic application of methylprednisolone acetate depot formulation could exert valuable clinical utility in knee arthrosis.

PATIENTS AND METHODS

The study had single blind, prospective, placebo-controlled design. The study was performed in Specialized Hospital „Vrnjacka Banja”, from September, 2005. to December, 2006. The sixty adult subjects with knee arthrosis were randomly assigned into two equal groups. The patients in both groups were treated with the same basic therapeutic protocol - application of drug therapy (NSAIL, other analgesics) and conventional physical agents: ultrasound 0.8 W/cm² with 5 minutes duration, TENS therapy, paraffin application, kinesitherapy.

In experimental group, iontophoresis of corticosteroids was applied which consisted of methylprednisolone acetate (LemoR depoR) in the dosage of 40 mg (prepared as liquid suspension in 1 mL), once daily, with duration of ten days. Methylprednisolone was applied in liquid solution, put on filter paper, from the negative pole of the electrode. For iontophoresis, milliampere dosage was applied with 120 – 150 mA*min/cm². According to individual sensitivity, the time of the procedure for each subject was adjusted. In another group of patients, beside the basic therapy, placebo was applied by iontophoresis, with the same current parameters of electrotheraphy as in experimental group.

For evaluation of the applied therapy effect in the patients with joint arthrosis, we used Visual Analogue Scale (VAS) for pain evaluation (12), Hubertus test (13), muscular test for quadriceps femoral muscle (14) measures of motion range in degrees and of treated knee joint, as well as reduction of the doses of NSAIDs. All parameters are measured three times: the time before therapy application (baseline), after ten therapeutic procedures and after a month of therapy. The study was approved by the Institutional Review Board.

The sample size was calculated for two independent arms in order to detect the significant difference in VAS score between treatment groups. The data for primary variable were based on previous research and medical history database at our institution. The statistical analysis included descriptive statistics as well as the hypothesis testing for continuous or categorical variables (15), according to the intention-to-treat principle. There were no missing data for outcome variables. Before testing, the Kolmogorov Smirnov test was used to examine the normal distribution of the data and then parametric or non-parametric statistics were used depending on distribution pattern. In general, t-test, one/two-way ANOVA and Pearson chi-square were primarily used. The probability of p = 0.05 for all statistical calculations was selected.

RESULTS

Sixty patients were allocated in two equal groups, which were comparable according to the main demographic and clinical variables (table 1). The differences in frequency of the following parameters were not significant: age (Mann Whitney U-test; p=0.362), gender (χ²-test; p=0.259), side of the disease (χ²-test; p=0.436), occupation (χ²-test; p=0.495), working experience (t-test; p=0.250), body height (t-test; p=0.920), distance knee-floor (t-test; p=0.305), foot length (size) (Mann Whitney U-test; p=0.940), body weight (t-test; p=0.529), time of the maximal pain (χ²-test; p=0.313), seasonal pain pattern (χ²-test; p=0.206), family history (χ²-test; p=0.796), target muscle hypotrophy (χ²-test; p=0.001 and p=0.002), synovitis (χ²-test; p=0.071), crepitating joint (χ²-test; p=0.002), and palpable tenderness (χ²-test; p=0.313).

Table 1. Demography and clinical properties of the patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Experimental group (n=30)</th>
<th>Control group (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>65.27±10.79</td>
<td>61.53±13.6</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>8 (26.7%)</td>
<td>10 (33.3%)</td>
</tr>
<tr>
<td>Female</td>
<td>22 (73.3%)</td>
<td>20 (66.7%)</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unilateral arthrosis</td>
<td>15 (50%)</td>
<td>18 (60%)</td>
</tr>
<tr>
<td>Bilateral arthrosis</td>
<td>15 (50%)</td>
<td>12 (40%)</td>
</tr>
<tr>
<td>Retired</td>
<td>11 (36.7%)</td>
<td>12 (40%)</td>
</tr>
<tr>
<td>Unemployed</td>
<td>13 (43.3%)</td>
<td>15 (50%)</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Merchants</td>
<td>2 (6.7%)</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>Nurses</td>
<td>1 (3.3%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Clerks</td>
<td>2 (6.7%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Pupils</td>
<td>1 (3.3%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Working experience (years)</td>
<td>31.87±5.68</td>
<td>29.5±5.35</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>163.4±8.92</td>
<td>164.5±9.51</td>
</tr>
<tr>
<td>Body distances, knee-floor (cm)</td>
<td>49.58±2.75</td>
<td>48.03±2.16</td>
</tr>
<tr>
<td>Body distances, foot length (cm)</td>
<td>24.75±1.96</td>
<td>24.58±1.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79.47±12.54</td>
<td>77.6±10.17</td>
</tr>
</tbody>
</table>

The values shown: represent the mean ± standard deviation or the number of patients (percent).

The analysis of the variables between the groups was made in relation to the time of examination in order to evaluate the influence of corticosteroid iontophoresis on patient’s health. During the application of methylprednisolone acetate, statis-
tically significant reduction in pain has been noticed, which was evaluated according to VAS pain scale (Friedman test; p<0.001). In a group of patients treated with iontophoresis with distilled water statistically significant improvement has been also noted (Friedman test; p<0.001). However, average value of pain before the therapy in experimental group was significantly higher than in comparator group (Mann-Whitney U test; p=0.026). After 10 days of therapy the VAS scores were comparable between groups (Mann-Whitney U test; p=0.072). Finally, reduction of pain at the end of the study was significantly bigger in experimental than in control group (Mann-Whitney U test; p=0.000). Therefore, overall VAS scores in active treatment group were much lower than in comparator group indicating better treatment outcome (table 2, figure 1).

Table 2. VAS scale in the study patients.

<table>
<thead>
<tr>
<th>VAS</th>
<th>Experimental group (n=30)</th>
<th>Control group (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>At baseline</td>
<td>7.67±1.9</td>
<td>7.1±1.35</td>
</tr>
<tr>
<td>After 10 days</td>
<td>5.33±0.96</td>
<td>6±1.51</td>
</tr>
<tr>
<td>After 30 days</td>
<td>4.37±1.13</td>
<td>5.77±1.36</td>
</tr>
</tbody>
</table>

Apart from reduction of pain after application of iontophoresis with methylprednisolone, functional improvement was also achieved; Hubertus test showed positive therapeutic effect in both groups (Friedman’s test; p<0.001) (table 3, figure 2). In experimental group, the values of the test, recorded before the therapy, were significantly lower than in control group (Mann Whitney U test; p=0.002). However, after 10 days of the therapy the difference between groups was not significant (Mann Whitney U test; p=0.693) as well as in the next 20 days (Mann Whitney U test; p>0.05).

Table 3. Values of Hubertus test in study subjects.

<table>
<thead>
<tr>
<th>VAS</th>
<th>Experimental group (n=30)</th>
<th>Control group (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>At baseline</td>
<td>23.73±2.3 (23)</td>
<td>26.2±3.08 (27)</td>
</tr>
<tr>
<td>After 10 days</td>
<td>29.93±2.98 (30)</td>
<td>30.37±3.76 (30)</td>
</tr>
<tr>
<td>After 30 days</td>
<td>30.63±2.92 (30)</td>
<td>29.57±6.75 (30)</td>
</tr>
</tbody>
</table>

The results of the test of femoral muscle strength as well as the magnitude of the affected knee contracture are showed in detail bellow, in tables 4 and 5 as well as in figures 3 and 4. The femoral muscle strength was significantly improved in the experimental group, (Friedman’s test; p=0.018), but not in the control group (Friedman’s test; p=0.097). The difference was noted after 10 days (Mann Whitney U test; p=0.012), and continued throughout the study (Mann Whitney U test; p=0.045).

Table 4. The values of the test for quadriceps femoral muscle.

<table>
<thead>
<tr>
<th>VAS</th>
<th>Experimental group (n=30)</th>
<th>Control group (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>At baseline</td>
<td>3.77±0.43</td>
<td>3.63±0.49</td>
</tr>
<tr>
<td>After 10 days</td>
<td>3.93±0.26</td>
<td>3.67±0.48</td>
</tr>
<tr>
<td>After 30 days</td>
<td>3.93±0.26</td>
<td>3.73±0.45</td>
</tr>
</tbody>
</table>

Frequency of subjects who gave the data about dose reduction of NSAIDs, in the period from 10th to 30th day from administration of the therapy, was significantly different between the tested groups (χ²-test; p<0.001).
During iontophoretic therapy administration, in the group with methylprednisolone acetate the dose reduction of NSAIL was found in 76.7% of the subjects, while in the group where distilled water was used, reduction of the drug dose was found in 20% of the patients, only. The difference was statistically significant ($\chi^2$-test; $p<0.001$).

During the study and in the follow up period adverse events, related to methylprednisolone (corticosteroid local or systemic effects) or iontophoresis itself like burns, or formation of undesirable vesicles and bullae in skin were not recorded.

Reduction of NSAIDs use and doses during the study (after 10 and 30 days) in the control and the experimental group were show in the figure 5 and 6.

### DISCUSSION

Iontophoresis augments penetration of electrically charged drugs through skin by administration of electric current. The two prerequisites for the treatment are: preparation of sufficiently charged drug in sufficient amount, and localisation of the disease at or near the body surface. Our results clearly point to valuable clinical advantages of methylprednisolone iontophoresis. Although our experimental drug, methylprednisolone acetate, was exceptionally used during iontophoresis due to difficulties of skin penetration of its suspensions (6), low water solubility and modest electrical behaviour (8), we confirmed its clinical utility. During application of methylprednisolone, statistically significant reduction in pain has been noted, much more than with placebo, as evaluated with VAS pain scale. It is known that, apart from characteristics of the drug, many other factors affect iontophoresis such us the current, formulation factors, biological factors and electrical and endo-osmotic flow (7, 16). Therefore, it is very likely that the properties of the pharmaceutical preparations of methylprednisolone acetate used in our study contribute to its utility recorded in our study. Some of the factors which might add to electrochemical behaviour of the preparation are: drug concentration, pH, ionic strength, and viscosity.

In addition, it is very likely that synergistic effects of drug and electrical current have been recorded. The significant part in reduction of the clinical symptoms might be, in fact, the effect of galvanic current itself. It is known that this physical agent has analgesic effect, especially „the anode galvanization”; positive pole of the electrode releases oxygen, makes acid reaction, vasoconstriction, produces analgesia and reduces bleeding and osmotic pressure (17).

Besides reduction of pain after therapeutic application of iontophoresis with methylprednisolone, functional improvement was also made. Although average value of Hubertus test was lower in control subjects at the end of 30-day period of testing, the difference was not statistically significant. Analysis of other clinical variables also support superiority of active treatment in comparison with the control. The patients treated with methylprednisolone experienced better quadriceps muscle strength and less contracture of the affected knee than patients treated with placebo.
Particular positive therapeutic effect of this methodology was reduction of NSAIDs doses after ten days. Frequency of subjects who reduced dose of NSAIDs was significantly different between the groups. In actively treated patients the dose reduction of NSAIL was found in about three quarter of subjects which is far more than in control group where the doses were reduced in a fifth of patients.

In conclusion, our results show that methylprednisolone acetate was superior to placebo when applied with iontophoretic method in the patients suffered from knee arthritis. Several subjective and objective parameters of disease activity were improved more by active treatment than by sham iontophoresis. The further, large-scale, randomized clinical studies should confirm our results before introduction of this method in routine practice.

REFERENCES


CLINICAL IMPORTANCE OF BIOCHEMICAL MARKERS OF CARDIAC DAMAGE IN HEMODIALYSIS PATIENTS

Dejan Petrovic1, Nikola Jagic2, Vladimir Miloradovic3, Biljana Stojimirovic4

1Center for Nephrology and Dialysis, Clinic for Urology and Nephrology, 2Department for Interventional Radiology, Center for Radiology Diagnostics, 3Department for Cardiology, Clinic for Internal Medicine, Clinical Center „Kragujevac“, Kragujevac; 4 Clinic for Nephrology, Institute for Urology and Nephrology, Clinical Center of Serbia, Belgrade, Serbia

KLINIČKI ZNAČAJ BIOHEMIJSKIH MARKERA SRČANOG OŠTEĆENJA KOD PACIJENATA NA HEMODIJALIZI

Dejan Petrovic1, Nikola Jagic2, Vladimir Miloradovic3, Biljana Stojimirovic4
1Centar za nefrologiju i dijalizu, Klinika za urologiju i nefrologiju, 2Odsek za interventnu radiologiju, Centar za radiološku diagnostiku, 3Odeljenje kardiolijek, Klinika za internu medicinu, Klinički centar „Kragujevac“, Kragujevac; 4Institut za urologiju i nefrologiju, Klinički centar Srbije, Beograd, Srbija

ABSTRACT

Cardiovascular diseases are the most frequent cause of morbidity and mortality in patients on regular hemodialysis. Cardiovascular mortality in this patients subset is approximately 9% per year, and among cardiovascular complications, the left ventricle hypertrophy, ischemic heart disease and congestive heart failure are the most prevalent. Risk factors for atherosclerosis and cardiovascular complications in hemodialysis patients are: high blood pressure, lipid metabolism disorder, oxidative stress, microinflammation, hypoalbunemia, anemia, hyperhomocysteinemia, high concentration of asymmetric dimethylarginine-ADMA and secondary hyperparathyroidism. Diagnostic strategy for early detection of patients with higher risk for cardiovascular complications should include the following: tests for cardiovascular risk factors detection (homocysteine, ADMA), tests for estimation of microinflammation, coronary artery plaque instability and vulnerability risks (CRP), tests for detection of markers of ischemia and damage of cardiac tissue, (cTnT, cTnl), as well as myocardial function tests (ANP, BNP, NT-proBNP). Precise detection of the most sensitive of high risk for cardiovascular complications enables right timing for adequate therapeutic strategy, which means high degree of survival of the patients with end stage of renal disease.

Key words: renal dialysis, cardiovascular diseases, morbidity, mortality, diagnosis

INTRODUCTION

Cardiovascular diseases are the most frequent cause of morbidity and mortality in patients on regular hemodialysis. Annual cardiovascular mortality in these patients is approximately 9% (1, 2), and among cardiovascular complications, the most prevalent are left ventricle hypertrophy, ischemic heart disease and congestive heart failure (1–6). Risk factors for cardiovascular complications in hemodialysis patients are: high blood pressure, lipid metabolism disorder, oxidative stress, microinflammation, hypoalbunemia, anemia, hyperhomocysteinemia, high concentration of asymmetric dimethylarginine-ADMA, high blood flow through the vascular access for hemodialysis and secondary hyperparathyroidism (table 1) (6–17).

| Table 1. Cardiovascular risk factors in hemodialysis patients. |
|-------------|-----------------|-----------------|
| CATEGORY | RISK FACTORS |
| TRADITIONAL | Cigarette Smoking, Hypertension, Hyperlipidemia, Diabetes mellitus, Obesity |
| HEMODYNAMIC | Arterial Retention of Na+ and H2O, AV fistula QAV > 1000 ml/min |
| NON TRADITIONAL | Metabolic | Hyperparathyroidism |

Modified according to reference (3).

Received/Primljen: 11. 12. 2007. Accepted/Prihvaćen: 27. 02. 2008.

SAŽETAK

Kardiovaskularne bolesti su najčešći uzrok morbiditeta i mortaliteta bolesnika koji se leče redovnim hemodializama. Stopa kardiovaskularnog mortaliteta kod ovih bolesnika iznosi približno 9% godišnje, a među kardiovaskularnim komplikacijama najveća je prevalencija hipertrofije leve komore, ishemijske bolesti srca i konjestivne srčane slabosti. U faktore rizika za razvoj ateroskleroze i kardiovaskularnih komplikacija kod bolesnika na hemodializaji spadaju: povišen arterijski krvni pritisak, poremećaj metabolizma lipida, oksidativni stres, mikroinflamacija, hipoalbunemija, anemija, hiperhomocisteinemija, povećana koncentracija asimetričnog dimetilarginina-ADMA i sekundarni hiperparatihoidizam. Diagnostička strategija za rano otkrivanje bolesnika sa povećanim rizikom za razvoj kardiovaskularnih komplikacija treba da uključi: testove za određivanje faktora kardiovaskularnog rizika (homocistein, ADMA), testove za procenu mikroinflamacije, nestabilnosti plaka koronarnih arterija i rizika njegovog prskanja (CRP), testove za određivanje pokazatelja ishemijske i oštećenja srčanog tkiva (cTnT, cTnl), kao i testove za određivanje pokazatelja funkcije miokarda (ANP, BNP, NT-proBNP). Utvrđivanje najosjetljivijih parametara visokog rizika za razvoj kardiovaskularnih komplikacija omogućava pravovremenu primenu odgovarajuće terapijske strategije, koja obezbeđuje visok stepen preživljavanja bolesnika sa završnim stadiumom tronične slabosti bubrega.

Ključne reči: hemodializa, kardiovaskularne bolesti, morbiditet, mortalitet, dijagnoza

LITERATURE REVIEW PREGLED LITERATURE LITERATURE REVIEW PREGLED LITERATURE
Metabolically active folate, 5-methyl tetrahydrofolate (5-MTHF), is included in the folate cycle. Re-methylation process has two ways: by re-methylation process or by trans-sulfuration. Homocystein, which can further be metabolized in two ways, is between 15–30 μmol/L, and true hyperhomocysteinemia is considered if plasma concentration of homocystein is above 100 μmol/L (23).

More than 80% of patients on hemodialysis has elevated plasma concentration of homocystein (16, 24). Hyperhomocysteinemia blocks activity of dimethylarginine dimethylhydrolase-DDAH enzyme, which has a specific role in the process of degradation of asymmetric dimethylarginine and contributes in accumulation of ADMA in endothelial cells and triggering of atherosclerotic process (21–26).

Hyperhomocysteinemia is the risk factor for atherosclerosis and cardiovascular complications in patients on hemodialysis (21–26).

Whole homocystein plasma concentration is independent predictor of cardiovascular mortality in patients on regular hemodialysis. Patients on hemodialysis with homocystein plasma concentration ≥ 37.8 μmol/L have 8.2 fold greater risk for cardiovascular mortality comparing to homocystein blood concentration bellow 22.9 μmol/L (27).

Asymmetric dimethylarginine is a result of degradation of methylated proteins (figure 3). Methylation of the arginine residues inside different proteins and/or polypeptides is done by means of N-methyltransferase I and II (methylase I and II). S-adenosylmethyonine serves as a methyl groups donor for the process of methylation of arginine residues inside different proteins and/or polypeptids is done by means of N-methyltransferase I and II (methylase I and II). S-adenosylmethyonine serves as a methyl groups donor for the process of methylation of arginine residues of proteins. As a result of methylating of arginine residues become S-adenosyl-L-homocystein. (SAH) and methylised proteins (proteins that contain ADMA) (9–12). Enzyme protein arginine methyltransferase I (PRMT I) takes part in the processes of asymmetric dimethylarginine-ADMA synthesis. By hydrolysis of methylated proteins ADMA is liberated. Asymmetric dimethylarginine is the most important endogenous blocking substance of Nitrous oxyde-NO synthesis in endothelial cells (eNOS) (9–12). In healthy population, normal concentration of ADMA in plasma is ≤ 1.0 μmol/L, in patients on hemodialysis ≤ 2.2 μmol/L, and if in concentrations between 3–15 μmol/L ADMA is blocking NO synthesis in endothelial cells and triggers the process of atherosclerosis. (12). Accumulation of ADMA in endothelial cells secondary leads to malfunction of the system of L-arginine/NO (9–12).
Main path of degradation of ADMA is processed by means of enzyme dimethylarginine dimethylhydrolase-DDAH. Upon the action of this enzyme ADMA is degrading till dimethylamine and L-citrulin (28). In hemodialysis patients elevated ADMA concentration is due to diminished activity of DDAH enzyme. Oxydative stress, microinflammation and hyperhomocysteinemia considerably diminish activity of this enzyme and elevate concentration of ADMA (28). Upon the enzyme aminotransferase dimethylarginine piruvate-DPT, one part of ADMA is metabolised into α-keto acids (28).

Patients on hemodialysis with left ventricle hypertrophy have highly significant statistically elevated plasma ADMA concentration comparing to the patients with normal left ventricle mass (10). By multivariant analysis it is proved that ADMA is independent risk factor for left ventricle hypertrophy (10). In hemodialysis patients ADMA is strong predictor of cardiovascular complications development and overall mortality (11). Every one μmol/l rise of ADMA in plasma is followed by overall risk mortality rise of 26% (11).

Microinflammation is independent risk factor for cardiovascular complications in patients on hemodialysis (29). Local and systemic inflammation have important role in pathogenesis of acute coronary syndrome. Inflammatory process has important role in prediction of plaque perspective, e.g. plaque stability. C-reactive protein, reactant of acute phase of inflammation, has important role in atherosclerosis process, progression and rupture of atherosclerotic plaque (29). Normal concentration of CRP in plasma is ≤ 5 mg/L, and concentration of CRP > 10 mg/L expresses elevated risk of development of cardiovascular complications in patients on hemodialysis (29).

Highly-sensitive CRP (hsCRP), serum amyloid A-SAA and other reactants of acute phase of inflammation and/or cytokines are used as a markers of inflammation and predictors of development of cardiovascular complications in hemodialysis patients (29, 30). Between hsCRP concentration and risks for coronary artery disease, there is statistically significant relation (29–31).

**Tests for estimation of plaque instability and risk of its rupture**

Elevated concentration of soluble CD-40 ligand indicates aggravated prothrombotic activity and possibility of development of coronary thrombosis. After thrombocyte activation there is significant rise and liberation of soluble fragments-CD40 ligands which express prothrombotic activity (sCD40L). Elevated concentration of sCD40L enables recruitment of certain subset of patients with elevated risk of acute coronary syndrom (18–21, 32).

(Myeloperoxidase)-MPO is an enzyme secreted by different inflammatory cells, including activated neutrophils and monocytes/macrophages, present in atherosclerotic plaques. Elevated myeloperoxidase concentration in serum is a predictor of development of acute coronary syndrome (18–21, 32).

Elevated activity of phospholipase D and choline liberation in plasma, is connected with atherosclerotic plaque rupture and onset of acute coronary syndrome Elevated concentration of choline in plasma, in patients with normal concentration of cardiac troponins, enables recruitment of certain subset of patients with elevated risk of unstable angina pectoris (18–21, 32).

Plasma Protein A bonded with pregnancy - PAPP-A is a glycoprotein of high molecular weight (200 kD), which is synthesised in syncyto-trophoblasts. Presence of this protein is proved in unstable atherosclerotic plaque of coronary arteries, and elevated concentration in plasma is a warning sign of possible development of acute coronary syndrome (18–21, 32).

**Tests for markers of ischemia and cardiac tissue damage**

Free fatty acids-FFAs in blood of patients with acute myocardial ischemia show early signs of myocardial damage. Albumin modified by ischemia-IMA (ishoemia-modified albumin) is another marker of early myocardial damage (18–21, 32).

Traditional enzymes, like CK and LDH, due to their high molecular weight (84 kD and 144 kD) do not penetrate membrane until the myocytes aren’t irreversibly destructed. (monophasic excretion) (19, 20, 32). Creatine kinase-CK is dimer composed of M and/or B subunits (CK-MM, CK-MB, CK-BB isoenzymes). Isoenzyme CK-MM is mostly in striated skeletal musculature (97% of whole CK) (32, 33). Isoenzyme CK-MB is mostly found in heart muscle, and accounts for 15–40% of total activity of Creatin kinase (32, 33). An insignificant amount of CK-MB is present in striated muscles as well (2–3% of total creatine kinase activity). Isoenzyme CK-BB is mostly present in brain, colon, ileum, stomach and urinary bladder (32, 33). Activity of whole creatine kinase-CK in plasma and concentration of isoenzyme CK-MB rise after 4–6 hours of myocardial damage, reaching the peak concentration after 12–24 hours, and after 48–72 hours it is getting back to normal values (32, 33). Isoenzyme MB creatine kinase (CK-MB) is more sensitive marker of myocardial damage than whole CK, but this isoenzyme concentration can be elevated after strited muscles damage as well (32, 33). Ratio CK-MB/total CK above 5% suggests myocardial infarction S(CK-MB/CK) x100 (%)C. Concentration of total creatine kinase-CK > 232 U/L and CK-MB > 16 U/L, as well as ratio CK-MB/CK > 5% suggests acute myocardial infarction (33). There are two CK-MB isoenzymes of creatine kinase: CK-MB1 and CK-MB2. In normal blood CK-MB isoenzymes are equally distributed, in ratio 1:1. Substantial CK-MB2:CK-MB1 ratio changes 2–4h after myocardial damage. CK-MB2:CK-MB1 ≥ 1.5 ratio is used as a diagnostic criterion of myocardial damage (34, 61).
CK-MB isoenzymes normalizes after 18–30h. Normal ratio of isoenzymes CK-MB2:CK-MB1 = 1, which stays still after 6h of the onset of chest pain, excludes the diagnosis of myocardial infarction (32, 33). But, in hemodialysis patients activity of CK-MB after myocardial damage is not fully reliable. Activity of CK-MB can be elevated in 5–50% in hemodialysis patients even in the absence of cardiac symptoms or any data on cardiac damage (34). Isoenzymes LDH, as α-HBDH (a-hydroxybutyrate dehydrogenase) and LDH isoenzyme 1, are more specific in diagnostic of myocardial damage compared to whole LDH (32, 33).

Myoglobin is protein of 17 kD of molecular weight, being in cytoplasm of heart and striated myocytes, and is easily liberated after cellular damage (33). Its concentration in blood elevates after 2–3 hours after myocardial damage. According to ESC/ACC (European Society of Cardiology/American College of Cardiology) myoglobin concentration and CK-MB in blood are used as early markers of myocardial damage (32, 33). Distribution of carbonic anhydrase III is limited to skeletal muscles, and its use in combination with myoglobin rises sensitivity of myoglobin in diagnostics of myocardial damage. Elevated ratio myoglobin/carbonic anhydrase III stresses myocardial damage (32, 33). Myoglobin concentration in blood is not used in routine clinical work (32, 33).

Cardiac troponins (cTnT and cTnI) mark myocardial cell destruction (20, 21). Complex of troponins consists of troponin-C (cTnC), troponin T (cTnT) and troponin I (cTnI), and its main function is regulation of contractility of heart muscle (33). Cardiac troponin I (cTnI) (molecule weight of 26 kD) blocks activity of activinomyosine ATP-ase. Troponin C (cTnC) (molecular weight 18 kD) is binding for cTnI, opposing the inhibiting effect of cTnI, and serves as a place for calcium binding, inevitable for the process of contraction. Troponin T (cTnT) (molecular mass of 39 kD) stabilises complex cTnC/cTnI and binds for actyn-myosyn filament (34, 61). A great deal of cardiac troponins (cTnI and cTnT) intracellularly are attached to myofibrilles, and small amount is free (6–8% cTnT and 3–4% cTnI). Cardiac troponins are excreted in phase of reversible (citrosolc form) and irreversible (citrosolc and structure form) myocardial ischemia and enables early detection of minimal myocardial damage. According to guidelines of ESC/ACC (European Society of Cardiology/American College of Cardiology) cardiac troponins are used as markers for evaluation of acute coronary syndrom, due to higher sensitivity and specificity compared to other markers (table 2) (32, 33).

Table 2. Characteristics of markers of cardiac damage.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Molecular weight</th>
<th>Early detection</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein-FA</td>
<td>12 kD</td>
<td>1.5 - 2.0 h</td>
<td>8 - 12 h</td>
<td>++</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>16 kD</td>
<td>1.5 - 2.0 h</td>
<td>8 - 12 h</td>
<td>++</td>
</tr>
<tr>
<td>CK-IAB</td>
<td>83 kD</td>
<td>2.0 - 3.0 h</td>
<td>1 - 7 days</td>
<td>+++</td>
</tr>
<tr>
<td>Troponin I</td>
<td>33 kD</td>
<td>3.0 - 4.0 h</td>
<td>2 - 14 days</td>
<td>+++</td>
</tr>
<tr>
<td>Troponin T</td>
<td>38 kD</td>
<td>3.0 - 4.0 h</td>
<td>2 - 14 days</td>
<td>+++</td>
</tr>
<tr>
<td>CK</td>
<td>96 kD</td>
<td>4.0 - 6.0 h</td>
<td>2 - 3 days</td>
<td>+++</td>
</tr>
<tr>
<td>sGOT</td>
<td>103 kD</td>
<td>6.0 - 10.0 h</td>
<td>2 - 3 days</td>
<td>+++</td>
</tr>
<tr>
<td>LOM</td>
<td>125 kD</td>
<td>6.0 - 10.0 h</td>
<td>2 - 3 days</td>
<td>+++</td>
</tr>
</tbody>
</table>

* hours after the symptom onset, CK=creatinine kinase, LDH-lactate dehydrogenase, sGOT-glutamate oxaloacetate transaminase.

Elevated concentration of cardiac troponins is found in as much as 40% of patients on hemodialysis, without symptoms of acute coronary syndrome (34–38). In hemodialysis patients troponin T elevation can be due to left ventricle hypertrophy, systolic dysfunction of left ventricle, voluminous left ventricle and myocardial stretching, coronary microcirculation disturbance, endothelial dysfunction, oxidative stress and microinflammation, episodes of hypotension during hemodialysis, myocardial damage due to calcium and oxalate precipitation, and/or disturbance in troponin fragmentation, as a result of chronic kidney weakness or inadequate hemodialysis (34–38).

In patients on regular hemodialysis, cardiac troponin T (cTnT), compared to troponin I (cTnI), is more sensitive marker of subclinical damage of myocardial cells (“minimal myocardial damage”-MMD) and proved as a better predictor of overall cardiovascular mortality (39). Patients on hemodialysis with troponin T concentration >0.10 ng/mL express statistically lower survival rate compared to patients with troponin T concentration <0.03 ng/mL (40). Correlation of cardiac troponin T with left ventricle mass indicates importance of this parameter in depiction of patients with left ventricle hypertrophy and systolic function disturbance (41). Patients with cardiac troponin T concentration > 55 ng/L in serum, have 3.47 fold higher risk of left ventricle hypertrophy, while patients with concentration of cTnT > 150 ng/L have 3.30 fold higher risk of left ventricle systolic function disturbance, compared to patients with concentration of troponin T < 150 ng/L (41). Concentration of cardiac troponin T in serum can serve as a reliable screening parameter for estimation of morphology and function of left ventricle in clinically stable hemodialysis patients (41). Between concentration of cardiac troponin T in serum, interventricular septum thickness, thickness of the posterior left ventricle wall and left ventricle mass, there is highly statistically significant positive correlation (42, 43). Patients with elevated concentration of cTnT (cTnT > 0.10 ng/mL) in serum have significantly higher left ventricle mass index compared to patients with normal concentration of cTnT (44).

Two year mortality in patients with concentration of cTnT < 0.01 ng/mL is 8.4%, 26% in patients with mild elevation of cTnT (cTnT ≥ 0.01 and cTnT < 0.04 ng/mL), 39% in patients with serum cTnT (≥ 0.04 and < 0.10 ng/mL), and 47% in patients with extreme elevation of serum cTnT (cTnT ≥ 0.10 ng/mL) (42). Patients with serum cTnT concentration ≤ 0.040 ng/mL have great survival rate, and significantly different compared to patients with concentration of serum cTnT (cTnT ≥ 0.10 ng/mL)(42, 45).

Cardiac troponin I has greatest importance in diagnostics of acute coronary syndrome. According to AHA (American Heart Association) Committee guidelines cardiac troponin I is used in diagnostics of myocardial damage in patients with unstable angina pectoris, without ST elevation. This enables diagnosis of myocardial damage before ECG registration of myocardial infarction (46, 47). After myocardial infarction cardiac troponin I can be detected in serum after 3–4 hours, and concentration remains elevated during 7–10 days (46, 47).

Troponin I is more sensitive marker of development of acute coronary syndrome compared to cTnT, and is used as a parameter for diagnostics and stratification of clinical difficulty of patients on hemodialysis developing acute coronary syndrome (48). Patients with concentration of troponin I - cTnI in serum ≥ 0.15 ng/mL are marked as a pos-
ative ones, while concentration of cTnl < 0.15 ng/mL is considered normal (34). Concentration of troponin I in serum > 0.8 ng/mL stresses marked damage caused by, and according to guidelines of ESC/ACC (European Society of Cardiology/American College of Cardiology) diagnosys of acute akiog myocard infarction includes concentration of cTnl ≥ 2.0 ng/mL, table 3 (48). Incidence of development of cardiovascular complications in patients on hemodialysis with concentration of cTnl > 0.15 ng/mL statistically highly significant compared to the group of patients with cTnl < 0.15 ng/mL (44). Patients with concentration of cTnl ≥ 0.3 μg/L have higher risk of ACS compared to the patients with a concentration of troponin I < 0.3 μg/L (49).

Table 3. Enzymes, isoenzymes, cardiac troponin I and their clinical importance in diagnosis of acute coronary syndrome.

Tests for detection of myocardial function markers

In population of patients without kidney disease, cardiac natriuretic peptides are the most important markers of left ventricle damage. These are used as a screening test for early detection of patients with asymptomatic left ventricle disturbance. Early detection of these patients enables timely treatment with angiotensin convertase I blockers and beta blockers, which both prevent congestive heart failure. In this population of patients natriuretic peptides are not used for prognosis and stratification of patients with congestive heart failure, but for the estimation of efficacy of applied therapy for congestive heart failure (50–52).

In patients with ESKD (End Stage Kidney Disease) on hemodialysis, natriuretic peptides (ANP, BNP, Nt-proBNP) have small sensitivity in early detection of patients with heart failure. High prevalence of disturbance of morphology of left ventricle (hypertrophy of left ventricle present in 75% of patients) and volume overload in interdialysis time, diminish diagnostic potential of BNP as a screening test in diagnostics of heart failure in these patients (52). In these patients BNP is independent predictor of death and left ventricle hypertrophy (53, 54). Patients on hemodialysis with concentration of BNP > 36.1 pmol/L (concentration of ANP > 34.8 pmol/L) have significantly lower death rate (overall and cardiovascular mortality) compared to the patients with concentration of BNP < 14.3 pmol/L, or concentration of ANP < 17.9 pmol/L (52, 53). Between concentration of BNP and Left Ventricle Mass index - LVMi there is statistically significant positive correlation (52–54). Serum BNP concentration is used for estimation of „dry” body mass in patients on hemodialysis (52).

Diagnostic strategy

End stage kidney disease is a situation with high risk of cardiovascular complications (55), and heart disease of these patients are leading cause of death in this population. Markers of early detection of myocardial damage (troponin I, troponin T) enable depiction of patients with high risk of acute coronary syndrom-ACS, which enables adequate therapy (platelet IIb/IIIa glycoproteins antagonists) (56, 57).

Pointing out the most sensitive parameters for detection of patients with high risk of cardiovascular complications enable proper timing for adequate therapeutic strategy, therefore making high survival rate in patients with end stage kidney disease (55–58). Biochemical markers play key role in diagnostics and therapy of the patients with ACS. Early depiction of myocardial ischemia in the absence of irreversible myocardial damage has a key role in prevention of ACS development. Exceptional importance belongs to the markers of early detection of myocardial ischemia/damage and to the markers of inflammation, coronary plaque instability and its rupture (18–21).

According to ESC/ACC (European Society of Cardiology/American College of Cardiology) Expert Committee guidelines, cardiac troponins (cTnT or cTnI) are used as a GOLD STANDARD in diagnostics of myocardial damage because of high specificity for heart tissue (34). Measuring concentration of cardiac troponins, cTn, in serum enables depiction of the subset of patients with elevated risk of main cardiovascular complications (34).

In patients with ESKD the use of multiple biomarker monitoring is inevitable for prediction of the outcome. C-reactive protein, homocystein, BNP and ADMA are high risk markers of cardiovascular complications in patients with ESKD (58–62). Simultaneous measurement of CRP and cTnl enables depiction of hemodialysis patients with elevated cardiovascular risk, in whom additional diagnostic monitoring and aggressive cardiovascular risk factor correction are necessary (58–62).

Primary strategy for lowering of cardiovascular mortality rate in hemodialysis patients should include antiaggregation therapy (Aspirin tabl. 100 mg/d), statins and beta-blockers, while secondary strategy includes coronary revascularization and percutaneous cardioverter defibrilator implantation (PCDs) (56).

Early detection of ESKD patients with high risk of cardiovascular complications enable adequate and timely therapy, thus lowering cardiovascular mortality rate and improving quality of life in these patients (62, 63).
REFERENCES


Ocular manifestations of chronic sarcoidosis

Svetlana Jovanović¹, Miroslav Vukosavljević², Milenko Jovanović³, Anka Stanojević Paović⁴
¹Clinic of Ophthalmology, Clinical Centre Kragujevac, Kragujevac, ²Center of Ophthalmology, Military Medical Academy, Belgrade, ³Optika „Vid“, Kragujevac, ⁴Clinic of Ophthalmology, Clinical Centre Serbia, Belgrade, Serbia

ABSTRACT

To investigate manifestations and clinical course of ocular sarcoidosis, diagnosed in childhood and adulthood, and to describe characteristics of patients who develop it. All patients examined in the authors’ referral practices for ocular sarcoidosis diagnosed after the age of 10 were identified. A review of their historical, clinical, laboratory investigations, (hypercalcemia with hypercalciuria, elevated angiotensin-converting enzyme (ACE), and other diagnostic tools (bronchoalveolar lavage, tuberculin skin test, HIV serological test) and fluorescein angiographic features was made. There were 15 patients with a mean age at diagnosis of 47 years (range, 10–65) and mean follow-up of 6.1 years (range, 0–15). These patients manifested many signs typical of ocular sarcoidosis, including the bilateral nature of the disease, and mutton-fat keratic precipitates, Koepp and Busacca iris nodules, white clumps of cells (snowballs) in the anterior inferior vitreous, linear or patchy retinal periphlebitis presents as sheathing. Cystoid macular edema, retinal neovascularisation, disc edema, and optic nerve granulomas also occur. One patient had bilateral orbital granulomas that did require treatment. Total exudative detachment of the retina was seen in one eye. In some patients (35%) regular monitoring may be all that is required, as a significant proportion of patients will show spontaneous improvement. On average, patients lost 3.4 lines of visual acuity during the follow-up period. Recognition of sarcoidosis includes compatible radiological and clinical presentation with histological evidence of noninfectious and noncaseating epithelioid cell granulomas. In severe cases, systemic corticosteroid therapy always constitutes the first approach. However, in patients that are refractory to corticosteroids, methotrexate has shown the most potential as alternative treatment.

Key words: sarcoidosis, uveitis, macular edema

INTRODUCTION

Sarcoidosis is a multisystem granulomatous disorder of unknown origin defined by the presence of non-caseating epithelioid granuloma and accumulation of T lymphocytes (1). The disease is further characterized by bilateral hilar lymph node enlargement, pulmonary infiltration, skin or eye lesions, an increase in serum levels of ACE and lysozyme, polyclonal B-cell activation, and cutaneousergy.

Ocular involvement is present in approximately 25% of patients (2), with common manifestations being a granulomatous anterior uveitis, choriorretinal inflammation, or
y and interleukin (IL)-2, and low levels of expression of Th2 cytokines such as IL-4 and IL-5. IL-12, an important regulator of Th1 immune response, has also been found to be upregulated at sites of inflammation in sarcoidosis (4). The presence of Th1 cytokines leads to the production of tumor necrosis factor and IL-6 by macrophages, triggering a cascade of inflammatory reactions culminating in fibrosis (5).

PATIENTS AND METHODS

All patients examined in the author’s referral practices for ocular sarcoidosis disease diagnosed after age of 10 years were identified. We took care to exclude any patient with a retinal vascular condition that can mimic some aspects of ocular sarcoidosis. A retrospective review of the records was performed, and historical, clinical and fluorescein angiographic features were included.

To be included in this study, a patient had to have Koepe and Busacca iris nodules, white clumps of cells (snowball) in the inferior anterior vitreous, orbital granulomas, nodular granulomas in both the retina and choroid, irregular nodular granulomas along venules (candle-wax drippings), linear or patchy retinal periphlebitic sheathing. Cystoid macular edema, retinal neovascularisation, disc edema, and optic nerve granulomas also occur.

Laboratory investigations for sarcoidosis are not specific but may contribute indirectly to diagnostic and clinical monitoring. Serum analysis of patients with sarcoidosis may indicate lymphopenia, hypercalciuria and elevated angiotensin-converting enzyme (ACE) levels. Other diagnostic tools include: bronchoalveolar lavage (useful to exclude granulomatous infection), tuberculin skin test (useful to exclude tuberculosis), HIV serological tests (to exclude HIV infection). We found “taches de boogie” (6) in posterior eye segment in our patients with sarcoidosis and we divided them in two clinical patterns: Demographic and clinical features of the patients were summarized. We recorded medical history, patient age and gender. All patients received a comprehensive eye examination, which included best corrected visual acuity (VA) determined with illuminated ETDRS charts (Early Treatment Diabetic Retinopathy Study chart, log MAR VA chart), slit-lamp biomicroscopy, dilated ophthalmoscopy, and, when possible, bilateral fundus photography, fluorescein angiography (FA), visual filed testing.

Decrease in visual acuity (VA) was defined as a final VA of >2 lines below acuity at diagnosis, as measured on logMAR VA chart. Improvement in visual acuity (VA) was defined as a final VA of >2 lines better than the VA at diagnosis. Stable VA was defined as a final acuity within 2 lines of the acuity at diagnosis.

RESULTS

There were patients with ocular sarcoidosis, at an age mean of 47±years (range, 10–65). The average follow-up period was 6.1 years (range, 1–10). One patient suffered from thyroid disease, confirming the well-known association between sarcoidosis and autoimmune thyroid disease, no one had diabetes mellitus (table 1). Systemic hypertension was diagnosed and treated in 3 of 15 patients (7%). Fifty-two percent of patients were male, 50% manifested unilateral disease and 37% patient had a positive family history. Individual patient data can be seen in the table 1. Ocular inflammation preceded any systemic sign of sarcoidosis by more than 1 year in 6 (40%) patients. In 35% of patients, irregular monitoring may be all that is required. In 30% of patients spontaneous improvement will ensue. The most common presenting complaint was: decrease in vision in 9 patients (60%), 8 (53%) had floaters, 2 (12%) had protrusio bulbi, and 1 was asymptomatic. At diagnosis, 87% patients had VA 1.0 log MAR (Snellen equivalent 0.1) or better. Only 13% of patient (3 eyes) had a VA below 1.0 log MAR (Snellen equivalent 0.1) at diagnosis.

Anterior uveitis has been registered in 6 (30%) patients. Iris nodules have been reported in 15% of patients. Posterior sineschiae have been reported in 20%, cataract in 10%, and glaucoma in 33% of patients. Connal band keratopathy developed in 5% of patients, and was associated with hypercalcaemia in this case. Scleritis is a relatively rare manifestation. We did not have any patient.

Table 1. Patient data.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>47 (10–65)</td>
</tr>
<tr>
<td>Average follow up period (years)</td>
<td>6.1</td>
</tr>
<tr>
<td>Male/female</td>
<td>62%/38%</td>
</tr>
<tr>
<td>Unilateral disease</td>
<td>50%</td>
</tr>
<tr>
<td>Bilateral disease</td>
<td>50%</td>
</tr>
<tr>
<td>Positive family history</td>
<td>37%</td>
</tr>
<tr>
<td>Anterior uveitis</td>
<td>30%</td>
</tr>
<tr>
<td>Posterior sineschiae</td>
<td>22%</td>
</tr>
<tr>
<td>Cataract</td>
<td>10%</td>
</tr>
<tr>
<td>Glaucoma</td>
<td>33%</td>
</tr>
<tr>
<td>Corneal band keratopathy</td>
<td>5%</td>
</tr>
<tr>
<td>Vitritis</td>
<td>60%</td>
</tr>
<tr>
<td>Intermediate uveitis</td>
<td>36%</td>
</tr>
<tr>
<td>Panuveitis</td>
<td>5%</td>
</tr>
<tr>
<td>Retinal vasculitis</td>
<td>30%</td>
</tr>
<tr>
<td>Periphlebitis</td>
<td>50%</td>
</tr>
<tr>
<td>CMO</td>
<td>20%</td>
</tr>
<tr>
<td>ERD</td>
<td>5%</td>
</tr>
<tr>
<td>Haemorrhthalinus</td>
<td>5%</td>
</tr>
</tbody>
</table>

Sarcoidosis-associated posterior uveitis was usually chronic. Involvement of the posterior segment was seen in 70% of patients with ocular sarcoidosis, and it can be the sole manifestation of the disease in 5% patients. The most common manifestation of sarcoidosis involving the posterior segment are vitritis, occurring in 60%, intermediate uveitis in 36%, panuveitis in 5%, retinal vasculitis in 30% of patients. Periphlebitis is a hallmark, although not pathognomonic, of sarcoidosis and may be associated with yellow periveneous exudates and candle wax drippings in middle and far periphery. Cellular infiltration of the vitreous may occur in clumps (snow balls), in the inferior vitreous or in chains (string of pearls).

We find „taches de boogie” in 50% patients with sarcoidosis-associated posterior uveitis and we divided them in two clinical patterns. The first type was associated with vitritis, segmental venous „sheathing” or periveneous exudates in 6 (40%) patients (figure 1).

Small, discrete white spots occur in clusters around retinal
venules, often limited to inferior quadrant. Visual acuity was 0.19 log MAR, because cataract has been reported in 10%. Visual prognosis was better in patients with the latter type. The second type was characterized by yellow-orange lesion located at the level of the choroid, predominantly in the posterior and nasal parts simulating lesions of birdshot chorioretinopathy (figure 2). These are discrete and depigmented but not atrophic. They are not associated with retinal vasculitis or retinal vascular obstruction. Visual acuity was 0.1 log MAR and peripheral area in 2 eyes of 2 patients (13%), with peripheral disease in one patient. Macular edema was noted clinically and/or angiographically in three eyes. Exudative RD was seen in one eye. Haemorrhage was seen in 25% patients, haemoptalmus in one eye. Bilateral macroaneurisms were noted in one patient (one eye with solitary macroaneurism and the other had more than three). One eye had visual complaints due to exudation toward the macula; the remaining silent microaneurisms in other eye were diagnosed during regular ophthalmologic examinations. The aneurisms occurred at a time of low uveitis activity; none of the patients had received systemic corticosteroids or periocular steroid injections in the affected eye in the year before the macroaneurisms were noted. Peripheral capillary closure is a feature of sarcoidosis in 3 patients (20%).

Decrease in VA over the follow-up period was noted in 3 patients and 6 eyes (20%). Stable acuity was seen in 8 eyes (27%), and improvement in vision was noted in 3 (6 eye) patients (20%), with the mean change 3, 4 lines of visual acuity during extended follow-up period. Only 7 patients (46%) had a final VA 0.19 log MAR (Snellen equivalent, between 0.7 and 0.8). Although there is benefit of laser photocoagulation in patients with ocular sarcoidosis, only one patient in this series was treated like that. Relatively few symptoms in diagnosis of ocular sarcoidosis in children often lead to late presentation to eye specialist and, frequently, with already established pathology and significant decrease in visual acuity. Other less frequent complications of sarcoidosis-associated uveitis include branch vein occlusion in one patient. Neurosarcoidosis is described in one case. Patient had posterior uveitis in one eye and panuveitis in second eye.

In severe cases, bilateral chronic uveitis, systemic corticosteroid therapy always constitutes the first approach (42%). However, in patients that are refractory to, corticosteroids, methotrexate has shown the most potential as alternative treatment (18%).

We initiate methotrexate therapy with a weekly dose of 2.5 mg to 10 mg administered orally, intramuscularly, or intravenously, as either a single or divided dose, in a 36 to 48-hour period. The dose is escalated gradually as dictated by the clinical response to a maximum of 50 mg/week. Methotrexate has delayed onset of action, requiring 3 to 6 weeks to take effect. Complete hemograms, with platelet and differential values, should be obtained before the onset of therapy and at intervals of 1 to 4 weeks. Similarly, pretreatment liver function tests, urinanalysis, and serum creatinine should be obtained, and tests should be repeated every 3 to 6 weeks. We divided our patients with „taches de boogie” in two clinical patterns (table 2) (7). First group with segmental venous „sheathing” or perivenular exudates and discrete white spots around retinal venules, has visual prognosis worst than second group (figure 1). The second type is characterized by yellow-orange lesion located at the level of the choroids; predominantly in the posterior and nasal funds simulating the lesions of birdshot chorioretinopathy (figure 2). Visual acuity is better because of absence of retinal inflammation. Corticosteroids have been the mainstay of treatment for sarcoid-associated uveitis. The uveitis associated with sarcoidosis is usually mild and typically resolves with topical steroids and cycloplegics. For uveitis resistant to topical steroids and for posterior uveitis, neovascularization, and orbital disease with visual symptoms or optic nerve involvement, periorbital and/or systemic corticosteroids may be used. However, in cases of chronic disease, prolonged corticosteroid therapy is often poorly tolerated, necessitating other steroid-sparing medications (table 3). Immunomodulatory drugs have been used in conjunction with steroids to control inflammation in patients refractory to steroids alone to prevent the onset and progression of complications of chronic inflammation and of chronic steroid use.

<table>
<thead>
<tr>
<th>Type</th>
<th>„taches de boogie” II</th>
<th>„taches de boogie” II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yitis</td>
<td>40%</td>
<td>/</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>„venous sheathing” 30%</td>
<td>/</td>
</tr>
<tr>
<td>Exudates</td>
<td>Small discrete white spots – perivenular</td>
<td>Yellow-orange lesion (depigmented, not atrophic, at the choroid)</td>
</tr>
<tr>
<td>Field</td>
<td>Inferior quadrant</td>
<td>Posterior and nasal fundus</td>
</tr>
<tr>
<td>Complication</td>
<td>Cataract 10%</td>
<td>0.1 log MAR</td>
</tr>
<tr>
<td>Visual acuity</td>
<td>0.19 log MAR</td>
<td>0.1 log MAR</td>
</tr>
</tbody>
</table>

For sarcoid-associated uveitis resistant to topical steroids and for posterior uveitis, neovascularization, and orbital disease with visual symptoms or optic nerve involvement, periorbital and/or systemic corticosteroids may be used (table 3). Methotrexate (MTX) is an antimetabolite, effective in the management of several systemic inflammatory diseases, including a variety of ocular inflammatory diseases, refractory to steroids and requiring 3 to 6 weeks to take effect.
DISCUSSION

Because it may require several years of repeated testing to confirm a diagnosis of sarcoidosis, some of these patients initially carry a diagnosis of idiopathic panuveitis with a clinical suspicion on sarcoidosis as ocular inflammation preceded any systemic signs of sarcoidosis by more than one year. Sarcoidosis-associated posterior uveitis was usually chronic, with late onset of the disease.

Sarcidosis is relatively rare in children and is an uncommon cause of childhood uveitis (8). Ocular sarcoidosis diagnosed in childhood and adulthood presents bilaterally, with vasculitis, macular edema, and retinal neovascularisation. As 77%-95% may have anterior uveitis and those of age 8 -15 have the same rate as adults; routine eye exams to rule out sarcoidosis should be scheduled annually, said Dr. Om P. Sharma, M.D. of the University of Southern California Medical Center in Los Angeles CA (9).

Patients often present with good vision and do not have extensive areas of exudation. In ocular sarcoidosis patients, vasculitis is often located in the end of periphery, between the equator and the ora serrata, and also juxtamacular region in the vast majority. Retinal vasculitis is a major sign of posterior segment involvement in sarcoidosis, although it predominantly involves the veins (10).

Peripheral capillary closure is a feature of sarcoidosis but tuberculosis, Eales disease, and in rare instances, multiple sclerosis, Behçet syndrome, and slow-flow retinopathy may have similar picture. The ability to identify retinal vasculitis as ischemic by fluorescein angiography has important implications for management. The neovascular response may occur secondary to widespread capillary shut-up or as direct consequence of intraocular inflammation. It is important to identify the presence or absence of retinal ischemia in this situation, because the management is different. In the former case, laser photocoagulation may be indicated (although there is a risk of exacerbating macula edema), whereas in the latter, adequate immunosuppression will usually induce regression of neovascular response.

Haemorrhage, less common in typical ocular sarcoidosis in adult patients, and occurred in young patients, with the bleeding localized to neovascularisations. Ocular sarcoidosis in adults seems to advance more than it does in children, with the majority of patients reaching a stable final VA. One of our patients did develop exudative RDs, and no patients developed neovascular glaucoma, but one patient developed secondary corticosteroid glaucoma.

Clinical evolution of the majority of patients with sarcoidosis is spontaneously favorable without any organ dysfunction. Thus, they require no treatment but only regular monitoring over a period of approximately 6 months. Such monitoring should include clinical and ophthalmological examination, chest x-ray, pulmonary function tests, blood cell count, hepatic enzy-mology and serum creatinine, calcium and ACE levels.

The visual prognosis was related to the course of uveitis, being better in patients exhibiting the monophasic tipe. Exacerbations of granulomatous uveitis are often associated with an appearance of fresh iris or retinal nodules. Control of the uveitis is followed with complete disappearance of gran-ulomas.

REFERENCES

THE IMPORTANCE OF Nd: YAG LASER IRIDOTOMY IN THE THERAPY OF THE CLOSED ANGLE GLAUCOMA

Svetlana Paunovic1, Zora Stankovic2, Milan Paunovic3
1Clinic of Ophthalmology, Clinical Centre Kragujevac, Kragujevac, 2Clinic of Ophthalmology, Clinical Centre Serbia, Belgrade, 3Center of Pediatric Surgery, Clinical Centre Kragujevac, Kragujevac, Serbia

ZNAČAJ Nd:YAG LASER IRIDOTOMIJE U TERAPIJI GLAUKOMA ZATVORENOG UGLA

Svetlana Paunovic1, Zora Stankovic2, Milan Paunovic3
1Klinika za oftalmologiju, Klinički centar Kragujevac, Kragujevac, 2Klinika za oftalmologiju, Klinički centar Srbije, Beograd, 3Centar za dečju hirurgiju, Klinički Centar Kragujevac, Kragujevac, Srbija

ABSTRACT
The final goal of the survey was to make a comparison between the efficiency of the drug, surgical and laser-wise treatment in patients with PACG in order to reach: a) normalization of IOP, b) maintaining the useful visual sharpness, c) stabilization of optic nerve papilla, and d) stabilization of visual field deterioration.

The patients treated at the Clinic for Eye Diseases of the Clinical Hospital Centre in Kragujevac in the period from June 1, 2004 until June 1, 2006. There were 81 patients in total, diagnosed with PACG who had been selected for this study. They were treated with: 1) medication, 2) Nd: YAG laser iridotomy and 3) operation. Ophthalmology check-ups have been introduced every one to three months, the vision field being tested twice a year.

During the monitoring period of 24 months, no statistically significant difference occurred in terms of changes of the visual sharpness among the three groups of examinees. The best IOP regulation was achieved after a laser treatment (45%), followed by a surgical treatment (35%) while the weakest was recorded in patients treated with medication (20%). The percentage of the visual field loss was the biggest in patients treated with medication (55%), and then in those treated with the laser (25%) while the least one occurred in patients with the surgical treatment (20%).

The laser iridotomy was proved to be efficient in 80,4% of patients with PACG, while the non-reactive were subjected to trabeculectomy.

Apart from the great efficiency of the Nd:YAG laser iridotomy in regulating IOP in patients suffering from PACG, the advantages of this method lie in outpatient departments' maneuvering, local anesthetic, being easy to bear and short performance time.

INTRODUCTION
Glaucoma is a multifactorial optical neuropathy characterized by the loss of retinal ganglion cells and atrophy of the optical nerve (1). The primary angular glaucoma is one of the rarest illnesses in medicine whose appearance could be largely predicted according to anatomical predispositions. The mechanism of increasing IOP during the PACG is: a) pupillary block, b) block of the chamber angle of the root part of iris, c) edema of the ciliary body and its thrusting forward. The risk factors are: a) age, b) sex, c) race, d) family anamnesis. The predisposing factors are: a) relatively anterior position of the iris-lens diaphragm, b) shallow front chamber, and c) narrow entrance into the angle of anterior chamber.

The Neodymium YAG laser was introduced into a clinical practice in 1981. It is a photodisruptor that emits...
radiation in the vicinity of the infrared part of the electromagnetic spectrum having a wavelength of 1064nm. There is no thermal but only mechanical tissue damage. Being used for capsulotomy and iridotomy, while rarely being used for cutting the membrane in a vitreal surgery. The mere purpose of the peripheral laser iridotomy is to reestablish the communication between anterior and posterior chambers by creating a hole on the outer edge, or rim of the iris, in case that less than 180 degrees of the angle being closed by peripheral front synchialts (2,3).

The indications for the Nd: YAG laser iridotomy are: 1) a primary angular glaucoma: acute, intermittent and chronic; 2) the other eye in patients suffering from acute glaucoma; 3) narrow angles suitable for closure; 4) secondary angle closure with a papillary block, and 5) POAG with a narrow angle and the glaucoma formed by combined mechanisms.

Nowadays a laser iridotomy method appears to be a matter of choice, except for the cases where there is an extremely shallow frontal eye chamber, wound leaking or inflammation (3). It is convenient (when possible) to treat the patient at an outpatient clinic, without general anaesthesia. Therefore the patient is pain-free, since the photo-disruption is easily bearable and does not take too much time (4,5,6).

The Complications of the Nd:YAG laser iridotomy treatment: 1) burns of cornea, 2) burns of macula and the blur of lenses, 3) damage of blood vessels, 4) ablation of retina, 5) short-term IOP, 6) iridotomy closure, 7) pain and blurred vision, dazzle related sensations and diplopia, 8) frontal eye chamber bleeding, 9) iritis, 10) pigment scattering (7,8,9). A proliferative diabetic retinopathy is a contraindication for the Nd:YAG laser usage.

PATIENTS AND METHODS

The patients were treated at the Clinic for Eye Diseases of the Clinical Centre in Kragujevac, in the period from June 1, 2004 until June 1, 2006. There was 81 patient in total, diagnosed with PACG, who had been selected for this study. A detailed ophthalmology checkup was made which included: 1) measuring of the visual sharpness, 2) IOP measurement, 3) bio-microscope examination, 4) gonioscopy, 5) ophthalmoscope fundus checkup and the three- mirror Goldmann glass checkup, and 6) perimetry.

Depending on a clinical stage of their illness, these patients were treated with: 1) drugs, 2) laser treatment (Nd: YAG laser iridotomy) and 3) surgical methods. The ophthalmology checkups were conducted every one to three months. The visual field was examined twice a year. There as 21 patient treated with drugs, while 11 out of these 21 was treated with the laser. In total there as 51 patient treated with the laser, while 10 of them were submitted to surgery. There were 30 patients in total who were surgically treated. There as 81 patient in total. An average age of these patients was 62, while the age range was 50 to 75.

Conditions and indications for performing iridotomy with the Nd:YAG laser were: 1) transparent eye mediums in front of the target tissue, 2) the existence of the frontal eye chamber with the minimal depth, 3) a patient is obliged to remove his contact lenses in the case he wears them at least one day prior to the intervention, 4) a medical doctor must not use the fluorescein dye to paint the cornea or measure the IOP prior to the photodisruptive process.

The laser iridotomy technique is as following: 1) the pupil has to be in miosis provoked by pilocarpin drops, 2) a local anesthetic is instilled, 3) a special Abraham lens is inserted (the lens diameter of 66D is 10mm), 4) an iridotomy spot is chosen, 5) a beam is directed at the angle which is not perpendicular, in order to avoid burns of macula, 6) the used energy for majority of people is 4 to 8 mJ, 7) a local steroid is prescribed that week, as well as acetazolamide (2-3 days).

RESULTS

During the follow-up period of 24 months, no statistical difference had been recorded in terms of changes in the visual acuity among these three groups (p>0,05) (tables 1 and 2).

Table 1. Visual acuity before the treatment.

<table>
<thead>
<tr>
<th>Visual acuity</th>
<th>L+</th>
<th>Cpr.</th>
<th>Laser</th>
<th>Med</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>0.05</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>0.06</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>0.1</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>0.2</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>0.3</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>0.4</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>0.7</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>0.8</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>0.9</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>1.0</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 2. Visual acuity after the treatment.

<table>
<thead>
<tr>
<th>Visual acuity</th>
<th>L+</th>
<th>Cpr.</th>
<th>Laser</th>
<th>Med</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>0.05</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>0.06</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>0.1</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>0.2</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>0.3</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>0.4</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>0.7</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>0.8</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>0.9</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>1.0</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>

The best regulation of IOP was achieved after the laser treatment (45%), i.e. there is a highly significant difference in IOP after the treatment, between the laser (p<0,01), and surgery (35%); there was also significant difference in IOP after the surgical procedure compared to preoperative values (p<0,01). The worse effect was observed in patients who were submitted to medical treatment (20%); however there was still significant difference compared to the IOP values prior to medical treatment (p<0,05), figure 1.

Figure 1. The IOP values before and after the treatment.

The visual field loss was the most pronounced in the patients with the medical treatment (55%), then in those treated by the laser (25%), while the least one was observed in the patients treated surgically (20%). (figure 2). In the group of 51 patient who were laser treated, 7 of them experienced increase in IOP; 2 of them experienced the iridotomy closure while 1 experienced iritis. The frequency of complications was 19,6%.
In the group of 30 patients who were surgically treated one of them had the narrow frontal chamber, 3 of them had cataract, 1 had hyphaema and 1 had infection. The overall frequency of complication was 20%.

The laser iridotomy was proved to be efficient in 80.4% eyes with PACG.

**DISCUSSION**

Nd: YAG laser iridotomy has major importance in the PACG treatment, especially with the acute glaucoma (3). The laser iridotomy is recommended for the glaucoma phase in the moment when edema of the cornea and congestion disappear (after 48 hours). In the meantime a prophylactic laser iridotomy should be carried out on the other eye. (5) It is efficient in 80% eyes with PACG. It is vital to confirm the angle is open after the laser iridotomy and when the IOP is normal (6,7).

The best regulation of IOP is achieved after the laser treatment compared to the surgical and medical treatment. Significant differences in changes of the visual field in these three groups were not observed, which is also vital for this survey. This is one of the reasons why we tend to choose the laser method, when possible. Even if there is no regulation of IOP, we are left with the option of surgical treatment (trabeculectomy) in those patients. The patients with the low risk of glaucoma progression should be treated with drugs, and laser iridotomy is also acceptable; the patients with high risk of glaucoma progression should be treated with the laser iridotomy or surgically (8,9).

Apart from the great efficiency of the laser iridotomy in the IOP regulation in eyes with PACG, the advantages of this method lie in possibility of outpatient treatment, local anaesthesia instead of general one, comfortability and efficiency.

**REFERENCES**

INSERTION OF NASAL SEPTAL BUTTON IN THE TREATMENT OF SEPTAL PERFORATION: A CASE REPORT

Branislav Belić1, Jasmina Stojanović2, Snežana Arsenijević2, Ivan Milojević2, Ljiljana Tadić2, Stevan Stojanović2
1Clinic of Otorhinolaryngology, Faculty of Medicine, University of Pristina, 2Clinic of Otorhinolaryngology, Clinical Center Kragujevac, Kragujevac, Serbia

ABSTRACT
Nasal septal perforation etiology varies to a degree, but it is most commonly associated with septal surgery. Penetrant nasal injuries, septal hematoma, nasotracheal intubation, nasal septal abscess, tuberculosis, syphilis, lupus erythematosus, Wegener’s granulomatosis, sarcoidosis, etc., as well as neoplasm can result in perforation.

Symptomatic perforations are commonly treated, and one way observe formation of crust layers, obstructions, presence of coloured secretion, paranasal pain, and whistling during inspiration. The first step to be taken is treatment of the basic illness which caused the perforation. If conservative treatment do not yield any beneficial results, the next step is to close the perforation, either by means of surgical or nonsurgical procedures. The surgical treatment represents rather difficult endeavour, and it is associated with various complications and failures. There are cases when the surgical approach is contraindicated either due to the patient’s age, his or her general and/or local condition, or due to the patient’s refusal to undergo surgical intervention. One of the nonsurgical methods which either temporary or permanently reduces, the symptoms of the nasal septal perforation, is insertion of the nasal septal button or obturator.

We have described the case of a patient with large symptomatic nasal septal perforation, to whom, by applying Kelly and Lee method, we performed the insertion of one-piece silicone nasal septal button under local anesthesia. The method of the preparation and one-piece nasal septal button insertion, described by Kelly and Lee, represents a simple, quick, easy method which is also quite comfortable for the patient in cases of nonsurgical management of nasal septal perforations.

Key words: nasal septum, injuries, prostheses and implants

INTRODUCTION
The etiology of nasal septal perforations is most commonly associated with septal surgery, especially with previously applied method of submucosal nasal septal resection. Besides, an overdue mucous membrane cauterization in cases of hemorrhage, as well as intranasal cryosurgery may result in perforation. Penetrant nasal injuries, septal hematoma, nasotracheal intubation, nasal septal abscess, tuberculosis, syphilis, lupus erythematosus, Wegener’s granulomatosis, sarcoidosis etc., various inhalation irritants like cocaine or occupational exposure to caustic or other industrial substances (especially chronic acid), as well as neoplasm can result in the ulceration of the mucous mem-

branes and cartilage ischaemia, the final outcome of which is the perforation itself. There is a number of perforations the etiology of which is yet unknown, and therefore are classified as idiopathic.

Nasal septal perforations are the most commonly asymptomatic. These would be the perforations with solid epithelial edges, with no bare cartilage or bone, not large in size, or those which are back localized thus not exposed to the air current effect. The most common symptoms are production of crust layers, epistaxis, obstruction, coloured secretion, paranasal pain, and when less acute whistling while inhaling. There is certain number of patients for whom the above symptoms are not too unpleasant, while, on the
other hand, there are patients which are subject to severe medical hindrances due to the symptoms. Crusting may lead to nasal breathing impediments or to severe fetor.

The surgical treatment represents a rather difficult endeavour, and is associated with various complications and the failure (1), while, on the other hand, the existence of numerous surgical methods only suggests the fact that there are no right ones among the many. Nonsurgical treatment is mainly based on nasal irrigation of the cavities. One of the surgical methods which reduces nasal septal perforation symptoms is insertion of the silicone or acryl nasal septal button or obturator. It diminishes drying of mucous membrane caused by air current passage through the nose (2). The insertion of the button may not always be such a simple procedure, and can sometimes be highly unpleasant for the patient.

We have demonstrated the case of a patient with symptomatic nasal septal perforation, with whom we installed one-piece silicone nasal septal button (figure 1), by the procedure demonstrated by Kelly and Lee (3).

**THE CASE**

A 65-year old patient was admitted at the Otorhinolaryngology Clinic, Clinical Center, Kragujevac, in April 2007, due to the obstructions in the nasal breathing, constant presence of coloured secretion in the nasal cavities, occasional nasal hemorrhage, acute postnasal drainage, the impression of „irregular nasal air passages”, as well as insomnia. Prior to admission to the Clinic, the patient had nasal septal surgical procedure fifteen years ago. The discomfort started immediately upon the surgery and became almost regular and unbearable in the last two or three years. By clinical examination we established the presence of perforation, of an irregular shape, 2 x 2.5 cm in size in the middle third of the nasal septum with crusts on the peripheral edge. By endoscopic nasal examination in the left nasal cavity we diagnosed synechia in the valvular region, as well as in the area of the upper peripheral edge of perforation between the mucous membrane in the medial part of the nasal shell and mucous membrane of the nasal septum 1 cm in length. The presence of tumor process was excluded upon removing the crust from the peripheral area perforation, as well as from its immediate surroundings (figure 2). Laboratory and microbiological tests were normal, and skin prick test to the standard set of inhalatory allergens was negative. The ultrasound examination showed a regular status of maxillary and frontal sinuses. Because of cardiological and pulmonary problems the patient was diagnosed as highly risky of receiving general anesthesia. Given the above, we decided that the patient was to undergo the synechia resection with radio frequency knife, as well as to be subject to the insertion of silicone nasal septal button in order to close the perforation on the nasal septum.

Thirty minutes before the procedure the patient was given 15 mg of midazolam and 0.5 mg of atropine sulfate, i.m. We started with the epimucous anesthesia of both nasal cavities, utilizing four sterile gauze strips (15 cm in length, and 1 cm in width), submerged into 2% solution of tetracaine chloride. Two gauze strips were placed in each of the nasal cavities (one in the upper, and the other in the lower nasal portions). After 10 minutes the gauze strips were removed and the patient continued to receive anesthesia by infiltrating 8 mL of 2% lidocaine chloride. We infiltrated 4 mL of the anesthetic per cavity in the area surrounding synechia and peripheral edge. We subsequently performed the resection of the synechia by applying monopolar radio frequency knife (Dr OPPEL ST-501, Radio Frequency Surgical Unit, Sometech Corporation, USA), which we simultaneously used for hemostasis. The silicone nasal septal button was initially trimmed and
Postoperatively, providing ambulatory medical care, we treated the sore surfaces of the synechia trimmed areas till the process of epithelization was fully completed. The next fourteen days the patient was subjected to everyday inhalation with calcium pantothenate and 0.9% NaCl solution, upon which he was advised to wash the nasal cavities with hypertonic buffered solution (1 L drinking water with 15 mg of salt without additives and 3–5 mg of baking soda), at least two times a day.

In postoperative monitoring of the patient during the following months, the patient tolerated well the septal button, there were no signs of infection, he had less insomnia, nasal breathing was significantly improved, the nasal secretion was still present, yet to a considerable smaller degree. What caused certain discomfort to the patient was occasional formation of crust layers upon the septal button edge, as consequence of the button edge being ill-placed upon the nasal septum, so that secretion accumulated in the space between the septal button and septum, forming the crust layer.

DISCUSSION

Majority of the septal perforations are asymptomatic and therefore no treatment is required. The most frequent symptoms are the ones related to the size and position of the perforation (4). With less acute perforations, major symptom is whistling, while with more acute ones the crust layers and hemorrhage prevail. The larger the perforation and the more it is frontally localized, the more acute the symptoms are.

The first step to be taken is treatment of the basic illness, which initially resulted in the perforation. In that way it is possible to achieve natural closure by treating the perforation in a conservative fashion. If the conservative treatment method yields no beneficial results and if the perforation is accompanied with acute symptoms, the next step is to close the perforation by either surgical or nonsurgical procedure (5). There are numerous surgical methods in the treatment of perforations, some of which were less or more successful. There are authors who demonstrated the application of rotational mucosal flap of the lower nasal shell in two-stage procedure (6), those who demonstrated the application of labial-buccal flap (7), or the nasal mucosal flap. Many authors suggest the application of free grafts, either that of nasal shell, concha auricle, tragus auricle or radial forearm fascial free flap, as well as the application of avascular human dermal allograft. The most likely to be successful is application of composite grafts (8).

The main issue when discussing the surgical approach are difficulties arising when resolving the perforations in direct correlation with their size. Likewise, surgical failure is more probable in a perforation with large diameter (9). What we should have in mind is the fact that the larger the perforation the less is the surface of the available nasal mucous membrane which is also frail with damaged vessels, therefore unsuitable for any kind of manipulation (10). There are cases when the surgical procedure is contraindicated, either due to the patient’s age, his or her overall or local condition, or due to the patient refusal to undergo surgical intervention. In such cases, septal obturator can be applied either as a permanent or as a temporary means of repair. Great number of studies described implantation of nasal septal button from various materials, such as: rubber, acrylate, resin, silicon. The advantages of septal

Figure 4. Septal button after the first tie was secured.

Figure 5. Nasal septal button immediately after insertion.
button insertion would be the following: a relatively simple implantation technique, one-day treatment and local anesthesia in most of the cases. Although the Luff et al. (11) suggested that, despite the symptoms being decreased, nasal septal button is not well tolerated in 50% of the patients. More recent studies found high level of tolerance, with symptoms significantly improved, with no indications of infection or any major local discomfort (12, 13).

We also detected no indications of infection in our patient, as well as no signs of button intolerance, whereby there also occurred a significant symptom improvement. The only complaint he had was related to occasional crust formation in the space between the septal button and nasal septum. The problem arose most probably due to irregular shape of the perforation and its size, and in-placement of the septal button upon the mucous membrane with secretion and crust formation. With cases like that this would be quite beneficial to utilize custom nasal septal buttons, designed according to the shape of the perforation itself (14, 15).

In conclusion, the method of inserting one piece silicone nasal septal button, as described by Kelly and Lee, is a quick, simple, easily performable and, for the patient, comfortable method, for the nonsurgical management of nasal septum perforations.

REFERENCES

INSTRUCTION TO AUTHORS
FOR MANUSCRIPT PREPARATION

Serbian Journal of Experimental and Clinical Research is a peer-reviewed, general biomedical journal. It publishes original basic and clinical research, clinical practice articles, critical reviews, case reports, evaluations of scientific methods, works dealing with ethical and social aspects of biomedicine as well as letters to the editor, reports of association activities, book reviews, news in biomedicine, and any other article and information concerned with practice and research in biomedicine, written in the English.

Original manuscripts will be accepted with the understanding that they are solely contributed to the Journal. The papers will be not accepted if they contain the material that has already been published or has been submitted or accepted for publication elsewhere, except of preliminary reports, such as an abstract, poster or press report presented at a professional or scientific meetings and not exceeding 400 words. Any previous publication in such form must be disclosed in a footnote. In rare exceptions a secondary publication will acceptable, but authors are required to contact Editor-in-chief before submission of such manuscript. the Journal is devoted to the Guidelines on Good Publication Practice as established by Committee on Publication Ethics-COPE (posted at www.publicationethics.org.uk).

Manuscripts are prepared in accordance with „Uniform Requirements for Manuscripts submitted to Biomedical Journals” developed by the International Committee of Medical Journal Editors. Consult a current version of the instructions, which has been published in several journals (for example: Ann Intern Med 1997;126:36-47) and posted at www.icmje.org, and a recent issue of the Journal in preparing your manuscript. For articles of randomized controlled trials authors should refer to the „Consort statement” (www.consort-statement.org). Manuscripts must be accompanied by a cover letter, signed by all authors, with a statement that the manuscript has been read and approved by them, and not published, submitted or accepted elsewhere. Manuscripts, which are accepted for publication in the Journal, become the property of the Journal, and may not be published anywhere else without written permission from the publisher.

Serbian Journal of Experimental and Clinical Research is owned and published by Medical Faculty University of Kragujevac. However, Editors have full academic freedom and authority for determining the content of the journal, according to their scientific, professional and ethical judgment. Editorial policy and decision making follow procedures which are endeavoring to ensure scientific credibility of published content, confidentiality and integrity of authors, reviewers, and review process, protection of patients’ rights to privacy and disclosing of conflict of interests. For difficulties which might appear in the Journal content such as errors in published articles or scientific concerns about research findings, appropriate handling is provided. The requirements for the content, which appears on the Journal internet site or Supplements, are, in general, the same as for the master version. Advertising which appears in the Journal or its internet site is not allowed to influence editorial decisions.

Address manuscripts to:
Serbian Journal of Experimental and Clinical Research
The Medical Faculty Kragujevac
PO. Box 124, Svetozara Markovica 69
34000 Kragujevac, Serbia
Tel. +381 (0)34 33 55 72; Fx. +381 (0)34 30 68 00
E-mail: medicus@medicus.medf.kg.ac.yu

MANUSCRIPT

Original and two anonymous copies of a manuscript, typed double-spaced throughout (including references, tables, figure legends and footnotes) on A4 (21 cm x 29,7 cm) paper with wide margins, should be submitted for consideration for publication in Medicus. Use Times New Roman font, 12 pt. Manuscript should be sent also on an IBM compatible floppy disc (3.5”), written as Word file (version 2.0 or later), or via E-mail to the editor (see above for address) as file attachment. For papers that are accepted, Medicus obligatory requires authors to provide an identical, electronic copy in appropriate textual and graphic format.

The manuscript of original, scientific articles should be arranged as following: Title page, Abstract, Introduction, Patients and methods, Results, Discussion, Acknowledgements, References, Tables, Figure legends and Figures. The sections of other papers should be arranged according to the type of the article.

Each manuscript component (The Title page, etc.) should begins on a separate page. All pages should be numbered consecutively beginning with the title page.

All measurements, except blood pressure, should be reported in the System International (SI) units and, if necessary, in conventional units, too (in parentheses). Generic names should be used for drugs. Brand names may be inserted in parentheses.

Authors are advised to retain extra copies of the manuscript. Medicus is not responsible for the loss of manuscripts in the mail.

TITLE PAGE

The Title page contains the title, full names of all the authors, names and full location of the department and institution where work was performed, abbreviations used, and the name of corresponding author.

The title of the article should be concise but informative, and include animal species if appropriate. A subtitle could be added if necessary.

A list of abbreviations used in the paper, if any, should be included. The abbreviations should be listed alphabetically, and followed by an explanation of what they stand for. In general, the use of abbreviations is discouraged unless they are essential for improving the readability of the text.

The name, telephone number, fax number, and exact postal address of the author to whom communications and reprints should be sent are typed at the end of the title page.
ABSTRACT
An abstract of less than 250 words should concisely state the objective, findings, and conclusions of the studies described in the manuscript. The abstract does not contain abbreviations, footnotes or references.

Below the abstract, 3 to 8 keywords or short phrases are provided for indexing purposes. The use of words from Medline thesaurus is recommended.

INTRODUCTION
The introduction is concise, and states the reason and specific purpose of the study.

PATIENTS AND METHODS/MATERIAL AND METHODS
The selection of patients or experimental animals, including controls, should be described. Patients’ names and hospital numbers are not used.

Methods should be described in sufficient detail to permit evaluation and duplication of the work by other investigators.

When reporting experiments on human subjects, it should be indicated whether the procedures followed were in accordance with ethical standards of the Committee on human experimentation (or Ethics Committee) of the institution in which they were done and in accordance with the Helsinki Declaration. Hazardous procedures or chemicals, if used, should be described in details, including the safety precautions observed. When appropriate, a statement should be included verifying that the care of laboratory animals followed accepted standards.

Statistical methods used should be outlined.

RESULTS
Results should be clear and concise, and include a minimum number of tables and figures necessary for proper presentation.

DISCUSSION
An exhaustive review of literature is not necessary. The major findings should be discussed in relation to other published works. Attempts should be made to explain differences between the results of the present study and those of the others. The hypothesis and speculative statements should be clearly identified. The Discussion section should not be a restatement of results, and new results should not be introduced in the discussion.

ACKNOWLEDGMENTS
This section gives possibility to list all persons who contributed to the work or prepared the manuscript, but did not meet the criteria for authorship. Financial and material support, if existed, could be also emphasized in this section.

REFERENCES
References should be identified in the text by Arabic numerals in parentheses. They should be numbered consecutively, as they appeared in the text. Personal communications and unpublished observations should not be cited in the reference list, but may be mentioned in the text in parentheses. Abbreviations of journals should conform to those in Index Medicus. The style and punctuation should conform to the Medicus style requirements. The following are examples:

Article: (all authors are listed if there are six or fewer; otherwise only the first three are listed followed by "et al.")


The authors are responsible for the exactness of reference data. For other types of references, style and interjection, the authors should refer to a recent issue of Medicus or contact the editorial staff.

Non-English citation should be preferably translated to English language adding at the end in the brackets native language source, e.g. (in Serbian). Citation in old language recognised in medicine (eg. Latin, Greek) should be left in their own. For internet sources add at the end in small brackets ULR address and date of access, e.g. (Accessed in Sep 2007 at www.medf.kg.ac.yu). If available, instead of ULR cite DOI code e.g. (doi: 10.1111/j.1442-2042.2007.01834.x)

TABLES
Tables should be typed on separate sheets with table numbers (Arabic) and title above the table and explanatory notes, if any, below the table.

FIGURES AND FIGURE LEGENDS
All illustrations (photographs, graphs, diagrams) will be considered as figures, and numbered consecutively in Arabic numerals. The number of figures included should be the least required to convey the message of the paper, and no figure should duplicate the data presented in the tables or text. Figures should not have titles. Letters, numerals and symbols must be clear, in proportion to each other, and large enough to be readable when reduced for publication. Figures should be submitted as near to their printed size as possible. Figures are reproduced in one of the following width sizes: 8 cm, 12 cm or 17 cm, and with a maximal length of 20 cm. Legends for figures should be given on separate pages.

If magnification is significant (photomicrographs) it should be indicated by a calibration bar on the print, not by a magnification factor in the figure legend. The length of the bar should be indicated on the figure or in the figure legend.

Two complete sets of high quality unmounted glossy prints should be submitted in two separate envelopes, and shielded by an appropriate cardboard. The backs of single or grouped illustrations (plates) should bear the first authors last name, figure number, and an arrow indicating the top. This information should be penciled in lightly or placed on a typed self-adhesive label in order to prevent marking the front surface of the illustration.

Photographs of identifiable patients must be accompanied by written permission from the patient.

For figures published previously the original source should be acknowledged, and written permission from the copyright holder to reproduce it submitted.

Color prints are available by request at the authors expense.

LETTERS TO THE EDITOR
Both letters concerning and those not concerning the articles that have been published in Medicus will be considered for publication. They may contain one table or figure and up to five references.

PROOFS
All manuscripts will be carefully reviewed by the publisher desk editor. Only in case of extensive corrections will the manuscript be returned to the authors for final approval. In order to speed up publication no proof will be sent to the authors, but will be read by the editor and the desk editor.

Je nastavak: Medicus (Kragujevac) = ISSN 1450 – 7994
ISSN 1820 – 8665 = Serbian Journal of Clinical and Experimental Research
COBISS.SR-ID 149695244