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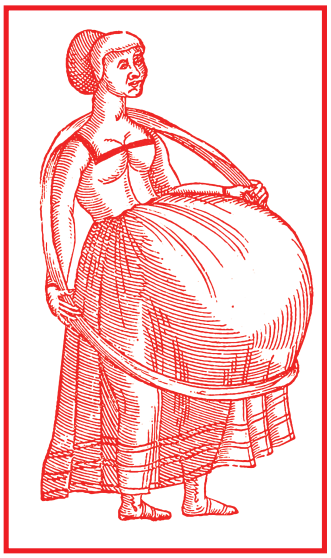
Dear readers,

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/inflamatory 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 interpratation 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!



UDK 618.177-089.888.11 / Ser J Exp Clin Res 2008; 9 (1): 5 – 8

INFLUENCE OF ASSISTED REPRODUCTIVE TECHNOLOGIES ON VITAL STATISTICS

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. (2, 3, 5, 7). The same problem is also evident in Slovenia, regardless of the economic state of its population.

Is it possible for us to accept the new values of individuals who more and more frequently believe that the number of descendants is not important while planning their life? How can we change our attitude towards life when family planning is no longer the highest priority? Do we have to accept the fact that a nation as well as every family may have no descendants any more? Why is there such concern? Is it because the lack of offspring means lower social security of the elderly who are now active members of the society?

The governments of European countries have adopted various „population policy" strategies to increase the birth rate among their population.

One of the possibilities to influence demographic changes is certainly the opportunity to treat infertility and the methods of assisted reproduction or assisted reproductive technology (ART). In the last decade there have been changes in the comprehension of infertility treatment and its effect on demographic changes. The basic principle of ART is to enable parenting to those who wish to have children but are unable to conceive. In this segment not one of the strategies of the population policy is useful and cannot turn the fertility trend to its own benefit. For a long time it was considered that the ART is a method for solving „individual" problems. The effect of ART on fertility rate seems minimal at first glance; however, it is possible to compare it with other measures of the population policy. Consequently, ART is not neglected and excluded from the „policy mix" for solving the demographic problem (4, 6, 8).

The analyses of the ART effect on the trends of demographic changes in Denmark and some other countries have clearly shown its significance. Nowadays, there are more than 6% of all children born in Denmark conceived by means of ART. Therefore, ART does not strictly belong to „medical" spheres any more but increasingly to the sphere of „population" policy. Since the prevalence of infertility is quite high (at least in 10% of population) and the inability to conceive a second child even higher (15%), it is clear that treating infertility can enormously influence the birth rate in a particular country (Slovenia, Denmark) and thus have a long-term effect on the economic state of a society (10,12).

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 characteristics of all European countries) but also poor prevention of pelvic tubal diseases caused by Chlamydia

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(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.

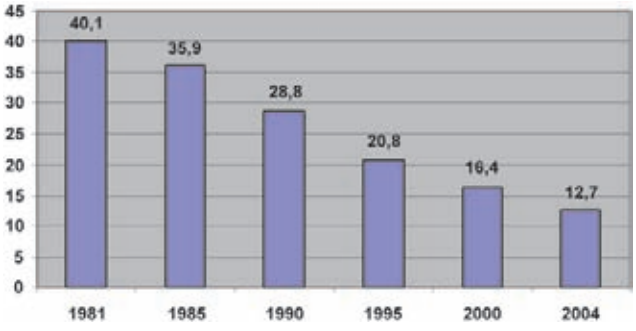

Figure 1. Allowed abortion rate per 1000 women in the reproductive period.

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%.

The results of epidemiological studies in European countries show that:

4% of couples who wish to have children remain with none

4–6% of couples are not able to conceive a second child

10–16% of couples have experience with treating primary infertility

6–17% of couples have experience with treating secondary infertility

approximately 15% of population comes in their fertile period of life for medical advice on infertility

In Slovenia around 500.000 women are in their fertile period. Assuming that three quarters of fertile women are in any kind of relationship, it means that 57.547 couples have problems with infertility (11% of population). Obviously, the problem is not rare or to be neglected.

This fact represents the central problem in lives of affected individuals and may cause an infertile couple to end their relationship.

Despite all, diagnosis and treatment of infertility are still not on the priority list of health care programs. Health care policy often does not follow the proclaimed principles. Some even do not treat infertility as a disease in the real sense of the word. The fact that we frequently cannot confirm the physical presence of a certain disease endangering our health or even our life contributes to such a perception of this „state".

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 spermatocytes 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

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).

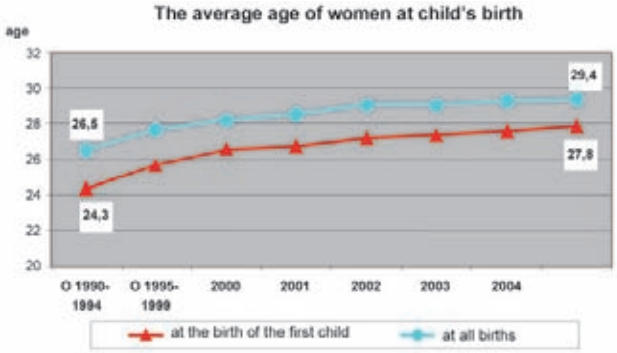

Figure 2. The average age of women at child's birth.

Since 1980 the birth rate of young women in Slovenia has been vividly and persistently decreasing – in the group of women from 20 to 24 years old it has dropped from 177 to 40 (more than four times) and in an even younger group from 56 to 6.5. Opposite to this, in the group of women from 30 to 34 years old, the birth rate has increased from 51 to 79 (55%). In the remaining age groups the changes in the birth rate are not so evident (figure 3).

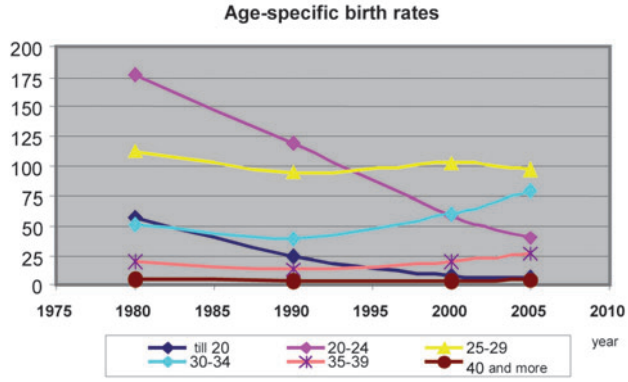

Figure 3. Age-specific birth rates.

A drastic decrease of the birth rate in Slovenia was first identified in cities where it is still today the lowest. From the second half of the previous century the number of births has decreased for 44%.

In 2000 the number of born children was for the first time lower than the number of the deceased and remained the same for the next five years (figure 4). The phenomenon of negative population growth rate (the so-called white plague) is of our great concern and still present in the new century Since there is lack of other mechanisms in the demographic policy, the treatment of infertility is an option to increase the number of births at least in the group of people who would like to have children but are unable to conceive and thus achieve a positive trend in population growth.

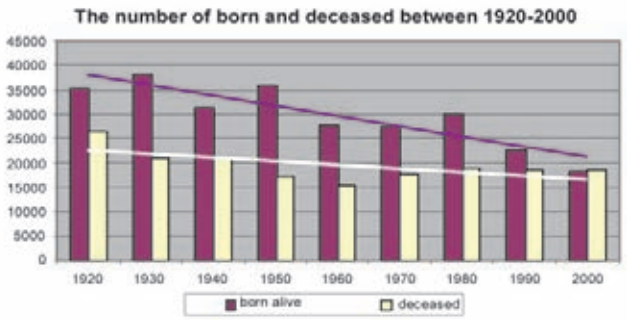

Figure 4. Trends in natural population growth rates between 1920–2000.

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

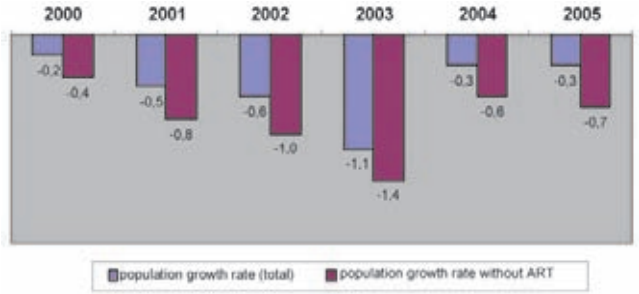

Figure 5. Population growth rate (total and without children born by means of ART) in Slovenia between 2000–2005.

REFERENCES

1. Andersen AN, Gossens GV, Gianaroli L, et al. The European IVF monitoring programme (EIM), for the European Society for Human Reproduction and Embriology (ESHRE) assisted reproductive technology in Europe, 2003. Results generated from European registers by ESHRE. Hum Reprod 2007; 22: 1513–25.

2. Bateman S. When reproductive freedom encounters medical responsibility: changing conceptions of reproductive choice. In: Vayena E, Rowe PJ, Griffin PD, eds. Current practise and controversies in assisted reproduction. Geneva: World Health Organization, 2002: 320–32.

3. Daar AS, Merli Z. Infertility and social suffering: the case of ART in developing counties. In: Vayena E, Rowe PJ, Griffin PD, eds. Current practise and controversies in assisted reproduction. Geneva: World Health Organization, 2002: 15–21.

4. Daya S. Cost-effective, evidence-based infertility care. Curr Opin Obstet Gynecol 2000; 12: 227–31.

5. Europen Parlament. Committee on Employment and Social Affairs. Draft report on demographic challenges and solidarity between the generations (2005/2147(INI)). (Accessed in Feb 2007 at http://www.europarl. europa.eu/meetdocs/2004_2009/documents/pr/579/579046/579046en.pdf).

6. Garceau L Henderson J, Davis LJ, et al. Economic implications of assisted reproductive techniques: a systematic review. Hum Reprod 2002: 17: 3090–109.

7. Grant J, Hoorens S, Sivadasan S, et al. Low fertility and population ageing. Causes, consequences and policy options. Santa Monica: RAND Corporation, 2004. (Accessed at http://rand.org/pubs/monographs/MG206).

8. Granberg M, Wykland M, Nilsson L. Couples willingness to pay for IVF/ET. Acta Obstet Gynecol Scand 1995: 74: 199–202.

9. Grant J, Hoorens S, Sivadasan S, Loo MV, Davanzo J, Hale L, Butz W. Trends in European fertility: should Europe try to increase its fertility rate… or just manage the consequences? Int J Androl 2006: 29; 17–24.

10. Granberg M, Strandell A, Thorburn J, et al. Economic evaluation of infertility treatment for tubal disease. J Assist Reprod Gen 2003; 20: 301–8.

11. Hardy E, Makuch MY. Gender, infertility and ART. In: Vayena E, Rowe PJ, Griffin PD, eds. Current practise and controversies in assisted reproduction. Geneva: World Health Organization, 2002: 272–80.

12. Hoorens S, Gallo F, Cave J, Grant J. Can assisted reproductive technologies help to offset population ageing? An assessment of the demographic and economic impact of ART in Denmark and UK: case report. Hum Reprod 2007; 22: 2471–5.

13. Informacijski sistem spremljanja fetalnih smrti v Sloveniji, 2004. IVZ RS. (Accessed in March 2007 at http://www.ivz.si/javne_datoteke/datoteke/26-fetalne_2004.pdf). (in Slovenian).

14. Ledger W, Gallo F, Hoorens S, Ziebe S, Connolly M. Present discounted value of children born using IVF compared with naturally conceived children: a simplified UK calculation. Hum Reprod 2006; 21(Suppl 1): i74-i75. O-188.

15. Stovall DW, Allen BD, Sparks AET, et al. The cost of infertility evaluation and therapy: findings of a self-insured university healthcare plan. Fertil Steril 1999; 72: 778–84.

16. Statistični urad Republike Slovenije. (Accessed in Feb 2007 at http://www.stat.si/tema_demografsko_prebivalstvo.asp). (in Slovenian).

17. Van Voorhis BJ, Stovall DW, Allen BD, Syrop CH. Cost-effective treatment of the infertile couple. Fertil Steril 1998; 70: 995–1005.

18. Vlaisavljević V. Zdravstveno-ekonomski vidiki zdrav-ljenja neplodnosti v Sloveniji. ISIS, 2006: 34–42. (in Slovenian).

19. Wright VC, Schieve LA, Raynolds MA, Jeng G. Assisted reproductive technology surveillance, United States 2002. MMWR Surveillance Summaries 2005; 54(S S02): 1–24.

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

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C677T POLIMORFIZAM GENA METILENTETRAHIDROFOLAT REDUKTAZE (MTHFR) KOD PACIJENATA SA HRONIČNOM LIMFOCITNOM LEUKEMIJOM I DIFUZNIM KRUPNOĆELIJSKIM B LIMFOMOM

Nataša Rakonjac[1], Vesna Ilić[1], Gordana Šupić[1], Bojana Cikota[1], Olivera Tarabar[2], Jovana Jovanović[1] i Zvonko Magić[1]
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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 677T in patients with chronic lymphocytic leukemia compared with control group (frequency of variant allele 677T 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 677T 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 of allele 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

SAŽETAK

Metilentetrahidrofolat reduktaza ima bitnu ulogu u metabolizmu folata, sintezi i metilaciji DNK, i samim tim predstavlja važan faktor u me hanizmu kancerogeneze. U prikazanoj studiji smo ispitali povezanost naj~učestalijeg polimorfizma C677T gena za metilentetrahidrofolat reduktazu sa etiologijom hronične limfocitne leukemije i difuznog krupnoćelijskog B limfoma. Pacijenti su upoređivani sa kontrolnom grupom koja je imala istu polnu i starosnu distribuciju kao i grupa pacijenata.

Naši rezultati su pokazali značajno nižu učestalost mutantnog 677TT alela kod pacijenata sa hroničnom limfocitnom leukemijom, poredeći sa kontrolnom grupom (frekvenca mutantnog alela 24% nasuprot 33%, redom). Razlika u distribuciji alela je statistički značajna (p=0,05). Rezultati su isti kada se poredi grupa pacijenata sa HLL i grupa pacijenata sa DLBCL (24% nasuprot 34,5%, p=0,05). Takvi rezultati su praćeni značajno većom frekvencom normalnog 677CC homozigota kod bolesnika sa HLL. Razlika u alelskoj distribuciji između bolesnika sa DLBCL i kontrolne grupe nije pokazala statističku značajnost (p=0,065).

Naši rezultati sugerišu da distribucija polimorfizma C677T MTHFR gena može da varira u okviru različitih grupa limfoproliferativnih oboljenja, kao i da 677CC genotip može da predstavlja faktor rizika za razvoj hronične limfocitne leukemije.

Ključne reči: metilentetrahidrofolat reduktaza, polimorfizam gena, B-ćelijska hronična limfocitna leukemija, krupnoćelijski difuzni B-limfom

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 lymphoma, chronic lymphocytic leukemia, etc.) and high progressive lymphoid neoplasms (acute leukemia, diffuse large B cell lymphoma, 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, chromosome damages 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

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of 5, 10- methylenetetrahydrofolate to 5, 10- metyltetrahydrofolate, 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 cancer predisposing gene (1).

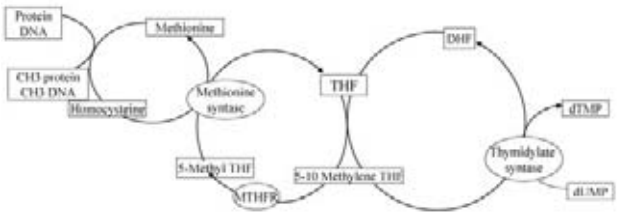

Figure 1. Methionine metabolizing and folate pathways. MTHFR catalyzes the reduction of 5,10-methylene THF to 5-methyl THF. Reduced activity of MTHFR results in the accumulation of 5,10-methylene THF, which accelerates methylation of dUMP to dTMP.

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; alanin→valin). 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 also of 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 methylenetetrahydrofolate 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.

Digestion

Amplified 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 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 1. Characteristics of study participants.

	Number	Gender		Age (years)	
		Female	Male	Median	Range
Controls	35	10	25	43	32–65
DLBCL	26	8	18	42	31–80
CLL	23	7	16	54	30–78

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).

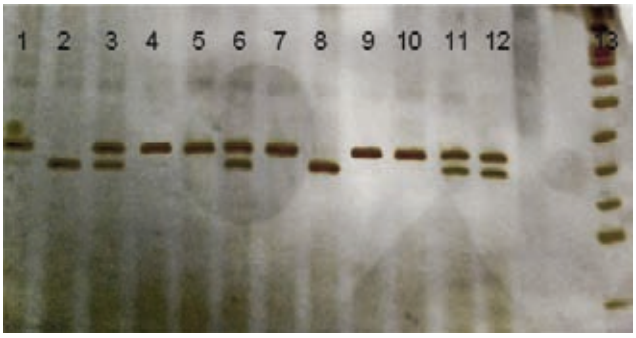

Figure 2. Polyacrylamide gel electrophoresis for C677T polymorphism of MTHFR gene. After digestion with Hinf I, the C allele produced 198 bp band and the T allele produced 175 and 23 bp bands. Homozygote 677CC→lines 1,4,5,7,9,10; homozygote 677TT→lines 2, 8; heterozygote 677CT→lines 3,6,11,12; PCR marker→line 13.

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.

	Number	677CC n (%)	677CT n (%)	677TT n (%)	T freq. (%)
Controls	35	16 (45,7)	15 (42,8)	4 (11,5)	33
CLL	23	13 (56,5)	9 (39,2)	1 (4,3)	24
DLBCL	26	11 (42,3)	12 (46,2)	3 (11,5)	34,5

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-methylenetetrahidrofolate. 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

1. Deligezer U, Akisik E, Yaman F, Erten N, Dalay N. MTHFR gene polymorphism in lymphoproliferative diseases. J Clin Lab Anal 2006; 20: 37–41.
2. Chiu BC, Weisenburger DD. An update of the epidemiology of non-Hodgkin's lymphoma. Clin Lymph 2003; 4: 161–8.
3. Fisher SG, Fisher RI. The epidemiology of non Hodgkin's lymphoma. Oncogene 2004; 23: 6524–34.
4. Bukowski JA, Huebner WW, Scnatter AR, Wojcik NC. An analysis of the risk of B lymphocyte malignancies in industrial cohorts. J Toxicol Environ Health A 2003; 66: 581–97.
5. Widschwendter M, Fiegel H, Egle D, et al. Epigenetic stem cell signature in cancer. Nat Genetic 2007; 39: 157–8.
6. Sharp L, Litlle J, Brockton N, et al. Polymorphism in the metylenetetrahydrofolate reductase MTHFR gene intakes of folate and related B vitamins and colorectal cancer: a case control study in population with relative low folate intake. Br J Nutr 2007; 23: 595–605.
7. Van Den Donk M, Van Engeland M, Pellis L. Dietary folate intake in combination with MTHFR C677T genotype and promoter methylation of tumor suppressor and DNA repair genes in sporadic colon adenomas. Cancer Epid Biom Prev 2007; 16: 327–33.
8. Ames BN. DNA damage from micronutrient deficiencies is likely to be an major cause of cancer. Mutation Res 2001; 475: 7–20.
9. Leclerc D, Rozen R. Molecular genetics of MTHFR: polymorphisms are not all benign. Medical Sci 2007; 23: 297–302.
10. Carrero J, Grimble R. Does nutrition have a role in peripheral vascular disease? Br J Nutr 2006; 2: 217–29.
11. Van der Linden I, Afman L, Heil S, Blom H. Genetic variation in genes of folate metabolism and neural-tube defect risk. Proc Nutr Soc 2006; 65: 209–21.
12. Ehrlich M, Woods CB, Yu MC, et al. Quantitative analysis of associations between DNA hypermethylation, hypomethylation, and DNMT RNA levels in ovarian tumors. Oncogene 2006; 25: 2636 –45.
13. Capaccio P, Ottaviani F, Cuccarini V, Censuales S, Cesana BM, Pignataro L. Association between methylenetetrahydrofolate reductase polymorphisms alcohol intake and oropharyngolaryngeal carcinoma in northern Italy. J Laryng Otol 2005; 119: 371–6.
14. Tan DJ, Barber J Shields P. Alcohol drinking and breast cancer. Breast Cancer Online 2006; 9(4): e15.
15. Gerhard DS, Nguyen LT, Zhang ZY, Borecki IB, Coleman BI, Rader IS. A relationship between methylenetetrahydrofolate reductase variants and the development of invasive cervical cancer. Gynecol Oncol 2003; 90: 560–5.
16. Rudd M, Sellick G, Allinson R, Matutes E, Catovsky D, Houlston R. MTHFR polymorphisms and risk of chronic lymphocytic leukemia. Cancer Epid Biom Prev 2004; 13: 2268–70.
17. Goldin LR, Pfeiffer RM, Li X, Hemminki K. Familial risk of lymphoproliferative tumors in families of patients with chronic lymphocytic leukemia: results from the Swedish Family-Cancer Database. Blood 2004; 104: 1850-4.
18. Nückel H, Frey UH, Dürig J, Dührsen U, Siffer W. Methylenetetrahydrofolate reductase MTHFR 677CT and 12998AC polymorphisms are associated with differential apoptosis of leukemic B cells in vitro and disease progression in chronic lymphocytic leukemia. Leukemia 2004; 18: 1816–23.
19. Matsuo K, Suzuki R, Hamajima N, et al. Association between polymorphisms of folate and methionine metabolizing enzymes and susceptibility to malignant lymphoma. Blood 2001; 97: 3205–9.
20. Skibola C, Forrest M, Coppede F, et al. Polymorphisms and haplotypes in folate metabolizing genes and risk of non Hodgkin's lymphoma. Blood 2004; 104: 2155–62.
21. Gemmati D, Ongaro A, Scapoli GL, et al. Common gene polymorphisms in the metabolic folate and methylation pathway and the risk of acute lymphoblastic leukemia and non Hodgkin's lymphoma in adults. Cancer Epid Biom 2004; 13: 787–94.
22. Krajinovic M, Lamothe S, Labuda D, et al. Role of MTHFR genetic polymorphism in the susceptibility to childhood acute lymphoblastic leukemia. Blood 2004; 103: 252–7.
23. Toffoli G, Rossi D, Gaidano G, Cecehin E, Boiocchi M, Carbone A. Methylenetetrahydrofolate reductase genotype in diffuse large cell lymphomas with and without hypermethylation of the DNA repair gene O6-metylguanine DNA metyltransferase. Int J Biol Markers 2003; 18: 218–21.
24. Wielmels JL, Smith RN, Taylor GM, Eden OB, Alexander FE, Greaves MF. Metylenetetrahydrofolate reductase (MTHFR) polymorphisms and risk factor of molecularly defined subtypes of childhood acute leukemia. Proc Natl Acad Sci USA 2001; 98: 4004–9.
25. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: A laboratory manual. 2nd ed. New York: Cold Spring Laboratory Press, 1989.
26. Wössman JL, Seidemann K, Mann G, et al. The impact of the methotrexate administration schedule and dose in the treatment of children and adolescence with B cell neoplasms: a report of the BFM Study Group NHL-BFM 95. Blood 2005; 105: 948–58.
27. Ordonez G, Carreira F, Alvarez F, et al. Normal frequencies of the C677T genotypes of the metylenetetrahydrofolate reductase (MTHFR) gene among lymphoproliferative disorders but not in multiple myeloma. Leuk Lymph 2000; 39: 607–12.
28. Nückel H, Frey U, Bau M, et al. Association of a novel regulatory polymorphisms C938A in the BCL2 gene promoter with disease progression and survival in chronic lymphocytic leukemia. Blood 2007; 109: 290–7.
29. Houlston RS, Sellick G, Yuille M, et al. Causation of chronic lymphocytic leukemia: insights of familial disease. Leuk Res 2003; 27: 871–6.
30. Auer RI, Jones C, Mullenbach RA, et al. Role of CCG trinucleotide repeats in the pathogenesis of chronic lymphocytic leukemia. Blood 2001; 97: 509–15.
31. Wahlfors J, Hiltunen H, Heinonen K, et al. Genomic hypomethylation in chronic lymphocytic leukemia. Blood 1992; 80: 2074–80.
32. Guillen VM, Collado M, Teror MJ, et al. Role of MTHFR (677, 1298) haplotype in the risk of developing secondary leukemia after treatment of breast cancer and hematological malignancies. Leukemia 2007; 21: 1413–22.
33. Pileri SA, Dirnhofer S, Went P, et al. Diffuse large B cell lymphoma: one or more entities? Present controversies and possible tools for its subclassification. Histopathology 2002; 41: 482–509.

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

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PRIMENA SUSPENZIJE METILPREDNOZOLONA JONTOFOREZOM KOD PACIJENATA SA ARTROZOM KOLENA

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ABSTRACT

The knee arthrosis is 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 attendant symptom of „activated knee arthrosis". Therapeutic regiment is based on applications of non-specific inhibitors of inflammation, non-steroidal 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 iontophoretic 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/cm^2, 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 iontophoretic 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

SAŹETAK

Gonartroza je vrlo često degenerativno reumatsko oboljenje. Ona se primarno odlikuje oštećenom zglobnom 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 medijatora, nesteroidnih inflamatornih lekova, i primeni fizikalne terapije. Kasnije se daju preparati hijaluronske kiseline. Primena jontoforeze kortikosteroida nije tako česta ukliničkoj praksi za razliku od intraartikularnog davanja kortizonskih preparata (npr. slabo rastvorljive suspenzije metilprednizolona i betametazona). U ovom radu je pokazan značaj primene kortikosteroida putem jontoforeze kod artroze kolenog zgloba. Optimalna primena metilpredizolon acetata putem jontoforeze kod artroze kolena je sprovedena po sledećem protokolu: aplikacija leka sa negativne elektrode, doza od 120mA*min/cm^2, a prosečno vreme aplikacije leka (u zavisnosti od individualne osetljivosti pacijenta) je oko 20 minuta. Poboljšanje simptoma i znakova i subjektivnih tegoba kod atroze kolena je mereno Hubertus testom i VAS skalom. Naši rezultati su pokazali da je kliničko i subjektivno poboljšanje bilo veće i dugotrajnije kod grupe koja je primala metilprednizolon putem jontoforetske aplikacije, nego u grupi lečenih placebom (destilovana voda).

Skraćenice: VAS - vizuelno analogna skala, NSAIL - nesteroidni antiinflamatorni lekovi, TENS - transkutana elektrostimulacija

Ključne reči: artoza kolena, kortikosteroidi, jontoforeza

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. Localization defines the type of joint arthrosis: medial, lateral or patellofemoral arthrosis. If all three forms are found, then we can talk about arthrosis of the whole knee joint (2).

Therapeutic regiment is based on application of inhibitors of inflammation nonsteroidal anti-inflammatory drugs and application of 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 antiinflamatory 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

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the most used corticosteroid agent during the iontophoretic 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 iontophoretic 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). Electrocatalytic oxidation of methylprednisolone was probably primary 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. Lemod 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 iontophoretic 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/cm^2 with 5 minutes duration, TENS therapy, paraffin application, kinesytherapy.

In experimental group, iontophoresis of corticosteroids was applied which consisted of methylprednisolone acetate (Lemod 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/cm^2. 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 electrotherapy 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 (χ^2-test; p=0.259), side of the disease (χ^2-test; p=0.436), occupation (χ^2-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 (χ^2-test; p=0.313), seasonal pain pattern (χ^2-test; p=0.206), family history (χ^2-test; p=0.796), target muscle hypotrophy (χ^2-test; p=0.001 and p=0.002), synovitis (χ^2-test; p=0.071), crepitating joint (χ^2-test; p=0.002), and palpable tenderness (χ^2-test; p=0.313).

Table 1. Demography and clinical properties of the patients.

Variable		Experimental group (n=30)	Control group (n=30)
Age (years)		65.27+10.79	61.53+13.6
Gender	male	8 (26.7%)	10 (33.3%)
	female	22 (73.3%)	20 (66.7%)
Diagnosis	unilateral arthrosis	15 (50%)	18 (60%)
	bilateral arthrosis	15 (50%)	12 (40%)
Occupation	retired	11 (36.7%)	12 (40%)
	unemployed	13 (43.3%)	15 (50%)
	merchants	2 (6.7%)	3 (10%)
	nurses	1 (3.3%)	0 (0%)
	clerks	2 (6.7%)	0 (0%)
	pupils	1 (3.3%)	0 (0%)
Working experience (years)		31.875+6.06	29.5+5.35
Height (cm)		164.34+8.92	164.59+9.51
Body distances, knee-floor (cm)		49.58+2.75	48.03+2.16
Body distances, foot length (cm)		24.75+1.96	24.58+1.6
Weight (kg)		79.47+12.54	77.6+10.17

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.

Visit	Experimental group (n=30)	Control group (n=30)
At baseline	7.87+0.9	7.1+1.35
After 10 days	5.33+0.96	6+1.51
After 30 days	4.37+1.13	5.77+1.36

The values shown: the mean± standard deviation

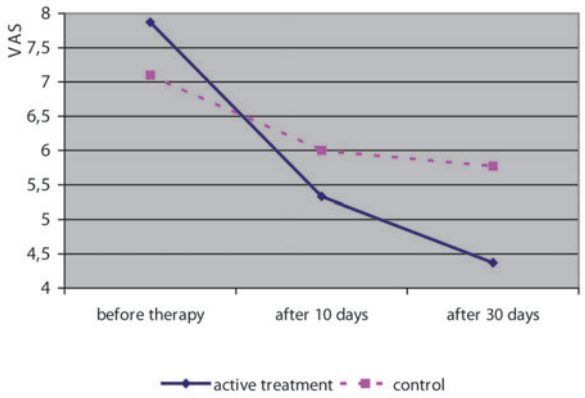

Figure 1. Pain evaluation according to VAS scale during the application of the therapy.

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.

Visit	Experimental group (n=30)	Control group (n=30)
At baseline	23.73+2.3 (23)	26.2+3.08 (27)
After 10 days	29.93+2.98 (30)	30.37+5.76 (30)
After 30 days	30.03+2.92 (30)	29.57+6.76 (30)

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; $p=0.045$).

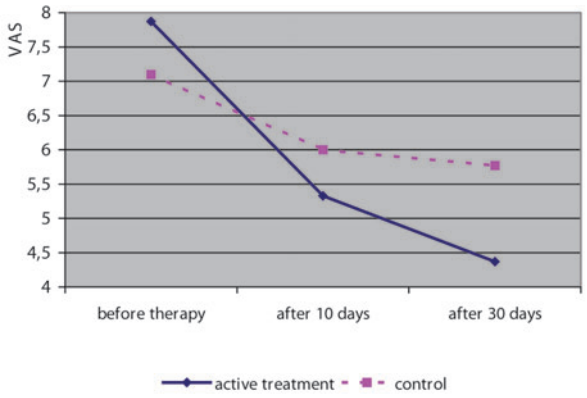

Figure 2. Values in Hubertus test in study subjects.

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

Visit	Experimental group (n=30)	Control group (n=30)
At baseline	3.77+0.43	3.63+0.49
After 10 days	3.93+0.26	3.67+0.48
After 30 days	3.93+0.26	3.73+0.45

The values shown: the mean± standard deviation

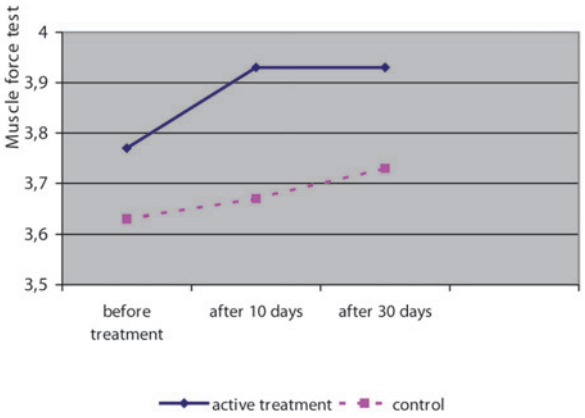

Figure 3. The values of muscle strenght (force test) for quadriceps femoral muscle.

On the other side, there improvement in the knee contracture was noted neither in the experimental (Friedman's test; $p=0.174$), nor in the control group (Friedman's test; $p=0.052$). The groups were similar at baseline (Mann Whitney U test; $p=0.577$), at 10th day (Mann Whitney U test; $p=0.401$), and at the study end (Mann Whitney U test; $p=0.361$).

Table 5. Contracture of treated knee joint, in degrees.

Visit	Experimental group (n=30)	Control group (n=30)
At baseline	6.17+10.72	3.28+6.16
After 10 days	5.67+9.8	2.67+5.04
After 30 days	5.5+9.4	2.33+4.5

The values shown: the mean± standard deviation

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 (χ^2-test; $p<0.001$).

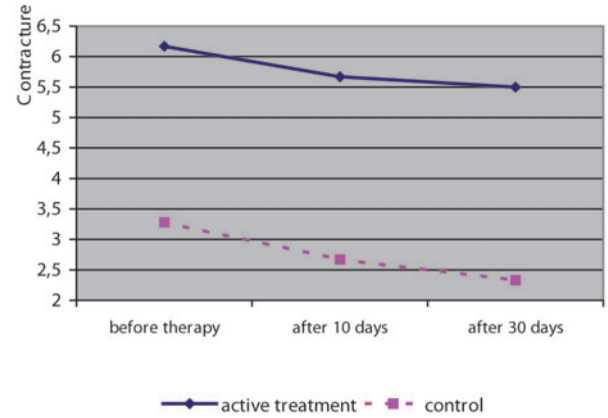

Figure 4. Contracture of the knee joint (degrees).

Table 6. Reduction of NSAIDs in tested groups.

Visit		Experimental group (n=30)	Control group (n=30)
After 10 days	Yes	27 (90%)	15 (50%)
	No	3 (10%)	15 (50%)
After 30 days	Yes	23 (76.7%)	6 (20%)
	No	7 (23.3%)	24 (80%)

The values shown: the number of patients (percent)

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 (χ^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.

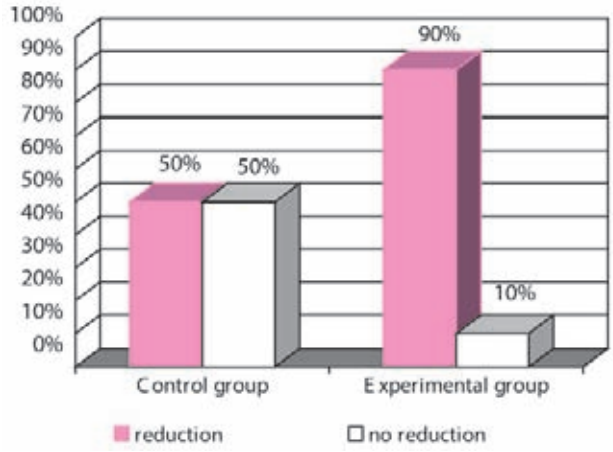

Figure 5. Reduction of NSAIDs use (first column) and dose (second column) after 10 days.

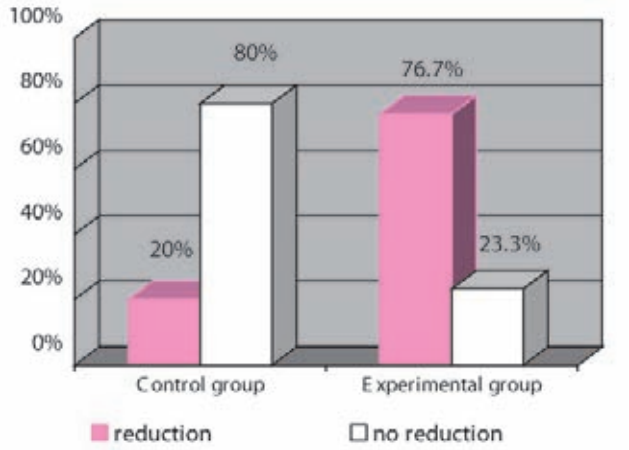

Figure 6. Reduction of NSAIDs use (first column) and dose (second column) between 10th and 30th day of the therapy in the subjects of the tested groups.

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 arthrosis. 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

1. Heilmann HH, Lindenhayn K, Walther HU. Synovial volumen gesunder und arthrotischer humaner Kniegelenke. Z Orthop Ihre Grenzgeb 1996; 134: 144–8. (in German).
2. Altman R, Asch E, Bloch D, et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. Arthritis Rheum 1986; 29: 1039–49.
3. Anderson CR, Morris RL, Boeh SD, Panus PC, Sembrowich WL. Effects of iontophoresis current magnitude and duration on dexamethasone deposition and localized drug retention. Phys Ther 2003; 83: 161–70.
4. Gokoglu F, Fndkoglu G, Yorgancoglu ZR, Okumus M, Ceceli E, Kocaoglu S. Evaluation of iontophoresis and local corticosteroid injection in the treatment of carpal tunnel syndrome. Am J Phys Med Rehabil 2005; 84: 92–6.
5. Glass JM, Stephen RL, Jacobson SC. The quantity and distribution of radiolabeled dexamethasone delivered to tissue by iontophoresis. Int J Dermatol 1980; 19: 519–25.
6. Gunther C, Kecskes A, Staks T, Tauber U. Percutaneous absorption of methylprednisolone aceponate following topical application of Advantan lotion on intact, inflamed and stripped skin of male volunteers. Skin Pharmacol Appl Skin Physiol 1998; 11: 35–42.
7. Gangarosa LPSr, Ozawa A, Ohkido M, Shimomura Y, Hill JM. Iontophoresis for enhancing penetration of dermatologic and antiviral drugs. J Dermatol 1995; 22: 865–75.
8. Goyala RN, Oyamab M, Umarb AA, Tyagic A, Bachhetia N. Determination of methylprednisolone acetate in biological fluids at gold nanoparticles modified ITO electrode. J Pharmaceut Biomed Anal 2007; 44: 1147–53.
9. Marques ALB, Li W, Marques EP, Zhang J. Electrocatalytic activity of surface adsorbed ruthenium-alizarin complexone toward the oxidation of benzyl alcohol. Electrochim Acta 2004: 49: 879–85.
10. Gindre CA, Berl V, Lepoittevin JP. Air oxidation of 17-hydroxycorticosteroids catalyzed by cupric acetate: formation of hemiacetal dimers. Steroids 2003: 68: 361–5.
11. Zgradic I. The technique of the intraarticular injection use. Belgrade: „Zgradic Branko“, 1995. (in Serbian).
12. Von Korff M, Ormel J, Keefe FJ, Dworkin SF. Grading the severity of chronic pain. Pain 1992; 50: 133–49.
13. Dorfmuller-Kuchlin S. Das Physiotherapeutische Assement. Krnakengymnastik 1996; 10: 1711–23. (in German).
14. Jevtic M. Clinical kinesytherapy. 2nd ed. Kragujevac: Medical Faculty University of Kragujevac, 2006. (in Serbian).
15. Altman DG. Practical statistics for medical research. 1st ed. London: Chapman and Hall, 1991.
16. Rai R, Srinivas CR. Iontophoresis in dermatology. Indian J Dermatol Venereol Leprol 2005; 71: 236–41.
17. Jevtic M. Physical medicine and rehabilitation. Kragujevac: Medical Faculty University of Kragujevac, 1999. (in Serbian).

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CLINICAL IMPORTANCE OF BIOCHEMICAL MARKERS OF CARDIAC DAMAGE IN HEMODIALYSIS PATIENTS

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KLINIČKI ZNAČAJ BIOHEMIJSKIH MARKERA SRČANOG OŠTEĆENJA KOD PACIJENATA NA HEMODIJALIZI

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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, hypoalbuminemia, anaemia, hyperhomocysteinemia, high concentration of asymetric 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 (homocystein, ADMA), tests for estimation of microinflamation, coronary artery plaque instability and voulnerability risks (CRP), tests for detection of markers of ischemia and damage of cardiac tissue, (cTnT, cTnI), 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 patiens with end stage of renal disease.

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

SAŽETAK

Kardiovaskularne bolesti su najčešći uzrok morbiditeta i mortaliteta bolesnika koji se leče redovnim hemodijalizama. Stopa kardiovaskularnog mortaliteta kod ovih bolesnika iznosi približno 9% godišnje, a među kardiovaskularnim komplikacijama najveća je prevalencija hipertrofije leve komore, ishemijske bolesti srca i kongestivne srčane slabosti. U faktore rizika za razvoj ateroskleroze i kardiovaskularnih komplikacija kod bolesnika na hemodijalizi spadaju: povišen arterijski krvni pritisak, poremećaj metabolizma lipida, oksidativni stres, mikroinflamacija, hipoalbuminemija, anemija, hiperhomocisteinemija, povećana koncentracija asimetričnog dimetilarginina-ADMA i sekundarni hiperparatireoidizam. Dijagnostička 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 ishemije i oštećenja srčanog tkiva (cTnT, cTnI), kao i testove za određivanje pokazatelja funkcije miokarda (ANP, BNP, Nt-proBNP). Utvrđivanje najosetljivijih parametara visokogm rizika za razvoj kardiovaskularnih komplikacija omogućava pravovremenu primenu odgovarajuće terapijske strategije, koja obezbeđuje visok stepen preživljavanja bolesnika sa završnim stadijumom hronične slabosti bubrega.

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

INTRODUCTION

Cardiovascular diseases are the most frequent cause of morbidity and mortality in patients on regular hemodialisys. 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, hypoalbuminemia, anaemia, hyperhomocysteinemia, high concentration of asymetric 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		Cigarete Smoking Hypertension Hyperlipidemia Diabetes mellitus Obesity
NON TRADITIONAL	HEMODYNAMIC	Anaemia Retention of Na+ and H2O AV fistula (QAV > 1000 mL/min)
	METABOLIC	Hypoalbuminemia Hyperhomocysteinemia Oxydative stress Microinflammation Secondary hyperparatireoidism

Modified according to reference (3).

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Laboratory tests in clinical cardiology

Four groups of laboratory tests are used in clinical cardiology: 1) tests for cardiovascular risk factors detection (homocystein, asymetric dimethylarginine-ADMA, C-reactive protein, lipids, oxidatively changed LDL lipoprotein-oxLDL, malondialdehid-MDA); 2) tests for estimation of microinflamation, (coronary artery plaque instability and voulnerability risks, choline, PAPP-A - protein A related with pregnancy); 3) tests for detection of markers of ischemia and damage of cardiac tissue, (IMA - Ischemia modulated albumin, Creatin kinase-CK, lactat dehydrogenase-LDH, SGOT-serum glutamat oxalat transaminase, cardiac troponin-cTn); 4) Myocardial function tests (ANP-atrial natriuretic peptid, BNP-brain natriuretic peptid, N terminal-pro BNP-Nt - proBNP) (figure 1) (18–21).

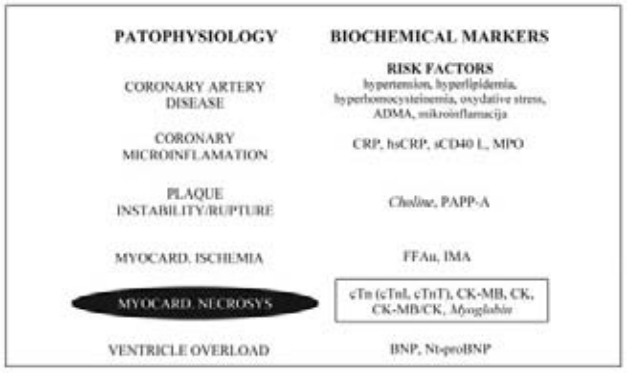

Figure 1. Biochemical markers for heart disease evaluation. Modified according to reference (18). CRP - C-reaktive protein, sCD40L - solubile CD40 ligand, MPO - myeloperoxydase, PAPP-A - protein A binded with pregnancy , FFA - free fatty acid, IMA - ischemia modulated albumin, CK - Creatine phosfokinase, CK-MB - Creatine kinase-isomer MB, cTnI - cardiac troponin I, cTnT - cardiac troponin T, BNP - brain natriuretic peptid, Nt-proBNP - N terminal fragment proBNP.

Test for risk factors determination and cardiovascular risk stratification

Homocystein is product of metabolism of essential aminoacid metionin (figure 2) (22). Metionin is demethylised into Sadenosylmetionin, which is important methyl group donor for different biological reactions. When S-adenosylmetionin lose methyl group it turns into homocystein, which can further be metabolised in two ways: by re-methylation process or by trans-sulfuration process (22–25). Re-methylation process has two ways. First way of re-methylation is included in the folate cicle. Metabolically active folate, 5-metyltetrahydrofolate (5-MTHF), in the presence of an enzyme from the folat cicle, reductase 5-MTHF, serves as a donor of the methyl groups, and the cofactor is vitamin B12. Other way of re-methylation uses betain as a methyl group donor, and doesn't depend on folate cicle, having rather small role in the re-methylation. By the process of trans-sulfuration, homocystein is transmitted into cystein, and for that reaction vitamin B6 is essential, as a cofactor, and enzyme β-cystation sintase (22–25). Average homocystein concentration in plasma of a healthy person is 6–12 μmol/L (22). In the patients who are treated with hemodialysis hyperhomocysteinemia is assumed if homocystein concentration in the plasma is ≥ 15 μmol/L (22). Mild hyperhomocysteinemia is assumed if homocystein concentration in the plasm is between 15–30 μmol/L, modest hyperhomocysteinemia is between 31–100 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 asymetric 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

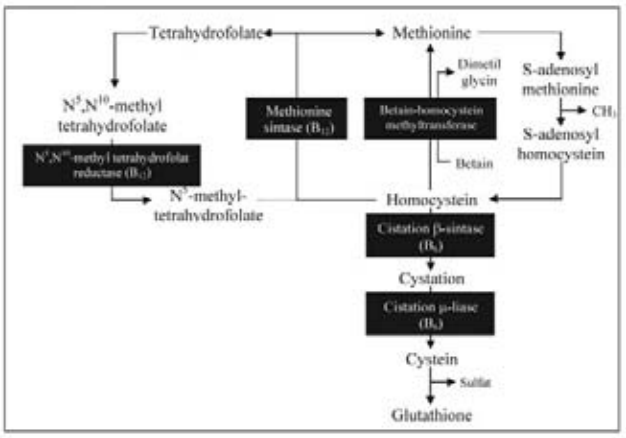

Figure 2. Biochemical paths of homocystein synthesis and degradation.

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). Asymetric dimethylarginine is a result of degradation of methylated proteins (figure 3). Methylation of the arginine residuals 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 residuals of proteins. As a result of methylation of arginine residuals 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 asymetric dimethylarginine-ADMA synthesis. By hydrolysis of methylated proteins ADMA is liberated. Asymetric 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 3–15μmol/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).

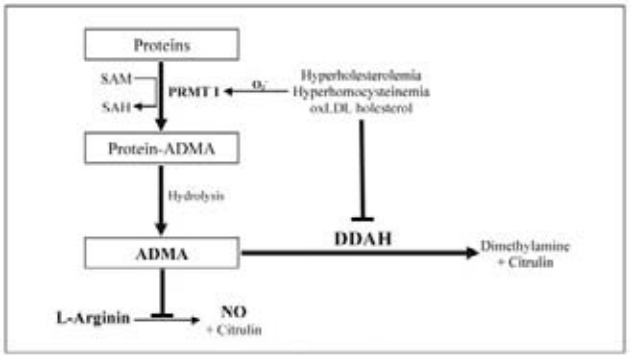

Figure 3. Biochemical paths of creation and degradation of asymmetric dimethylarginine-ADMA. SAH-S-adenosyl-L-homocystein, DDAH-dimethylarginine dimethylhydrolase, PRMT I-protein arginine methyltransferase I, NO-nitrous oxyde.

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, microinflamation 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 independant risk factor for cardiovascular complications in patients on hemodialysis (29). Local and systemic inflammation have important role in pathogenesis of acute coronary syndrom. Inflammatory process has important role in prediction of plaque perspective, eg. plaque stability. C-reactive protein, reactant of acute phase of inflammation, has imortant role in the 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 statisticaly significant relation (29–31).

Tests for estimation of plaque instability and risk of its rupture

Elevated concentration of solubile CD-40 ligand indicates aggravated prothrombotic activity and possibility of development of coronary thrombosis. After thrombocyte activation there is significant rise and liberation of solubile 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 inlammatory cells, including activated neutrophils and monocytes/macrophages, present in aterosclerotic plaques. Elevated myeloperoxydase 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 aterosclerotic 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 binded with pregnancy - PAPP-A is a glycoprotein of high molecular weight (200 kD), which is synthetised in syncytio-trophoblasts. Presence of this protein is proved in unstable aterosclerotic 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 (ishaemia-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 untill the myocites aren't ireversibly 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 sceletal 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 striated muscles damage as well (32, 33). Ratio CK-MB/total CK above 5% suggests myocardial infarction Š(CK-MB/CK) x100 (%)Ć. 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). Ratio of

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 diagnosys 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 (α -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 myocites, 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 actinomyosine ATP-ase. Troponin C-cTnC (molecular weight 18 kD) is binding for cTnI, oposing 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 bindes for actyn-myosyn filament (34, 61). A great deal of cardiac troponins (cTnI I cTnT) intracellulary are attached to myofibriles, and small amount is free (6–8% cTnT and 3–4% cTnI). Cardiac troponins are excreted in phase of reversible (citosolyc form) and irreversible (citosolyc 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.

Proteins	Molekul weight	Early detection*	Duration	Sensitivity	Specificity
Protein-FA	12 kD	1.5 - 2.0 h	8 - 12 h	+++	++
Myoglobin	16 kD	1.5 - 2.0 h	8 - 12 h	+++	+
CK-MB	83 kD	2.0 - 3.0 h	1 - 2 days	+++	+++
Troponin I	33 kD	3.0 - 4.0 h	7 - 14 days	++++	++++
Troponin T	38 kD	3.0 - 4.0 h	7 - 14 days	++++	++++
CK	96 kD	4.0 - 6.0 h	2 - 3 days	++	++
sGOT	~103 kD	6.0 - 10.0 h	3 - 5 days	++	+
LDH	135 kD	6.0 - 10.0 h	5 - 7 days	++	+

* hours after the symptom onset, CK-creatine 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 disfunction of left ventricle, voluminous left ventricle and myocardial stretching, coronary microcirculation disturbance, endothelial disfunction, oxydative stress and microinflammation, episodes of hypotension during hemodialysis, myocarial 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 greatest 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 Associatonion) 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 syndrom compared to cTnT, and is used as a parameter for diagnostics and stratification of clinical difficulty of patients on hemodilaysis developing acute coronary syndrom (48). Patients with concentration of troponin I - cTnI in serum ≥ 0.15 ng/mL are marked as a pos-

itive ones, while concentration of cTnI < 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 aktnog myocardial infarction includes concentration of cTnI ≥ 2.0 ng/mL, table 3 (48). Incidence of development of cardiovascular complications in patients on hemodialysis with concentration of cTnI > 0.15 ng/mL statistically highly significant compared to the group of patients with cTnI < 0.15 ng/mL (44). Patients with concentration of cTnI ≥ 0.3 μg/L have higher risk of ACS compared to the patietns with a concentration of troponin I < 0.3 μg/L (49).

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

LABORATORY PARAMETER	TIME of DETECTION	CLINICAL IMPORTANCE
Whole creatin kinase - CK	4.0 - 6.0 h	CK > 232 U/L
Isoenzym MB Creatin kinase - CK-MB	2.0 - 3.0 h	CK-MB > 16 U/L
CK and isoenzymes CK-MB ratio	2.0 - 4.0 h	CK-MB/CK > 5%
Isoenzymes Creatinin kinase MB ratio	2.0 - 4.0 h	CK-MB 2/CK-MB 1 ≥ 1.5
Cardiac troponin I	2.0 - 4.0 h	cTnI ≥ 2.0 ng/mL

Tests for detection of myocardial function markers

In population of patients without kidney disease, cardiac natriuretic peptids are the most imortant 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 tretament with angiotensin convertase I blockers and beta blockers, which both prevent congestive heart failure. In this population of patients natriuretic peptids are not used for prognosis and stratification of patients with congestive heart failure, but for the estimation of eficacy of applied therapy for congestive heart failure (50–52).

In patients with ESKD (End Stage Kidney Disease) on hemodialysis, natriuretic peptids (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, diminsh 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 corelation (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 cardiovacular 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 therapeutical 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 abscence of ireversible 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 complicatons (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 cTnI enables depiction of hemodialysis patients with elevated cardiovascular risk, in whom additional diagnostic monitoring and agressive cardiovascular risk factor correction are neccessary (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

1. Parfrey PS. Cardiac disease in dialysis patients: diagnosis, burden of disease, prognosis, risk factors and management. Nephrol Dial Transplant 2000; 15(Suppl 5): 5868.
2. London GM. Left ventricular alterations and end-stage renal disease. Nephrol Dial Transplant 2002; 17(Suppl 1): 29–36.
3. Rigatto C, Parfrey PS. Uraemic cardiomyopathy: an overload cardiomyopathy. J Clin Basic Cardiol 2001; 4: 93–5.
4. London GM. Cardiovascular disease in chronic renal failure: patophysiologic aspects. Semin Dial 2003; 16: 85–94.
5. London GM, Guerin AP, Marchais SJ. Hemodynamic overload in end-stage renal disease patients. Semin Dial 1999; 12: 77–83.
6. Goldsmith DJA, Covic A. Coronary artery disease in uremia: Etiology, diagnosis, and therapy. Kidney Int 2001; 60: 2059–78.
7. Vidt DG. Inflammation in renal disease. Am J Cardiol 2006; 97(2 Suppl 1): 20–7.
8. Wanner C, Zimmermann J, Schwedler S, et al. Inflammation and cardiovascular risk in dialysis patients. Kidney Int 2002; 61(Suppl 80): 99–102.
9. Fliser D, Kielstein JT, Haller H, Bode-Böger SM. Asymmetric dimethylarginine: a cardiovascular risk factor in renal disease? Kidney Int 2003; 63(Suppl 84): 37–40.
10. Zoccali C, Mallamaci F, Maas R, Benedetto FA, Tripepi G, Malatino LS, et al. Left ventricular hypertrophy, cardiac remodeling and asymmetric dimethylarginine (ADMA) in hemodialysis patients. Kidney Int 2002; 62: 339–45.
11. Zoccali C, Bode-Böger SM, Mallamaci F, Benedetto FA, Tripepi G, Malatino LS, Cataliott A, et al. Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: a prospective study. Lancet 2001; 358: 2113–7.
12. Böger RH. The emerging role of asymmetric dimethylarginine as a novel cardiovascular risk factor. Cardiovasc Res 2003; 59: 824–33.
13. Zoccali C, Mallamaci F, Tripepi G. Novel cardiovascular risk factors in end-stage renal disease. J Am Soc Nephrol 2004; 15(Suppl 1): 77–80.
14. Zoccali C, Mallamaci F, Tripepi G. Traditional and emerging cardiovascular risk factors in end-stage renal disease. Kidney Int 2003; 63: 105–10.
15. Balović G, Petrović D. Secondary hyperparathyreoidism - a risk factor for development of uremic cardiomyopathy in patients on hemodialysis. Medicus 2005; 6: 82–5. (in Serbian).
16. Petrović D, Stojimirović B. Prevalence of risk factors for development of cardiovascular complications in hemodialysis patients. In: Radenković S, ed. Cardionephrology 3. Naiss: GIP „PUNTA", 2007: 35–43. "in Serbian".
17. Petrović D, Stojimirović B. Vascular access blood flow for hemodialysis - a risk factor for development of cardiovascular complications in hemodialysis patients. Med Pregl 2007; 60: 183–6. (in Serbian).
18. Panteghini M. Role and importance of biochemical markers in clinical cardiology. Eur Heart J 2004; 25: 1187–96.
19. Majkić-Singh N. Biochemical markers in diagnosing acute coronary syndrome. Jugoslov Med Biohem 2003; 22: 289–301. (in Serbian).
20. Majkić-Singh N. The choice of biochemical markers in diagnosing acute coronary syndrome. Jugoslav Med Biochem 2005; 24: 1–13. (in Serbian).
21. Panteghini M. Biochemical markers of cardiac disease. Jugoslav Med Biochem 2004; 23: 201–11.
22. Friedman AN, Bostom AG, Selhub J, Levey AS, Rosenberg IH. The kidney and homocysteine metabolism. J Am Soc Nephrol 2001; 12: 2181–9.
23. Culleton BF, Bostom AG. Hyperhomocysteinemia in chronic renal disease. In: Loscalzo J, London GM, eds. Cardiovascular disease in end-stage renal failure. New York: Oxford University Press, 2000: 211–28.
24. Petrović D, Stojimirović B. Homocisteine as risk factor for cardiovascular complications in hemodialysis patients. In: Radenković S, ed. Cardionephrology 2. Naiss: GIP „PUNTA", 2005: 31–6. (in Serbian).
25. Petrović D, Radovanović M, Nikolić A, Poskurica M, Stojimirović B. Correlation between homocysteine and left ventricular hypertrophy in hemodialysis patients. Medicinski Časopis 2006; 40: 12–8. (in Serbian).
26. Massy ZA. Potential strategies to normalize the levels of homocysteine in chronic renal failure patients. Kidney Int 2003; 63(Suppl 84): 134–6.
27. Mallamaci F, Zoccali C, Tripepi G, et al. Hyperhomocysteinemia predicts cardiovascular outcomes in hemodialysis patients. Kidney Int 2002; 61: 609–14.
28. Cooke JP. Asymmetrical dimethylarginine: the uber marker? Circulation 2004; 109: 1813–8.
29. Lacson E, Levin NW. C-Reactive protein and end-stage renal disease. Semin Dial 2004; 17: 438–48.
30. Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease. Circulation 2003; 107: 499–511.
31. Rao M, Jaber BL, Balakrishnan VS. Inflammatory biomarkers and cardiovascular risk: association or cause and effect? Semin Dial 2006; 19: 129–35.
32. Jaffe AS, Babuin L, Apple FS. Biomarkers in acute cardiac disease. J Am Coll Cardiol 2006; 48: 1–11.
33. Kemp M, Donovan J, Higham H, Hooper J. Biochemical markers of myocardial injury. Br J Anaesth 2004; 93: 63–73.
34. Beciani M, Tedesco A, Violante A, et al. Cardiac troponin I (2nd generation assay) in chronic haemodialysis patients: prevalence and prognostic value. Nephrol Dial Transplant 2003; 18: 942–6.
35. Wood GNI, Keevil B, Gupta J, et al. Serum troponin T measurement in patients with chronic renal impairment predicts survival and vascular disease: a 2 year prospective study. Nephrol Dial Transplant 2003; 18: 1610–15.
36. Iliou MC, Fumeron C, Benoit MO, et al. Factors associated with increased levels of cardiac troponins T and I in chronic haemodialysis patients: Chronic Haemodialysis And New Cardiac Markers Evaluation (CHANCE) study. Nephrol Dial Transplant 2001; 16: 1452–8.
37. Iliou MC, Fumeron C, Benoit MO, et al. Prognostic value of cardiac markers in ESRD: Chronic Hemodialysis and New Cardiac Markers Evaluation (CHANCE) study. Am J Kidney Dis 2003; 42: 513–23.
38. Babuin L, Jaffe AS. Troponin: the biomarker of choice for the detection of cardiac injury. CMAJ 2005; 173: 1191–202.
39. Ishii J, Nomura M, Okuma T, et al. Risk stratification using serum concentrations of cardiac troponin T in patients with end-stage renal disease on chronic maintenance dialysis. Clin Chim Acta 2001; 312: 69–79.
40. Conway B, McLaughlin M, Sharpe P, Harty J. Use of cardiac troponin T in diagnosis and progression of cardiac events in patients on chronic haemodialysis. Nephrol Dial Transplant 2005; 20:2759–64.
43. Mallamaci F, Zoccali C, Parlongo S, et al. Diagnostic value of troponin T for alterations in left ventricular mass and function in dialysis patients. Kidney Int 2002; 62: 1884–90.
42. Apple FS, Murakami MM, Pearce LA, et al. Predictive value of cardiac troponin I and T for subsequent death in end-stage renal disease. Circulation 2002; 106: 2941–5.

43. Petrovic D, Obrenovic R, Radovanovic M, Majkic-Singh N, Stojimirovic B. Left ventricular hypertrophy in hemodialysis patients-correlation with cardiac troponins. Nephrol Dial Transplant 2006; 21(Suppl 4): 189.

44. Mallamaci F, Zoccali C, Parlongo S, et al. Troponin is related to left ventricular mass and predicts all-cause and cardiovascular mortality in hemodialysis patients. Am J Kidney Dis 2002; 40: 68–75.

45. Dierkes J, Domröse U, Westphal S, et al. Cardiac troponin t predictors mortality in patients with end-stage renal disease. Circulation 2000; 102: 1964–75.

46. Roberts MA, Fernando D, Macmillan N, et al. Single and serial measurements of cardiac troponin I in asymptomatic patients on chronic hemodialysis. Clin Nephrol 2004; 61: 40–6.

47. Boulier A, Jaussent I, Terrier N, et al. Measurement of circulating troponin Ic enhances the prognostic value of C-reactive protein in hemodialysis patients. Nephrol Dial Transplant 2004; 19: 2313–8.

48. Hussein M, Mooij J, Roujouleh H, Shenawi OA. Cardiac troponin-I and its prognostic significance in a dialysis population. Hemodialysis Int 2004; 8: 332–7.

49. Troyanov S, Ly QH, Schampaert E, et al. Diagnostic specificity and prognostic value of cardiac troponins in asymptomatic chronic haemodialysis patients: a three year prospective study. Heart 2005; 91: 1227–8.

50. Ritz E, Dikow R, Adamzcak M, Zeier M. Congestive heart failure due to systolic dysfunction: the cinderella of cardiovascular management in dialysis patients. Semin Dial 2002; 15: 135–40.

51. Schrier RW. Role of diminished renal function in cardiovascular mortality. J Am Coll Cardiol 2006; 47: 1–8.

52. Mark PB, Petrie CJ, Jardine AG. Diagnostic, prognostic, and therapeutic implications of brain natriuretic peptide in dialysis and nondialysis-depedent chronic renal failure. Semin Dial 2007; 20: 40–9.

53. Mallamaci F, Zoccali C, Tripepi G, et al. Diagnostic potential of cardiac natriuretic peptides in dialysis patients. Kidney Int 2001; 59: 1559–66.

54. Zoccali C, Mallamaci F, Benedetto FA, et al. Cardiac natriuretic peptides are related to left ventricular mass and function and predict mortality in dialysis patients. J Am Soc Nephrol 2001; 12: 1508–15.

55. Nolan CR. Strategies for improving long-term survival in patients with ESRD. J Am Soc Nephrol 2005; 16(11 Suppl 2): 120–7.

56. Herzog CA. Cardiac arrest in dialysis patients: approaches to alter an abysmal outcome. Kidney Int 2003; 63(Suppl 84): 197–200.

57. Herzog CA, Apple FS. Cardiac biomarkers in the new millennium. Semin Dial 2001; 14: 322–3.

58. Ooi DS, Zimmerman D, Graham J, et al. Cardiac troponin T predicts long-term outcomes in hemodialysis patients. Clin Chem 2001; 47: 412–17.

59. Apple FS, Murakami MAM, Pearce LA, Herzog A. Multi-biomarker risk stratification of N-terminal pro-B-type natriuretic peptide, high-sensivity C-reactive protein, and cardiac troponin T and I in end-stage renal disease for all-cause death. Clin Chem 2004; 50: 2279–85.

60. De Filippi C, Wasserman S, Rosanio S, et al. Cardiac troponin T and C-reactive protein for predicting prognosis, coronary atherosclerosis, and cardiomyopathy in patients undergoing long-term hemodialysis. JAMA 2003; 290: 353–9.

61. Zoccali C, Tripepi G, Mallamaci F. Predictors of cardiovascular death in ESRD. Semin Nephrol 2005; 25: 358–62.

62. Mallamaci F, Tripepi G, Cutrupi S, Malatino LS, Zoccali C. Prognostic value of combined use of biomarkers of inflammation, endothelial dysfunction, and myocardiopathy in patients with ESRD. Kidney Int 2005; 67: 2330–37.

63. Dikow R, Adamczak M, Henriquez DE, Ritz E. Strategies to decrease cardiovascular mortality in patients with end-stage renal disease. Kidney Int 2002; 61(Suppl 80): 5–10.

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OCULAR MANIFESTATIONS OF CHRONIC SARCOIDOSIS

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OFTALMOLOŠKE MANIFESTACIJE HRONIČNE SARKOIDOZE

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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, (hypercalcaemia 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, Koeppe 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 epitheloid cell granulomas. In severe cases, systemic corticosteroid therapy always constitutes the first approach. However, in patents that are refractory to corticosteroids, methotrexate has shown the most potential as alternative treatment.

Key words: sarcoidosis, uveitis, macular edema

SAŽETAK

Prikaz manifestacija i kliničke slike okularne sarkoidoze dijagnostikovane kod odraslih i dece sa opisom karakteristika pacijenata kod kojih se pojavila. Svi pacijenti ispitani od strane autora su imali dijagnostikovanu okularnu sarkoidozu i bili su stariji od 10 godina. Dat je prikaz kliničke slike sa anamnezom i laboratoratorijom (hiperkaciemija, sa kalciurijom, povišen angiotenzin-konvertujući enzim (ACE)), kao i drugim dijagnostičkim pretragama (bronhoalveolarna lavaža, tuberkulin kožni test, HIV seroloski test) i fluoresceinska angiografija. Ukupno je bilo 15 pacijenata prosečne starosti 47 g. (rang, 10–65) sa prosečnim praćenjem 6.1 godine. (rang, 0–15). Kod ovih pacijentata su uočeni brojne promene tipične za okularnu sarkoidozu, uključujući bilateralnu prirodu bolesti, slaninaste precipitate na endotelu rožnjače, Kepeove i Busaka nodule, snežne lopte u prednjem vitreusu, znake linearnog i „krpastog" periflebitisa, prisutno kao tzv. omotavanje. Cistoidni makularni edem, neovaskularizacija retine, edem papile, i granulom papile takođe su bili prisutni. Kod jednog pacijenta gde je dijagnostikovan bilateralni orbitalni granulom, bila je potrebna dodatna terapija. Totalna eksudativna ablacija je uočena takođe kod jednog pacijenta. Kod 35% pacijenata je bio potreban samo redovan monitoring, i to je bilo u proporciji sa brojem pacijenata koji pokazuju spontano poboljšanje. U vremenskom periodu u kom su bili praćeni pacijetni su u proseku gubili 3,4 linije u vidnoj oštrini. Prepoznavanje sarkoidoze podrazumeva radiološku i kliničku dijagnostiku sa histološkom potvrdom neinfektivnog i nekazeoznog granuloma epiteloidnih ćelija. Kod težih slučajeva sistemski kortiko preparati su bili prva linija terapije. Međutim kod reftrakternih slučajeva metotreksat se pokazao kao veoma potentan u tzv. alternativnoj terapiji.

Ključne reči: sarkoidoza, uveitis, edem makule

INTRODUCTION

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

Ocular involvement is present in approximately 25% of patients (2), with common manifestations being a granulomatous anterior uveitis, chorioretinal inflammation, or both. Posterior segment lesions include periphlebitis with perivascular sheathing, vitritis, chorioretinitis, and choroidal and optic nerve granulomas (3).

Because of persistent antigen exposure, exuberant cellular immune response occurs, leading to the development of granulomas, which are accumulations of T lymphocytes, mononuclear phagocytes, epithelioid cells, and multinucleated giant cells. Angiotensin-converting enzyme is derived from the epithelioid cells of the granulomas and reflects the granuloma load in the patient.

In sarcoidosis, granulomatous inflammation is characterized by dominant expression of Th1 cytokines such as interferon-

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γ 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 Koeppe 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 cart, log MAR VA carts), slit-amp biomikroscopy, dilated ophtalmoscopy, 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 log-MAR 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 a mean age 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%). Sixty-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 bellow 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 sinechiae have been reported in 20 %, cataract in 10%, and glaucoma in 33% of patients. Corneal band kerathopathy 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.

Variable	Value
Mean age (years)	47 (10–65)
Average follow up period (years)	6.1
Male/female	62%/38%
unilateral disease	50%
positive familiar history	37%
Anterior uveitis	30%
Posterior sinechiae	20%
Cataracta	10%
Glaucoma	33%
Corneal band kerathopathy	5%
Vitritis	60%
Intermediate uveitis	36%
Panuveitis	5%
Retinal vasculitis	30%
Periphlebitis	50%
CMO	20%
ERD	5%
Haemophtalmus	5%

Sarcoidosis-associated posterior uvetis 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 perivenous 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 uvetis and we divided them in two clinical patterns. The first type was associated with vitritis, segmental venous „sheathing" or perivenular exudates in 6 (40%) patients (figure 1). Small, discrete white spots occur in clusters around retinal

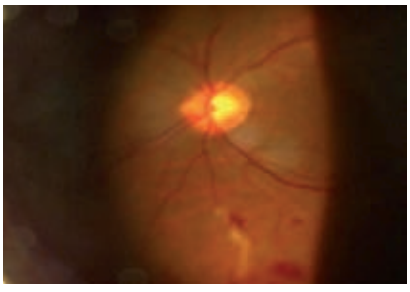


Figure 1. „Taches de boogie" I.

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, haemophtalmus 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%).

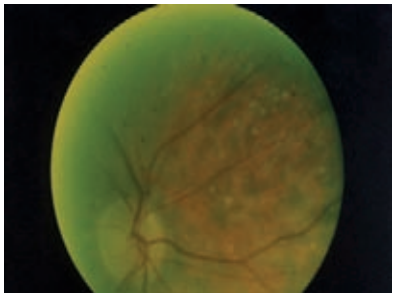


Figure 2. „Taches de boogie" II.

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 i 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 panuvetis in second eye.

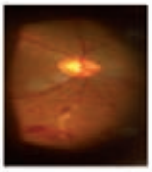
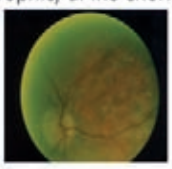
In severe cases, bilateral chronic uveitis, systemic corticosteroid therapy always constitutes the first approach (42%). However, in patents that are refractory to, corticosteroids, methotrexate has shown the most potential as alternative treatment (18%).

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 2. „Taches de boogie" sarcoidosis-associated posterior uvetis divided in two clinical patterns.

Sarcoidosis-associated posterior uveitis 50%		
Type	„taches de boogie" I	„taches de boogie" II
Vitritis	40%	/
Vasculitis	„venous scheathing" 30%	/
Exudates	Small discrete white spots –perivenular 	Yellow-orange lesion (depigmented, not atrophic) at the chorioid 
Field	Inferior quadrant	Posterior and nasal fundus
Complication	Cataract 10%	/
Visual acuity	0.19 log MAR	0.1 log MAR

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.

Table 3. Treatment of sarcoidosis-associated uvetis.

Treatment mode	Percent
Regular monitoring	35 %
The patients will show spontaneous improvement	30 %
The patients were treated with corticosteroid	65 %
Corticosteroid-sparing systemic immunomodulatory therapy (metotrexat)	18 %
Systemic corticosteroids (bilateral chronic uveitis)	42 %
Topical corticosteroids and periocular inections of corticosteroids (acute anterior inflammation and severe anterior uveitis)	57 %

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 uvetis 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, vasculits 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 fotocoagulation may be indicated (although there is a risk of exacerbating macula edema), whereas in the latter, adequate immunosuppression will usually induce regression of neovascular resoponse.

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 stabile 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 ophtalmological examination, chest x-ray, pulmonary function tests, blood cell count, hepatic enzymology 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 granulomas.

REFERENCES

1. Karma A. Ocular sarcoidosis-a diagnostic challenge: 434-04. Symposium. Acta Ophthalmol Scand 2006; 84(238 Supplement): 111.
2. Hosoda Y, Yamaguchi M, Hiraga Y. Global epidemiology of sarcoidosis. What story do prevalence and incidence tell us? Clin Chest Med 1997; 18: 681-94.
3. Braswell RA, Kline LB. Neuro-ophthalmologic manifestations of sarcoidosis. Int Ophthalmol Clin 2007; 47: 67-77.
4. Ooi KG, Galatowicz G, Calder VL, Lightman SL. Cytokines and chemokines in uveitis: is there a correlation with clinical phenotype? Clin Med Res 2006;4: 294-309.
5. Katatya YP, Holter JF. Immunology of sarcoidosis. In: James DG, ed: Sarcoidosis and other granulomatous disorders. New York: Marcel Dekker, 1994: 181.
6. Obenauf CD, Shaw HE, Syndor CF, et al: Sarcoidosis and its ocular manifestations. Am J Ophthalmol 1978; 86: 648-55.
7. Jabs Da, Johns CJ. Ocular involvement in chronic sarcoidosis. Am J Ophthalmol 1986; 102: 297-301.
8. Ohara K, Okubo A, Saski H. Branch retinal vein occlusion in a child with ocular sarcoidosis. Am J Ophthalmol 1995; 119: 806-7.
9. Sarcoidosis Network 2004; 12(2). (Accessed in May 2007 at http://www.sarcoidosisnetwork.org/documents/Mar_Apr_2004.pdf).
10. Stavrou P, Linton S, Young DW, Murray PI, et al. Clinical diagnosis of ocular sarcoidosis. Eye 1997; 11: 365-70.

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

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ZNAČAJ Nd:YAG LASER IRIDOTOMIJE U TERAPIJI GLAUKOMA ZATVORENOG UGLA

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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%). In the laser-treated group of 51 patient, the frequency of complications was 19, 6%, while out of 30 patients who had been surgically treated, the frequency of complications was 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.

Abbreviations: Nd: YAG, Neodymium: Yittrium-Aluminum Garnet; PACG, Primary angle-closure glaucoma; IOP, Intraocular pressure; POAG, Primary open-angle glaucoma

Key words: laser, iridotomy, glaucoma

SAŽETAK

Cilj ispitivanja je bio poređenje efikasnosti medikamentnog,hirurškog i laserskog tretmana kod pacijenata sa PACG u postizanju: a) normalizacije IOP-a, b) zadržavanja korisne vidne oštrine, c) ne napredovanja promena na papili vidnog živca i d) ne napredovanja funkcionalnih oštećenja koje utvrđujemo pregledom vidnog polja.

Analizirani su bolesnici koji su lečeni u Klinici za očne bolesti KC Kragujevac u periodu od 01.06.2004. do 01.06.2006.god. Ukupno 81 pacijent sa dijagnozom PACG, su izabrani za ovu studiju. Tretirani su :1) medikamentno, 2) Nd:YAG laser iridotomijom i 3) operativno .Oftalmološke kontrole obavljane su na 1 do 3 meseca, vidno polje je testirano dva puta godišnje.

U periodu praćenja u trajanju od 24 meseca, nije bilo statistički značajne razlike u promeni vidne oštrine između tri grupe ispitanika. Najbolja regulacija IOP-a je postignuta posle laserskog tretmana (45 %), zatim posle operativnog (35 %), a najslabija kod medikamentno tretiranih pacijenata (20 %). Procenat ispada u vidnom polju je najveći kod medikamentno tretiranih (55 %), zatim kod laserom tretiranih (25 %), a najmanji kod operisanih pacijenata (20 %). U grupi laserom tretiranih od ukupno 51 pacijenta učestalost komplikacija je 19,6 % , dok je kod operisanih od ukupno 30 pacijenata učestalost komplikacija 20 %. Laserska iridotomija se pokazala efikasnom kod 80,4 % pacijenata sa PACG, a kod nereaktivnih je rađena trabekulektomija.

Pored velike efikasnosti Nd:YAG laser iridotomije u regulisanju IOP-a kod pacijenata sa PACG pogodnost ove metode lečenja je ambulantni rad, lokalna anestezija, vrlo lako se podnosi i kratko traje.

Skraćenice: Nd: YAG, Neodymium: Yittrium-Aluminum Garnet; PACG, Primarni glaukom zatvorenog ugla; IOP, intra okularni pritisak; POAG, Primarni glaukom otvorenog ugla.

Ključne reči: laser, iridotomija, glaukom

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

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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 synechials (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) ophtalmoscope 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.

Visual acuity	L+	0,01	0,05	0,06	0,1	0,2	0,3	0,4	0,7	0,8	0,9	1,0
Opr.		3	3		5	5	6	4	2	2		
Laser		5	20			8	7	8	2	1		
Med					2		5	6	3	5		

Table 2. Visual acuity after the treatment.

Visual acuity	L+	0,01	0,05	0,06	0,1	0,2	0,3	0,4	0,7	0,8	0,9	1,0
Opr.	3	3	1		4	5	6	4	2	2		
Laser		6	19			9	6	8	2	1		
Med					3	1	4	5	3	5		

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.

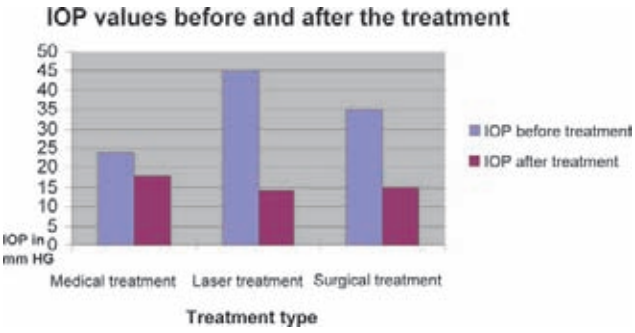

Figure 1. The IOP values before and after the treatment.

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%.

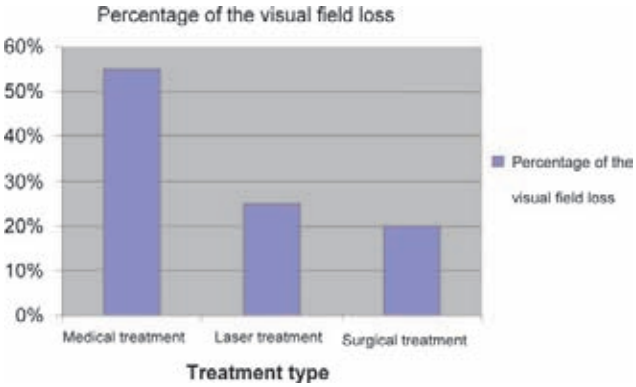

Figure 2. Percentage of the visual field loss.

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. Significan 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 regulaton 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

1. Foster PJ, Johnson GJ. Glaucoma in China: how big is the problem? Br J Ophthalmol 2001; 85: 1277–82.
2. Friedman DS. Who needs an iridotomy ? Br J Ophthalmol 2001; 85:1019–21.
3. American Academy of Ophthalmology. Laser peripheral iridotomy for pupillary-block glaucoma. Ophthalmology 1994; 101: 1749–58.
4. Wang N, Wu H, Fan Z. Primary angle closure glaucoma in Chinese and Western populations. Chin Med J (Engl) 2002; 115: 1706–15.
5. He M, Foster PJ, Ge J, et al. Prevalence and clinical characteristics of glaucoma in adult Chinese: a population-based study in Liwan district. Invest Ophthalmol vis Sci 2006; 47: 2782–8.
6. Foster PJ, Buhrmann R, Quigley HA, Johnson GJ. The definition and classification of glaucoma in prevalence surveys. Br J Ophthalmol 2002; 86: 238–42.
7. Nolan WP, Foster PJ, Devereux JG, et al. YAG laser iridotomy tretment for primary angle closure in east Asian eyes. Br J Ophthalmol 2000; 84: 1255–9.
8. Hsiao CH, Hsu CT, Shen SC, Chen HS. Mid-term follow-up of Nd:YAG laser iridotomy in Asian eyes. Ophthalmic Surg Lasers Imaging 2003; 34: 291–8.
9. Ang LP, Aung T, Chew PT. Acute primary angle closure in an Asian population: long- term outcome of the fellow eye after prophylactic laser peripheral iridotomy. Ophthalmology 2000; 107: 2092–6.

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

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INSERCIJA SEPTALNOG OPTURATORA U TRETMANU PERFORACIJE NOSNE PREGRADE: PRIKAZ SLUČAJA

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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

SAŽETAK

Etiologija perforacija nosne pregrade je različita, ali se najčešće vezuje za septalnu hirurgiju. Penetrantne povrede nosa, hematomi septuma, nazotrahealna intubacija, zatim oboljenja poput apscesa nosne pregrade, tuberkuloze, sifilisa, lupusa eritematozusa, Wegenerove granulomatoze, sarkoidoze i dr., kao i različiti inhalatorni iritansi, te neoplazme takođe mogu da dovedu do perforacije.

Leče se uglavnom samo simptomatske perforacije koje se manifestuju stvaranjem krusti, epistaksom, opstrukcijom, prisustvom kolorisanog sekreta, paranazalnog bola, a kada su manje i zviždanjem pri inspiraciji. Prvi korak u tretmanu je lečenje osnovne bolesti koja je dovela do perforacije. Ukoliko konzervativni tretman ne da rezultate, sledeći korak je zatvaranje perforacije bilo hirurškim bilo nehiruruškim putem. Hirurško lečenje perforacija je težak zadatak i povezano je sa komplikacijama i neuspesima. U nekim slučajevima hirurški pristup je kontraindikovan bilo zbog godina pacijenta, njegovog opšteg i/ili lokalnog stanja, ili zbog odbijanja pacijenta da se podvrgne hirurškoj intervenciji. Jedna od nehirurških tehnika kojom se u tim slučajevima kao privremeno ili trajno rešenje redukuju simptomi perforacije nosne pregrade je insercija septalnog opturatora.

Prikazali smo slučaj pacijenta sa velikom simptomatskom perforacijom nosne pregrade, kod koga smo metodom po Kelly-ju i Lee-ju učinili inserciju jednodelnog septalnog silikonskog opturatora u lokalnoj anestezji.

Tehnika pripreme i insercije jednodelnog septalnog opturatora, opisana od strane Kelly-ja i Lee-ja, predstavlja jedan brz, jednostavan, lako izvodljiv i za pacijenta komforan metod, za nehirurško zbrinjavanje perforacija nosne pregrade.

Ključne reči: nosni septum, povrede, proteze i implantati

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 erythematodes, Wegener's granulomatosis, sarcoidosis etc, various inhalation irritants like cocaine or occupational exposure to caustic or other industrial substances (especially chromic acid), as well as neoplasm can result in the ulceration of the mucous membrane 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

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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).

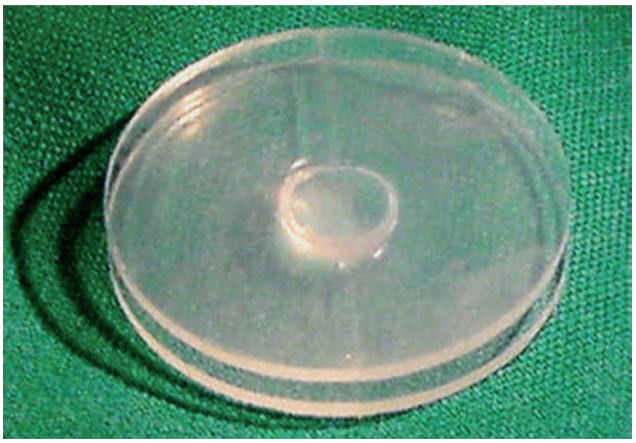

Figure 1. Nasal septal button, silicone (standard shape - 30 mm in diameter).

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 admittion 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 synehia 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.

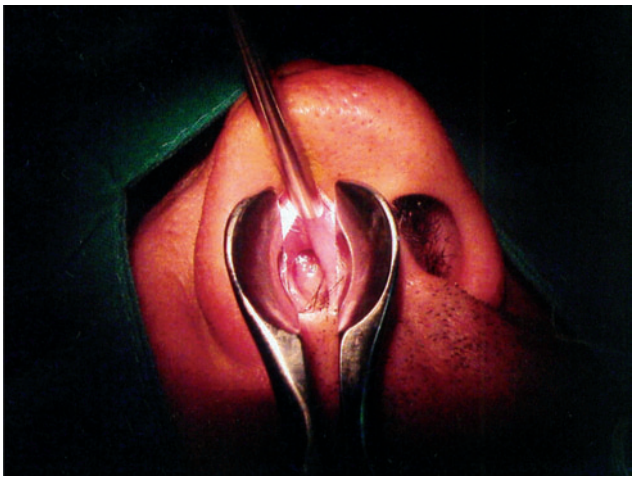

Figure 2. Rhinoscopic view from right nostril. An large septal perforation is visible (findings after crusts removal).

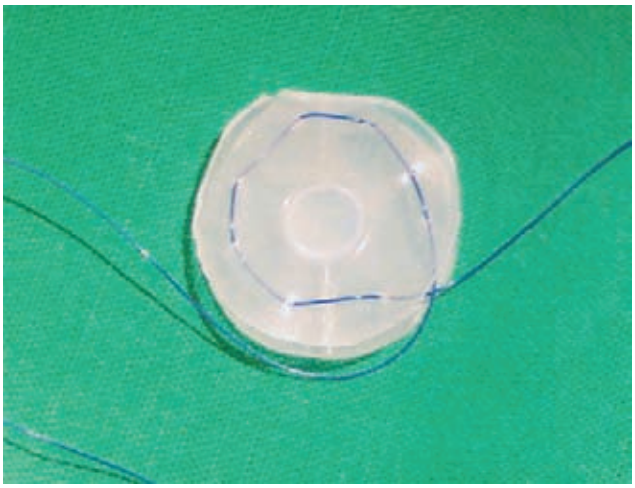

Figure 3. Septal button after modeling and placement of pursestring stitch.

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

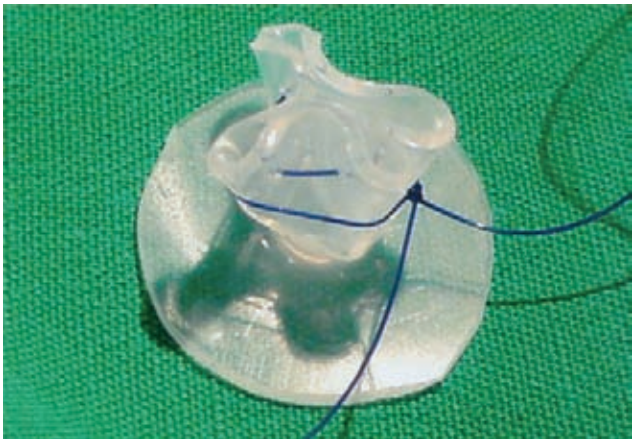

Figure 4. Septal button after the first tie was secured.

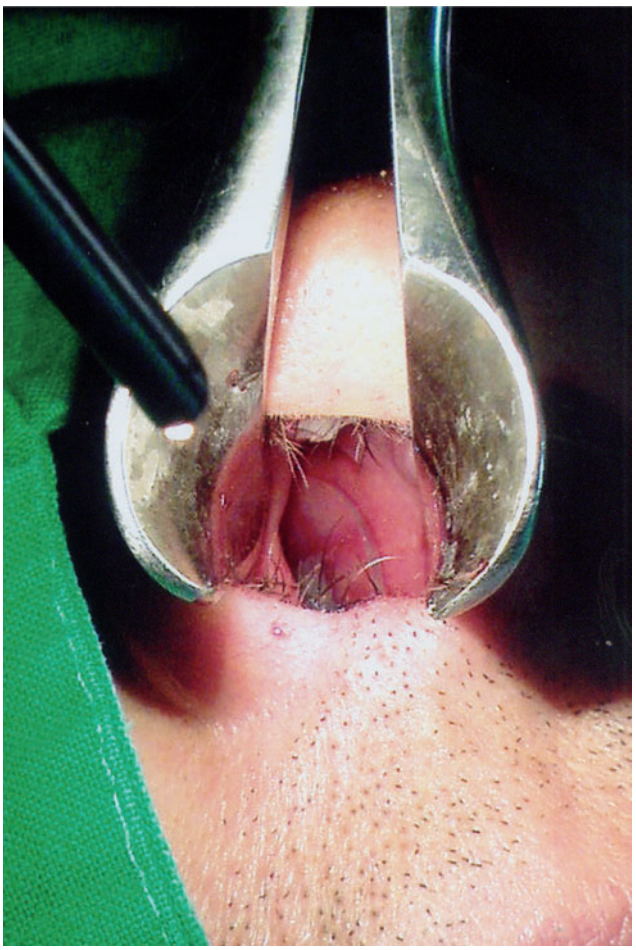

Figure 5. Rhinoscopic view from the right nostril. One-piece silicone nasal septal button immediately after insertion.

adjusted according to the perforation shape, and was subsequently prepared for the insertion according to the Kerry and Lee method (figure 3 and 4). The button was easily installed into the perforation (figure 5). The patient underwent the entire procedure quite well, complaining of no pain or any other kind of discomfort, whatsoever. Two gauze strips with antibiotic ointment were placed in the synechia resection region, which were removed two days after the procedure, when the patient was discharged from the hospital.

Postoperatively, providing ambulatory medical care, we treated the sore surfaces of the synechia trinned 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 of 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 frontaly 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 implatation of nasal septal button from various materials, such as: rubber, acrylate, resin, silicon. The advantages of septal

button insertion would be the following: a relatively simple implatation 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

1. Papay FA, Eliachar I, Risica R, Carroll M. Large septal perforations. Repair using inferior turbinate sliding advancement flap. Am J Rhinol 1989; 3: 185–9.
2. Mckinstry RE, Johnson JT. Acrylic nasal septal obturators for nasal septal perforations. Laryngoscope 1989; 99: 560–3.
3. Kelly G, Lee P. A New Technique for the insertion of a silastic button for septal perforation. Laryngoscope 2001; 111: 539–40.
4. Brain D. Septo-rhinoplasty: closure of septal perforation. Laryngol Otol 1980; 94: 495–505.
5. Osma U, Cureoglu S, Akbulut N, Meric F, Topcu I. The results of septal button insertion in the management of nasal septal perforation. Laryngol Otol 1999; 113: 823–4.
6. Haye R. Septal perforation – prosthetic and surgical treatment. Rhinology 1989; 27: 11–5.
7. Tipton JB. Closure of large septal perforations with a labial-buccal flap. Plast Reconstr Surg 1970; 46: 514–5.
8. Woolford TJ, Jones NS. Repair of nasal septal perforations using local mucosal flaps and a composite cartilage graft. J Laryngol Otol 2001; 115: 22–5.
9. Brain D. The nasal septum. In: Kerr AG, ed: Scott-Brown's Otolaryngology. London: Butterworth & Co, 1989: 154–7.
10. Romo T3d, Sclafani AP, Falk AN, Toffel PH. A graduated approach to the repair of nasal septal perforations. Plast Reconstr Surg 1999; 103: 66–75.
11. Eliachar I, Mastros NP. Improved nasal septal prosthetic button. Otolaryngol Head Neck Surg 1995; 112: 347–9.
12. Price DL, Sherris DA, Kern EB. Computed tomography for constructing custom nasal septal button. Arch Otolaryngol Head Neck Surg 2003; 129: 1236–9.
13. Luff DA, Kam A, Bruce IA, Willatt DJ. Nasal septum buttons: symptom scores and satisfaction. J Laryngol Otol 2002; 116: 1001–4.
14. Berry S, Brandrick J. Septal perforation – are septal buttons better?: a retrospective analysis. The Internet Journal of Otorhinolaryngology, 2005; 4: 1–4. (Accessed in Oct 2007 at http://www.ispub. com/ostia/index. php? xml-FilePath=journals/ijorl/vol4n1/septal.xml).
15. Mullace M, Gorini E, Sbrocca M, Artesi L, Mevio N. Management of nasal septal perforation using silicone nasal septal button. Acta Otorhinolaryngol Ital 2006; 26: 216–8.

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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.

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

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ACKNOWLEDGMENTS

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