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S A D R Ž A J / C O N T E N T S

Stavnovnici Beograda ispisuju poruke podrške i saosećanja sa narodom Japana koji je pretrpeo velike ljudske i materijalne žrtve u nedavnom katastrofalnom zemljotresu praćenom cunamijem. Nastala oštećenja na nuklearnoj elektrani u oblasti Fukušima zapretila su novom katastrofom, tačno četvrt veka posle černobiljskog akcidenta (26.4.1986), čije posledice po zdravlje stanovništva gotovo cele severne zemljine hemisfere još uvek nisu do kraja sagledane (vidi Uvodnik, str. 299–300).

Citizens of Belgrade, Serbia, write messages of support and compassion for people of Japan who suffered a huge human and material loss in the recent catastrophic earthquake fallowed by tsunami. The consequential damages to the Nuclear Power Plant in the area of Fukushima are threatening by a new disaster exactly 25 years after the Chernobyl accident (April 26, 1986)the consequence of which to the health of almost whole Northern Hemisphere have not yet been completely recognized (See Editorial, page 299–300).

E D I T O R I A L / U V O D N I K

Japan in our hearts

Japan u našim srcima

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It seems like we have recently written an Editorial for the May issue of *Vojnosanitetski Pregled* dedicated to the 20th Anniversary of the Chernobyl accident, but it was five years ago and we are now approaching April 2011 and marking the 25th Anniversary of the largest peacetime nuclear accident [1, 2]. Instead of reporting readership on better situation in Belarus and Ukraine, this Editorial remains under the shadow of a new nuclear accident in the Nuclear Power Plant (NPP) in Fukushima, Japan.

April, it is said to be the most beautiful month in Japan. It is the time of *Sakura Matsuri* – Cherry Blossom Holidays, the Holidays of spring and flowering. At evenings, in Ueno Park, Tokyo, paper lanterns are switched on, and under their flickering light pink flowers of over a thousand cherry trees can be seen even during the night, to welcome the new cycle and new life coming.

This year, citizens of Japan expect April overwhelmed with grief over the loss of their loved ones in one of the greatest natural disasters. On March 11, in a series of devastating earthquakes and tsunami, over 20 000 Japanese lost lives. The whole settlements were destroyed. And, as that was not enough, came the news that the NPP Fukushima was damaged, and a superhuman effort was undertaken to prevent melting of the reactor and a large-scale nuclear disaster [3].

The whole world was frightened. It is thought about the scale of the accident intensity, how much radioactive material could be discharged into the environment, where could the radioactive cloud be carried by winds, how long to arrive, estimation of contamination of individual territories, which measures should be taken... The news arrived that the strength and type of these facilities are not similar to those at Chernobyl, and that the accident could not be anywhere near as in Chernobyl, or perhaps still can spread radioactive cloud...

All the time 50 devoted workers of the NPP Fukushima, risking their lives, tried to keep the reactor under control and prevent a large-scale contamination. Government carried out evacuation of vulnerable people settled within a radius of 20 km, and then 30 km from the NPP. Industrious workers of Japan, in just seven days restored the access roads and transmission lines, providing electricity needed for the reactor cooling [4]. During that time citizens of Japan peacefully, with dignity, in quiet pain were trying to find their loved ones in ruins, patiently waited in queues for the necessary supplies, paying their bills in supermarkets without the staff and expressing full confidence in government and the future of their country. At each step, they express deep appreciation and respect for members of rescue teams.

These days we are getting news on a reactor set under control, on the risk of nuclear accidents reduced to much lesser extent, and that there will be need for substantial resources and time-consuming work for the full restoration of this accident. At the same time, there are information about the problems in Chernobyl. The cost for maintaining the safety of the damaged reactor at Chernobyl, only this year, is estimated to 92 million dollars. The additional funding will be needed to finish a new sarcophagus by 2014, which will provide adequate protection of the population of Ukraine and Europe. The international community has to provide these funds, because Ukraine cannot do it by itself [5]. Environmental problems, economic collapse, social and psychological problems are still present there. The efforts and costs of rehabilitation of the consequences on health of the exposed population are referred by every European country as part of their program for the treatment and control of malignant disease [6].

During both accidents citizens of Serbia expressed amazing extent of humanity, desire to provide help and support the people of both countries. And if we, 25 years before, thought that our sensitivity was the result of understanding and compassion for similar people, today, when the impoverished citizens of Serbia are collecting donations, making thousands of paper cranes for good luck and form a flag of Japan from their bodies, it is clear that the similarity of na-

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tions is not the only reason. The citizens of Serbia remember and respect the understanding and help sent by people of Japan in previous years.

We are so different from the Japanese people. In our Editorial written five years ago we discussed about the need to organize a National Network for the case of accident, change legislation in the field of radiation protection and nuclear safety, establish a center for emergency situations, harmonize with EU regulations, introduce standard procedures, etc. Today, five years later we still think so. However, we have established the Agency for Radiation Protection and Nuclear Safety, which has no proper support yet, the Center for Emergency Situations, which is not completely functional, but still trying to change legislation and harmonize it with that of the EU, we did not organize a National Network, and still have many concerns and unresolved problems.

During these five years, surrounding NPP were getting older, the new ones were built, potential problems multiplied. And while we listen to reports from Japan and admire experts and people of Japan, we are inspired to make effort to solve our problems. As if we were ashamed of the lessons given by people of Japan.

We hope that personal tone of this Editorial will not be criticized. These days, since March 11, watching the tragic events in Japan, we have been thinking of our teachers and friends, especially, in July 2010 in the Military Medical Academy, (MMA) Belgrade, IAEA representatives Prof. Akashi, MD and Ms. Tominaga MD, who presented the paper on "Medical Response to Radiological Mass Casualty Event". Their lectures, the valuable experience and benevolent attitude of Prof, Akashi will remain in the indelible memory of the MMA team.

On that occasion, we exchanged little gifts. We received a souvenir marked with three symbols: a huge wave, proud Japanese woman in the traditional kimono and *Sakura*. Only recently we have understood (or we just think so) the meaning of these symbols: "When life encounters a major problem, knowledge, dignity and patience will help you win and go on living with faith in better future". According to the behaviour of people of Japan, they seem to know this very well. These days the rest of the world have the opportunity to learn from them. Very generous from Prof. Akashi and the people of Japan. We thank them for that.

We wish all the best to people of Japan. They earn and deserve that. Most of all, we wish them victory in this battle, with as few losses as possible. And, of course, a peaceful, this year's *Sakura Matsuri*, with no more threats.

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ORIGINAL ARTICLES

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The influence of CD40 ligation and interferon-γ on functional properties of human monocyte-derived dendritic cells activated with polyinosinic-polycytidylic acid

Uticaj povezivanja CD40 molekula i interferona-γ na funkcionalna svojstva dendritičnih ćelija monocitnog porekla aktivisanih poliinosinsko-policitidilinskom kiselinom

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Abstract

Background/Aim. Ligation of a Toll-like receptor (TLR) by specific TLR agonists is a powerful tool for maturation induction of monocyte-derived dendritic cells (MoDCs). Studies so far have shown that the treatment of dendritic cells (DCs) with a TLR3 ligand, polyinosinic-polycytidylic acid [Poly(I:C)], may be an appropriate activation agent for obtaining mature MoDCs, competent to prime effective immune responses. However, little is known about how subsequent interaction of MoDCs with T cell-derived stimuli, such as CD40 or interferon-γ (IFN-γ), modulates MoDC functions. Therefore, this problem was the main objective of this study. **Methods.** Immature MoDCs were prepared by cultivation of monocytes from peripheral blood mononuclear cells with granulocyte macrophage-colony stimulating factor (GM-CSF) and interleukin (IL)-4 for 5 days. After that, maturation was induced by the treatment of these cells with Poly(I:C) for 2 days. At day 6, immature MoDCs and Poly(I:C)-activated MoDCs were incubated either with CD40 ligand (L)-transfected J558 cells or IFN-γ for additional 24 hours. Cytokine production was measured by ELISA and FlowCytomix Human T helper Th1/Th2 11plex. Allostimulatory capability of MoDCs was tested using an allogeneic mixed leukocyte reaction (MLR) assay. **Results.** Immature MoDCs showed a moderate potential for stimulation of proliferation of $CD4^+$ T cells, which was enhanced by the treatment with Poly(I:C). Ligation of CD40 or treatment with IFN-γ of immature or Poly(I:C)-treated MoDCs significantly up-regulated their allostimulatory activity. MoDCs matured in the presence of Poly(I:C) up-regulated the production of IL-12 and IL-10, which was followed by increased levels of IFN-γ and decreased levels of IL-5 in co-cultures with allogeneic $CD4^+$ T cells. Ligation of CD40 on immature MoDCs up-regulated the production of IL-12 and IL-23 which was accompanied by increased secretion of IFN-γ in co-culture. Stimulation of CD40 on Poly(I:C)-treated MoDCs significantly enhanced the production of IL-12, IL-23 and IL-10. However, such treated MoDCs decreased the production of IFN-γ and IL-10 and up-regulated the secretion of IL-17. Immature MoDCs treated with IFN-γ up-regulated IL-12, but lowered the production of IL-5 and IL-17 by $CD4^+$ T cells. Treatment of Poly(I:C)-activated MoDCs with IFN-γ down-regulated the production of IL-12 and up-regulated IL-10 by these cells and increased/decreased the levels of IL-10/IFN-γ, respectively, in co-culture with $CD4^+$ T cells. **Conclusion.** Treatment with Poly(I:C) or ligation of CD40 on immature MoDCs induces maturation of these cells into a phenotype that supports Th1 response. Activation of CD40 on Poly(I:C)-treated MoDCs shifts the immune response towards Th17. Treatment of immature MoDCs with IFN-γ down-regulated Th2 and Th17 responses. However, addition of IFN-γ to Poly(I:C)-activated MoDCs down-regulated Th1 response and promote T regulatory mechanisms. Each of these results may have functional and therapeutic implications.

Key words:
dendritic cells; CD40 ligand; interferon-gamma; poly I-C.

Apstrakt

Uvod/Cilj. Poliinosinsko-policitidilinska kiselina [*Polyinosinic-polycytidylic acid* – Poli (I:C)] stimuliše funkcionalno i fenotipsko sazrevanje dendritičnih ćelija (DC). Međutim, malo je podataka o modulaciji funkcije DC tokom interakcije sa T-limfocitima posredovanoj receptorom CD40 i interferonom-γ (IFN-γ), što je bio cilj ovog istraživanja.

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Metode. Nezrele DC dobijene su kultivacijom monocita (Mo) iz periferne krvi u prisustvu faktora stimulacije granulocitno-makrofagnih kolonija (*Granulocyte-Macrophage Colony-Stimulating Factor* – GM-CSF) i interleukina (IL)-4 tokom pet dana. Sazrevanje je indukovano dvodnevnom inkubacijom MoDC sa Poli(I:C). Poslednja 24 časa, nezrele i zrele MoDC kultivisane su sa ćelijama J558 koje su transfektovane ligandom CD40 ili u prisustvu IFN-γ. Produkcija citokina određivana je ELISA metodom, a alostimulatorna sposobnost u mešanoj leukocitnoj kulturi. **Rezultati.** Stimulacija nezrelih MoDC sa Poli(I:C) povećala je sekreciju IL-12, njihovu alostimulatornu sposobnost i produkciju IFN-γ u kokulturi sa $CD4^+$ T limfocitima. Slični rezultati dobijeni su povezivanjem CD40 molekula ili tretiranjem nezrelih MoDC sa IFN-γ. Međutim, stimulacija CD40 molekula na MoDC koje su aktivisane sa Poli(I:C) povećala je produkciju IL-12, IL-23 i IL-10 što je pospešilo produkciju IL-17, a snizilo produkciju IFN-γ i IL-10 u MoDC/$CD4^+$ kokulturi. Suprotno tome, IFN-γ snizio je produkciju IL-12, a povećao produkciju IL-10 od strane MoDC aktivisanih sa Poli(I:C), što je bilo povezano sa sniženjem IFN-γ, a porastom nivoa IL-10 u ćelijskoj kokulturi. **Zaključak.** Poli(I:C), IFN-γ i povezivanje CD40 molekula su aktivatori sazrevanja MoDC i stimulatori Th1 imunog odgovora. Ligacija CD40 molekula na MoDC aktivisanim sa Poli(I:C) usmerava u pravcu Th17, a inhibira Th1 imuni odgovor. U istom modelu IFN-γ inhibira Th1 odgovor, a stimuliše imunoregulatorne mehanizme. Svaki od dobijenih rezultata može imati specifične funkcijske ili terapeutske implikacije.

Ključne reči:
ćelije, dendritične; CD40 ligand; interferon-gama; poli I-C.

Introduction

Dendritic cells (DCs) are bone marrow-derived cells that function as antigen-presenting cells (APCs). Immature DCs in the periphery capture and process antigens and have a low T cell stimulatory capability. These potent APCs express a wide variety of pattern recognition receptors (PRRs) by which they recognize a conserved groups of molecules, collectively known as molecular patterns (MPs). Activation of PRRs triggers signaling pathways resulting in phenotypic changes and functional maturation of DCs. An important group of PRRs are Toll-like receptors (TLRs) which are crucial proteins that link innate and adaptive immunity [1].

Upon encounter inflammatory cytokines, bacterial or viral products, DCs enter a crossroad where their fate, migratory type or cytokine-producing type is determined. At this stage DCs express costimulatory molecules, migrate to lymphoid organs and secrete cytokines to initiate immune responses [2, 3]. Inflammatory and innate cytokines create the environment in which antigen-specific adaptive T cells expand and differentiate into different effector $CD4^+$ T cells such as T helper (Th1, Th2, Th17) and various subsets of T cells with regulatory activities (Tregs) [4].

Dendritic cells are also important in antitumor immunity and DC-based cancer vaccines have given the encouraging results [5]. Human monocyte-derived DCs (MoDCs) are currently the major source of DCs used in clinical vaccination protocols for the treatment of cancer [6]. MoDCs can be easily prepared by plastic adherence of monocytes from peripheral blood mononuclear cells (PBMCs) and subsequent incubation of the cells for several days in granulocyte macrophage-colony stimulating factor (GM-CSF) and interleukin (IL)-4 containing medium [7]. *In vivo*, human DCs have been shown to be more efficient than immature DCs in inducing specific antitumor antigen proliferative and cytotoxic T cell responses [8,9]. Therefore, an important goal in immunotherapy is to identify an optimal protocol for DC maturation. *In vitro* generated mature DCs should produce IL-12 after migration to the lymph nodes and upon subsequent contact with T cell in order to stimulate Th1 immune response and thus maximize clinical efficacy [10].

Ligation of different TLRs by specific TLR agonists is a powerful tool for induction of DC maturation both *in vitro* and *in vivo*. Polyinosinic-polycytidylic acid – Poly(I:C), a synthetic analogue of dsRNA and a TLR3 agonist, triggers the maturation of MoDCs into a phenotype that strongly supports the Th1 responses [11]. Poly(I:C)-treated DCs show a mature phenotype with high expression of costimulatory molecules and a maturation marker, CD83 [12]. Therefore, Poly(I:C) may be an appropriate maturation agent for obtaining stable homogenous mature DCs that are potentially competent to prime effective immune responses *in vivo*. This is supported by the experiments showing that such treated DCs retain the ability to secrete bioactive IL-12 in lymph nodes which is initiated during the *ex vivo* maturation step [10].

CD40 is a cell surface receptor that belongs to the tumor necrosis factor-R (TNF-R) family. Ligation of CD40 on DCs plays an important role in an enhanced survival of these cells, secretion of cytokines and enzymes as well as in enhanced tumoricidal activity and NO synthesis. CD40 ligand (CD40L), is mainly expressed on activated $CD4^+$ T cells. CD40:CD40L interaction has shown the complexity and importance in T cell-dependent humoral immune responses, in acquired cellular immune responses as well as in innate immunity. DC:T-cell interaction via CD40:CD40L upregulates the expression of costimulatory and adhesion molecules on DCs and triggers DCs to secrete IL-12 [13, l4]. These data suggest that ligation of CD40 on DCs may be an additional way to enhance IL-12 production and Th1 immune response. So far, therapies targeting CD40 have been designed to trigger CD40 signaling and thus boost the immune response against tumor. It should be noted that biologically relevant production of IL-12 by DCs is not induced by CD40 engagement alone but requires a second signal [15] which can be provided by other stimuli such as IFN-γ, a key Th1 cytokine.

Interferon-γ is one of the most powerful DC potentiating agent. This cytokine, which is produced by natural killer (NK) and by Th1 cells [16, 17] promotes specific cytotoxic im-

munity by up-regulation of costimulatory and adhesion molecules, chemokines, antigen processing and presentation. IFNγ is a necessary costimulus for IL-12 production in MoDCs [15] and this amplification may be important in stabilization of the Th1 response.

Dendritic cells constatly receive multiple signals and need to integrate them to give a response appropriate to extracellular milieu. The involved factors (TLR3 ligand, CD40L, IFN-γ) may be of crucial importance for modulation of *ex vivo* generated DCs. However, little is known whether their combination may act sinergistically or antagonistically on DC functions and this scientific problem was the principle aim of this study.

Methods

Medium and reagents

Human MoDCs were cultured in RPMI 1640 medium (ICN, Costa Mesa, CA, USA) supplemented with 2 mM L-glutamine, 20 μg/mL gentamicin, 50 μM 2-mercaptoethanol (2-ME) and 10% heat inactivated fetal calf serum (FCS). Recombinant human IL-4 was purchased from Roche Diagnostics GmbH (Mannheim, Germany). Recombinant human GM-CSF (Leucomax, spec. activity 4.44×10^6 UI) was obtained from Schering-Plough (Basel, Switzerland). Final concentrations of Poly(I:C) (Sigma-Aldrich, Munich, Germany) and IFN-γ (R&D Systems, Minneapolis, USA) were 25 μg/mL and 5 ng/mL, respectively. The number of CD40L-expressing J558 cells was 1.8 x 10^6/mL.

Cell preparation and MoDC cultures

MoDCs were generated from PBMCs. Briefly, PBMCs from buffy coats of six healthy volunteers were isolated by density centrifugation on Lymphoprep gradient (Nycomed, Oslo, Norway), resuspended in 5 ml of 10% FCS with 2-ME in RPMI medium and allowed to adhere to plastic flasks. After 2 h at 37°C, non-adherent cells were removed and adherent cells were cultured in 5 ml of control medium containing GM-CSF (100 ng/mL) and IL-4 (20 ng/mL). At day 3, 2.5 mL of medium was removed and replaced by the same volume of fresh medium containing GM-CSF and IL-4. After 6 days MoDCs were replated (5 x 10^5 cells/mL) in medium with a GM-CSF/IL-4 and Poly(I:C). At day 7, half of each of these cultures were incubated with J558 cells or with IFN-γ for additional 24 hours. After 8 days, cell-free supernatants were collected and stored at − 20°C for the subsequent determination of cytokine levels.

Allogeneic T-cell activation

The ability of T cells to proliferate was tested in an allogeneic mixed leukocyte reaction (MLR). $CD4^+$ T cells were used as responders in MLR, after their isolation from PBMCs using immunomagnetic sorting with $CD4^+$ isolation kits (MACS technology, Myltenyi Biotec, Bergish Gladbach, Germany) following instructions of the manufacturer. After loading the cell suspension onto a column placed in the magnetic field of a MACS Separator, unlabeled cells run through and this cell fraction consists mainly of the $CD4^+$ T-cell subset as determined by flow cytometry using an anti-CD4 FITC (Serotec, Oxford, UK).

Purified $CD4^+$ T cells (1×10^5 cells/well) were cultivated for 5 days with different numbers of allogeneic MoDCs in complete RPMI medium with 10% FCS in 96-well round-bottomed cell culture plates. Different DC: T cells ratios were used. To assess cell proliferation, cells were pulsed with [^{3}H]-thymidine for the last 18 h (1 μCi/well, Amersham, Books, UK). Labeled cells were harvested onto glass fiber filters and the incorporation of the radionuclide into DNA was further measured by β-scintillation counting (LKB-1219 Rackbeta, Finland). Results were expressed as count per minute (cpm) ± SD of triplicates.

Cytokine assays

After 8 days MoDCs were treated with PMA (20 ng/mL) and ionomycin (500 ng/mL) for 8 hours to stimulate excretion of the synthesized cytokines. A similar procedure was used for stimulation of MoDC/$CD4^+$ T cell coculture after a 5 day incubation period. Cells were harvested, centrifugated and cell-free supernatants were collected and stored at − 20°C for the subsequent determination of cytokine levels. The levels of IL-12p70, IL-23, IL-17 and IL-10 were measured by sandwich ELISA assays from R&D Systems (Minneapolis, USA), following the manufacturer's instructions. The leveles of IFN-γ and IL-5 cytokines were evaluated using FlowCytomix Human Th1/Th2 11plex Kit from Bender MedSystems (Vienna, Austria).

Statistical analysis

Data were analyzed for significant differences using Student`s paired *t*-test ($p < 0.05$ was considered statistically significant).

Results

Effects of CD40 ligation and IFN-γ treatment on the cytokine production by MoDCs

Immature MoDCs were generated by incubating monocytes with GM-CSF and IL-4 for 5 days. After that, maturation was induced by the treatment of these cells with Poly(I:C) for 2 days. At day 6 immature MoDCs and MoDCs induced to mature with Poly(I:C) were incubated either with CD40L-transfected J558 cells or IFN-γ for additional 24 hours. The levels of IL-12, IL-23 and IL-10 were detected in culture supernantans.

The results presented in Figure 1 show that immature MoDCs produced a very small quantity of all three cytokines. Poly(I:C) treatment significantly enhanced the production of IL-12 and IL-10, whereas the production of IL-23 was not significantly changed. Ligation of CD40 on immature DCs was followed by up-regulation of IL-12 and IL-23. However, such treatment of Poly(I:C)-stimulated MoDCs resulted in about 2-fold, 20-fold and 3-fold increase in the production of IL-12, IL-23 and IL-10, respectively. The addition of IFN-γ to immature MoDCs exerted similar stimulatory effect on IL-12 production, as Poly(I:C) did. No significant effect was observed regarding IL-23 and

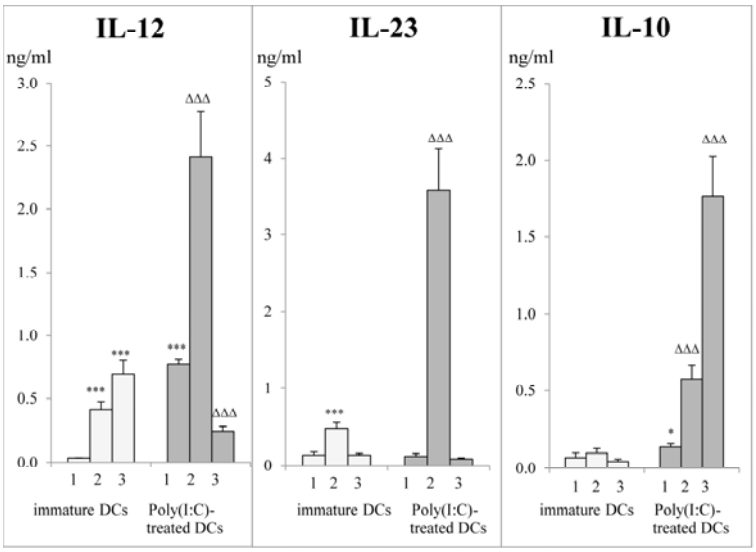

Fig. 1 - Cytokine production by immature human monocyte-derived dendritic cells (MoDCs) and polynosinic-polycytidylic acid – Poly(I:C)-treated MoDCs activated by CD40 ligation and interferon (IFN)-γ

Supernatants of immature and Poly(I:C)-treated MoDCs challenged with CD40L-transfected J558 cells or IFN-γ were collected and processed to determination of cytokine levels using sandwich ELISA assays.
Data represent mean values of six different experiments ± standard deviations (six donors).
Treatment: 1- control; 2- +CD40L; 3- +IFN-γ
***p* < 0.05, ****p* < 0.005 compared with immature MoDCs**
$^{\Delta\Delta\Delta}p$ < 0.005 compared with Poly(I:C)-treated MoDCs

IL-10 production. However, the addition of IFN-γ to the cultures of Poly(I:C)-treated MoDCs down-regulated the production of IL-12 and subsequently up-regulated (7-fold increase) the production of IL-10.

Effects of CD40 ligation and IFN-γ treatment on the allostimulatory activity of MoDCs

The influence of CD40 ligation and IFN-γ on the allostimulatory potential of immature and Poly(I:C)-treated MoDCs was examined in a MLR, where allogeneic $CD4^+$ T-cells were used as responders. The results are presented in Figure 2.

Immature MoDCs showed a moderate potential for stimulation of $CD4^+$ T-cell proliferation, which progressively decreased with lowering the number of DCs as stimulators. MoDCs matured in the presence of Poly(I:C) enhanced the allostimulatory activity of MoDCs at the highest (1 : 10) DC:$CD4^+$ T-cells ratio. Ligation of CD40 on immature MoDCs or IFN-γ treatment of these cells was followed by significant up-regulation in their allostimulatory activity. Such treatment of Poly(I:C)-activated MoDCs additionally enhanced the proliferation of allogeneic $CD4^+$ T-cells.

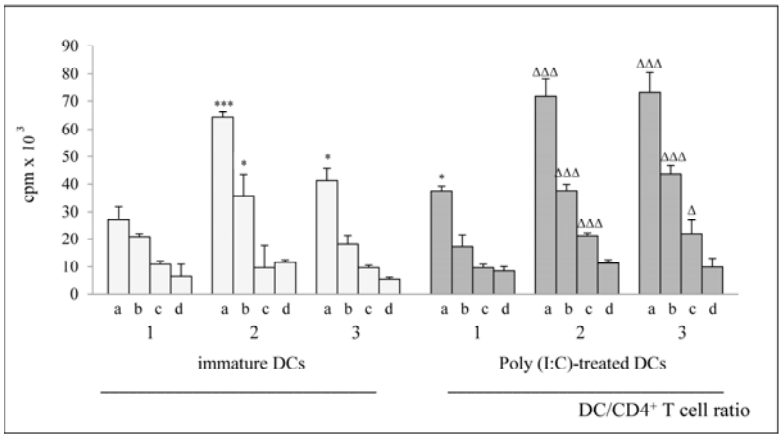

Fig. 2 - Allostimulatory activity of immature human monocyte-derived dendritic cells (MoDCs) and polynosinic-polycytidylic acid – Poly(I:C)-treated MoDCs stimulated with CD40L-transfected J558 cells and interferon (IFN)-γ

The ability of $CD4^+$ T cells to proliferate was tested in allogeneic mixed leukocyte reaction. Different rations of MoDC/$CD4^+$ T cells were used (a- 1:10; b- 1:20; c- 1:40; d- 1:80). After five days of culture cells were pulsed with $[H]^3$-thymidine (1 μCi/well) for the last 18 h. Incorporation of the radionuclide into DNA was measured by β-scintillation counting.
Data represent the mean value of triplicates ± standard deviations.
Treatment: 1- control; 2- +CD40L; 3- +IFN-γ.
***p* < 0.05, ****p* < 0.005 compared with control immature MoDCs**
$^{\Delta}p$ < 0.05, $^{\Delta\Delta\Delta}p$ < 0.005 compared with control Poly(I:C)-treated MoDCs

Effects of CD40 ligation and IFN-γ treatment on the Th polarization capability of MoDCs

The effect of MoDCs on the polarization of Th immune responses was measured by production of cytokines in DC/CD4$^+$ T-cell co-cultures.

As shown in Figure 3 treatment of MoDCs with Poly(I:C) up-regulated the production of Th1 cytokine (IFN-γ), and down-regulated the production of Th2 cytokine (IL-5), while the levels of IL-17 and IL-10 were not changed. Treatment of immature MoDCs with CD40L-transfected cells exerted similar effect on cytokine production as Poly(I:C) did, except that the production of IL-5 was not significantly changed. In contrast, ligation of CD40 on Poly(I:C)-treated DCs enhanced Th17 response and down-regulated Th1 response and production of IL-10. The addition of IFN-γ to immature MoDCs showed no significant effect on the production of IFN-γ, but lowered the production of IL-17 and IL-5. IFN-γ treatment of MoDCs, matured in the presence of Poly(I:C), significantly enhanced the levels of IL-10 and decreased production of IFN-γ, whereas the secretion of IL-17 and IL-5 was not significantly modulated.

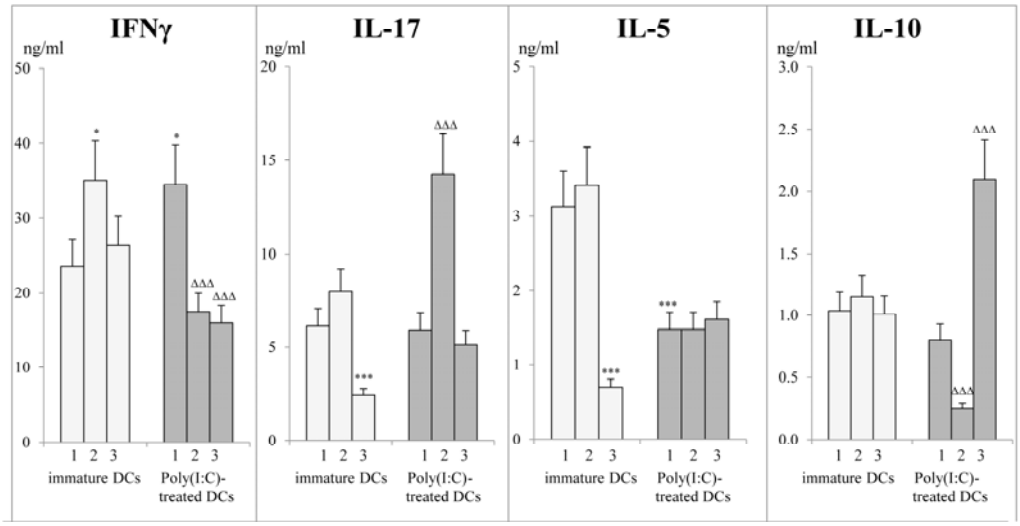

Fig. 3 - Polarization of Th immune response by immature human monocyte-derived dendritic cells (MoDCs) and polynosinic-polycytidylic acid – Poly(I:C)-treated MoDCs activated with CD40L-transfected cells and interferon (IFN)-γ
Production of cytokines in MoDC/CD4$^+$ T-cell cocultures was measured using sandwich ELISA assays and FlowCytomix Human Th1/Th2 11plex. Data represent mean values of three experiments ± standard deviations (three donors). Similar differences between groups were obtained with three other donors. However, the levels of all cytokines in these cultures were significantly lower (data not shown).
Treatment: 1- control; 2- +CD40L; 3- +IFN-γ.
***$p < 0.05$, *** $p < 0.005$ compared with control immature MoDCs**
$^{\Delta\Delta\Delta}p < 0.005$ compared with control Poly(I:C)-treated MoDCs

Discussion

Dendritic cells are professional APCs with an unique ability to prime naive T cells upon antigen presentation, regulate the type of T cell–mediated immune response, but also to induce immunological tolerance [18]. In our study we generated DCs *in vitro* from peripheral blood monocytes with GM-CSF and IL-4. It has been confirmed, similarly as in our previous study [19] and numerous other publications [10, 12, 20, 21] that such MoDCs are immature, triggered moderate allogeneic T cell response in MLR and produced low levels of IL-12 and IL-23, dominant Th1 and Th17 polarizing cytokines, respectively. Such characteristics are in accordance with the knowledge that the capacity of immature DCs to stimulate the immune response is rather weak and thus limits their clinical efficacy, especially as tumor vaccines [22].

Poly(I:C) is a synthetic analog of double-stranded RNA that binds to TLR3, a PRR highly expressed in immature MoDCs [23]. This TLR3 agonist behaves like MP and upon binding to TLR3 signals the presence of infectious agent, followed by activation of DCs and induction of protection to viral cytopathic effects [11]. Activation of DCs leads to induction of inflammatory cytokines and activation of IFN-β promoter, NF-κB and MAP kinases through engagement of the TRIF adaptor protein that cause DCs to mature [24, 25]. It is known that Poly(I:C) induces phenotypic maturation of MoDCs by up-regulation of co-stimulatory molecules (CD80, CD86 and CD40), and maturation marker, CD83 [10, 12]. This could be a dominant mechanism of increased allostimulatory activity of Poly(I:C)-treated MoDCs in our experiments. Poly(I:C) is also a very potent stimulator of IL-12 production and subsequent activator of the Th1 immune response both *in vitro* and *in vivo*, the properties desirable for induction of anti-tumor immunity [26]. This is confirmed in our present study, too. Therefore, Poly(I:C) may be an appropriate maturation agent for obtaining stable homogenous mature DCs that are potentially competent to prime effective immune responses *in vivo*. This is also supported by the experiments showing that Poly(I:C)-treated DCs retain the ability to secrete bioactive IL-12 in lymph

nodes which is initiated during the *ex vivo* maturation step [10]. We also showed that Poly(I:C)-treated MoDCs down-regulated the Th2 immune response, whereas the Th17 immune response was not significantly changed. Down-regulation of Th2 immune response is in agreement with the current concept of reciprocal regulation of Th1/Th2 balance [27].

Ligation of CD40 on DCs plays an important role in maturation and functional modulation of these cells [28, 29]. We used a CD40L-transfected cell line to simulate CD40:CD40L bidirectional crosstalk between DCs and T cells that provides reciprocal regulation of both lymphocytes and DCs [28, 29]. We showed that ligation of CD40 on immature or Poly(I:C)-treated MoDCs significantly up-regulated their allostimulatory activity, most probably as a consequence of increased expression of adhesion and co-stimulatory molecules, such as ICAM-1, HLA-DQ, CD80 and CD86 [30]. It has already been shown that the engagement of CD40 on immature MoDCs as a single signal induces high levels of Th1 polarizing cytokine IL-12 [15] and subsequent production of IFN-γ. We confirmed such results in our study. Moreover, we demonstrated that ligation of CD40 on immature MoDCs was followed by an increased production of IL-23 and IL-17, a phenomenon that has not been described so far. The production of IL-17 was additionally enhanced following ligation by CD40 on Poly(I:C)-treated MoDCs. IL-17 is a signature cytokine of the Th17 subset of $CD4^+$ T cells, whose expansion and maturation is promoted by IL-23 [31]. The Th17 immune response was potentiated after ligation of CD40 on Poly(I:C)-treated MoDCs, but this was followed by down-regulation of Th1 immune response.

Th1 cells were considered as the most important CD+ T cell subset for generating antitumor immunity because of their potential to enhance cytotoxic function of $CD8^+$ cells by producing IFN-γ, as a key activating factor. Recent publications shed new light on potential benefits of Th17 cells in rejection of tumors [32, 33]. Athough first it was considered that the effects of Th17 cells were dependent on IFN-γ and independent of IL-17 and IL-23, due to conversion of Th17 to Th1 [32], the protective function of Th17 cells, with maintained cytokine expression profile, against tumors have been confirmed [33]. The properties of Th17 cells, such as the ability to enhance inflammatory responses and to increase antigen presentation by DCs, promotion of leukocyte homing to tumors, facilitation of $CD8^+$ T cell priming and effector differentiation offer new possibilities for developing the Th17 cell-based therapy for tumors. Regarding the results of our study which are consistent with new insights of tumor immunotherapy, Poly(I:C) together with CD40 ligation generates desirable $CD4^+$ T cell subsets with suitable cytokine mileu for the treatment of tumors. However, such hypothesis needs further testings *in vivo* because we showed that signaling through CD40 on Poly(I:C)-treated MoDCs decreased the Th1 immune response. At the moment it is not known whether such balance between Th1 and Th17 immune response is optimal for antitumor immune response or not. It is known that the Th1 type of immune response could be harmful if exaggerated [34] and thus CD40 signaling could be protective and immunomodulatory.

Interferon-γ is classified as type II IFN in accordance with its receptor specificity and sequence homology [35]. IFNs were initially described as agents that interfere with viral replication [36]. IFN-γ is produced by NK cells and possibly by APCs during the early course of infection, while T lymphocytes became a major source of this cytokine in the adaptive immune response [37]. Cytokine increases antigen processing, presentation and APC costimulatory molecules [35]. We showed in this work that the treatment of immature or Poly(I:C)-activated MoDCs with IFN-γ also enhanced the proliferative activity of allogeneic $CD4^+$ T cells. The allostimulatory potential of MoDCs decreased by lowering the DC:$CD4^+$ T cell ratio. At higher ratios MoDCs showed lesser proliferative capability. One explanation could be that stimulatory effects of costimulatory and adhesion molecules and suitable levels of IL-12 are abrogated by low numbers of producing cells.

A significant finding of this study was related to the dual role of IFN-γ on IL-12 production: stimulation by immature MoDCs; suppression by Poly(I:C)-treated MoDCs. The increased production of IL-12 was followed by increased IFN-γ production and down-regulation of IL-5 and IL-17 production by $CD4^+$ T cells in co-culture. Up-regulation of IL-12 by immature MoDCs is in agreement with previous results [17]. The produced IL-12 attracts and activates T cells and NK cells to produce IFN-γ [14] which, in return, stimulate further production of IL-12 by amplifying loop. Down-regulation of IL-5 production could be explained by reciprocal regulation of Th1 and Th2 immune response [27] and by direct inhibitory effect of IFN-γ on the growth of Th2 cells [35]. The reason why IFN-γ-treated MoDCs inhibited IL-17 production without significant changes of IL-23 production is not clear. Since IL-23 predominantly acts on already differentiated Th17 cell subset [31], it is possible that IFN-γ-treated MoDCs inhibited the differentiation of $Th17^+$ cells by modulating the production of Th17 differentiation cytokines such as TGF-β, IL-1β, IL-6 and IL-21 [38]. Therefore, this hypothesis should be tested in the next experiment.

The inhibition of IL-12 production and stimulation of IL-10 production by IFN-γ- and Poly(I:C)-treated MoDCs is an important finding which could be relevant for down-regulation of Th1 immune response and promotion of an IL-10-mediated immunoregulatory milieu. It is not completely clear whether IFN-γ primarily acts on down-regulation of IL-12 by Poly(I:C)-activated MoDCs or on up-regulation of IL-10 production. It is known that IL-10 is a potent anti-inflammatory and immunosuppresive cytokine that inhibits the production of IL-12 by MoDCs [39]. Therefore, IL-10 is a very important cytokine for self-limiting Th1 cell-mediated immunopathology in conditions of strong inflammatory stimuli [40, 41].

Recently it has been shown that IFN-γ, beside amplifying production of pro-inflammatory cytokines during activation of DCs, also triggers an immunosuppressive enzyme indoleamine 2,3-dioxygenase (IDO) activity in DCs [42]. It is known that IDO^+ DCs exert immunoregulatory potential which is important for down-regulation of the immune response [43]. In addition, cytokine can also induce the development of adaptive

regulatory T cells [42]. Cumulatively, our results support the concept that IFN-γ, as a dominant Th1 effector cytokine, with the pro-inflammatory properties could be also an important down-regulator of strong immune response.

Conclusion

Treatment with Poly(I:C) or ligation of CD40 on immature MoDCs induced maturation of these cells into a phenotype that supports Th1 response. Activation of CD40 on Poly(I:C)-treated MoDCs shifted the immune response towards Th17. Treatment of immature MoDCs with IFN-γ down-regulated Th2 and Th17 responses. However, addition of IFN-γ to Poly(I:C)-treated MoDCs down-regulated Th1 response and promoted immunoregulatory mechanisms by induction of IL-10, thus limiting the exaggerated and potentially harmful immune response.

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ORIGINAL ARTICLE

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The frequency of sensitization to inhalatory allergens and concomitant rhinitis in asthmatic patients

Učestalost senzibilizacije na inhalatorne alergene i pridruženog rinitisa kod obolelih od astme

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Abstract

Background/Aim. Asthma is one of the most common chronic pulmonary diseases. The number of asthmatics has been continuously increasing all over the world. Depending on its causing agent, asthma is classified as allergic and nonallergic. Asthma is often associated with other allergic diseases, and it is most commonly preceded by the symptoms of rhinitis. The aim of this study was to establish the type and frequency of allergic sensitization to inhalatory allergens, frequency of concomitant rhinitis, gender and age-related distribution of asthma, and the presence of some risk factors in patients with diagnosed asthma. **Methods.** This retrospective and partially prospective analysis included 733 patients with asthma diagnosed in the Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica over the period January, 2004–December, 2008. The obtained date were statistically processed. **Results.** Females were significantly more often affected by asthma ($p < 0.05$), most frequently at 20–29 years of age. A hereditary predisposition to the diseases in terms of atopy was registered in 34.9% of the examined subjects. Most patients had allergic asthma (79.5%). Sensitization to internal and external inhalatory allergens was verified in 77.5% and 67.6% of the patients respectively, and combined hypersensitivity to both allergen types in 48.8% of the patients. Rhinitis was registered in 63.9% and 28% of the patients with allergic and nonallergic asthma, respectively. Rhinitis symptoms preceded the occurrence of asthma in 60% of the patients, with the precedence of rhinitis ranging from 1 to 27 years. A high correlation between rhinitis and asthma was established for the disease of both allergic ($r = 0.92$) and nonallergic ($r = 0.88$) etiology. **Conclusion**. The majority of the patients have allergic asthma, and they are females at 20–29 years of age. Senstization to internal allergens is most common, and then to external ones. Rhinitis is the most common concomitant disease, usually preceding the occurrence of asthmatic symptoms.

Key words:
asthma; rhinitis; allergens; comorbidity.

Apstrakt

Uvod/Cilj. Astma je jedna od najčešćih hroničnih plućnih bolesti. Broj obolelih neprestano se povećava u celom svetu. U odnosu na uzročni faktor astma se klasifikuje na alergijsku i nealergijsku. Često postoji udruženost astme i drugih alergijskih bolesti, a najčešće njenoj pojavi prethode simptomi rinitisa. Cilj ovog rada bio je da se utvrde vrsta i učestalost alergijske senzibilizacije na inhalatorne alergene, postojanje drugih pridruženih alergijskih bolesti, distribucija astme u odnosu na pol i starost i postojanje nekih rizičnih faktora kod osoba sa dijagnostikovanom astmom. **Metode.** Retrospektivno, delom prospektivno, analizirano je 733 bolesnika sa dijagnostikovanom astmom u Institutu za plućne bolesti Vojvodine u Sremskoj Kamenici, u periodu od januara 2004. do decembra 2008. godine. Dobijeni podaci statistički su obrađeni. **Rezultati.** Značajno češće od astme boluju žene ($p < 0,05$), a starost bolesnika najčešće je od 20 do 29 godina. Genetska predispozicija u smislu atopije postoji kod 34,9% ispitanika. U najvećem procentu dokazana je alergijska astma (79,5%). Senzibilizacija na unutrašnje inhalatorne alergene verifikovana je kod 77,5%, na spoljašnje kod 67,6%, a kombinovana na obe vrste alergena kod 48,8% ispitanika. Kod osoba sa alergijskom astmom rinitis postoji 63,9%, a kod onih sa nealergijskom astmom 28%. Simptomi rinitisa kod 60% obolelih javljaju se pre pojave astme u periodu od 1 do 27 godina. Visoka povezanost rinitisa i astme postoji i kod alergijske ($r = 0,92$) i kod nealergijske etiologije bolesti ($r = 0,88$). **Zaključak.** Najveći broj bolesnika ima alergijsku astmu, ženskog je pola i starosti od 20 do 29 godina. Najzastupljenija je senzibilizacija na unutrašnje alergene, a potom na spoljašnje. Najčešća pridružena alergijska bolest je rinitis, koji se većinom javlja znatno pre ispoljavanja simptoma astme.

Ključne reči:
astma; rinitis; alergeni; komorbiditet.

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Introduction

Allergic diseases have been constantly increasing in number over the last two decades. Their higher frequency has been particularly evident in children, young adults and in economically undeveloped countries. These diseases are therefore regarded as the epidemic of the 21st century and the modern civilisation diseases. The allergic diseases include the respiratory tract ones, food allergies and atopic dermatitis. The most common of them are allergic diseases of the respiratory tract: allergic rhinitis and asthma [1].

In 2004, the World Health Organisation (WHO) reported about 300 million people in the world suffering from asthma. Parallel to the development of modern society and rapid industrialisation, further increase of the prevalence of asthma is predicted all over the world (it has been estimated that additional 100 million people will be affected until 2025). Due to these facts, WHO experts call asthma „the quiet tsunami". It has been assessed that about 24% of the total worlds' population suffer from allergic rhinitis. It is not a severe, life-threatening disease, but can considerably disturb the quality of life of the affected subjects [2, 3].

Asthma is a chronic inflammation of the airways in which the important role is played by a variety of cells, including mastocytes, eosinophils, T-lymphocytes and epithelial cells. In hypersensitive subjects, this inflammation induces recurrent episodes of wheezing, suffocation, chest tightness and cough, particularly during the night and/or early in the morning. These symptoms are associated with a disseminated and changing, spontaneously or by drugs, at least partially reversible obstruction of air flow through the airways. Inflammation also causes an elevated reactivity of the airways to diverse stimulants, which may be present even when neither the disease symptoms, nor bronchial obstruction are manifested. Depending on its inducing agents, asthma is classified as allergic (extrinsic) and nonallergic (intrinsic). Allergic rhinitis is an inflammation of the nasal mucosa, mediated by the immunoglobulin (Ig) E mechanism. It is clinically manifested by sneezing, nasal secretion, blocked and itching nose [3, 4].

Risk factors for allergic respiratory tract diseases may be internal or external, acting usually in concomitance. Internal risk factors include genetic predisposition (atopy and hypersensitivity) and gender. Atopy is a predisposition of an organism to produce larger quantities of IgE antibodies in response to diverse substances from the external environment. External factors are allergens, infections, working environment, tobacco smoke, air pollution, live style and diet habits, food additives, drugs, and others [3, 4].

Establishing the diagnosis of asthma is a continual process. The basic procedures include history taking, physical examination, lung function tests (spirometry, bronchial challenge, bronchodilation test), confirmation of hypersensitivity to inhalant allergens by cutaneous tests or measuring the levels of specific antibodies. The cutaneous prick test is painless, easy to perform and very sensitive and highly specific for establishing allergy [1, 4].

The aim of this study was to define the frequency and type of sensitization to inhalant allegens in asthmatic subjects, as well the age-related distribution over the examined period, the presence of other allergic disorders, gender and age structure, as well as the presence of some predisposing and contribution factors for the disease genesis.

Methods

The study was retrospective and prospective in character. The data were collected from the files of 733 patients in the Allergology Unit of the Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica, where the patients with asthma were diagnosed and controlled. The investigation was done for the period from January 1, 2004 to December 31, 2008. The inclusion criterion was that a patient had the diagnosis of asthma established by routine diagnostic procedures applied in this highly specialised health institution.

The following parameters were examined and analysed: annual distribution, sex structure, age structure, smoking habits, genetic predisposition, type of asthma depending on its etiological factor, concomitance of other allergic diseases (allergic rhinitis, food allergy and atopic dermatitis), time correlation of the genesis of allergic rhinitis and asthma, type and frequency of hypersensitivity to standard inhalatory allergens established by prick testing.

The obtained results were statistically processed by the Microsoft Excel 2003 software for statistical and tabular calculations. Common statistical methods were applied – Student's *t*-test i Pearson's correlation analisis.

Results

In the examined 5-year period, there were 733 patients with the established diagnosis of asthma registered in the Allergy Unit of the Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica. All the patients lived in Vojvodina, mostly in Novi Sad community.

The number of asthmatic patients registered per year, and the patients' gender structure in each year are given in Figure 1 and 2, respectively. A statistically significant difference ($p < 0.05$) was registered between the number of affected male and female patients. Females are more frequently affected with asthma than males.

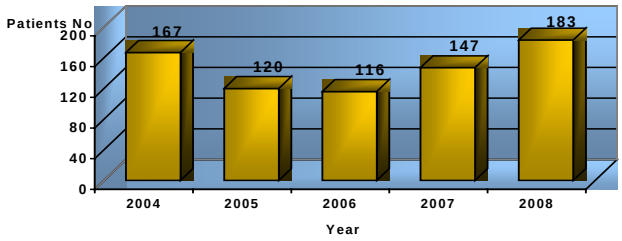

Fig. 1 – The number of the patients (n = 733) with the established diagnosis of asthma in a 5-year period

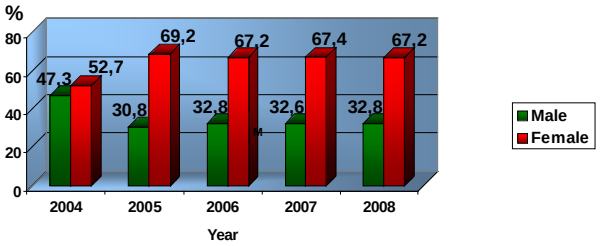

Fig. 2 – Distribution of the patients with the established diagnosis of asthma (n = 733) by gender in a 5-year period

The age structure of the affected patients is given in Figure 3. The age group from 20 to 29 is most frequently affected.

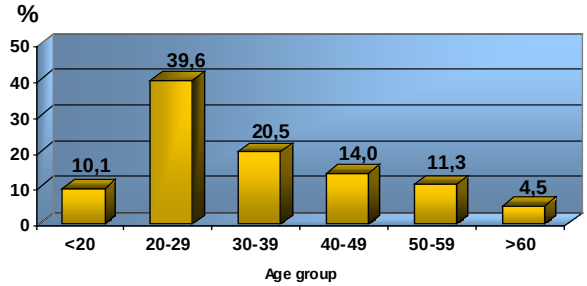

Fig. 3 – Age structure of the patients with the established diagnosis of asthma (n = 733)

The data on patients' smoking habits, genetic predisposition in terms of atopy, the distribution of nonallergic and allergic asthma established by cutaneous prick testing to standard inhalant allergens, concomitant allergic diseases, time correlation of allergic rhinitis and asthma occurrence, and frequency of rhinitis symptoms depending on asthma type are presented in table 1.

Table 1

Parameters examined in the patients with the established diagnosis of asthma (n = 733)

Parameters	% of patients
Smoking habits	
non-smokers	73.9
smokers	13.9
ex-smokers	12.2
Genetic predisposition	
positive atopy	34.9
negative atopy	65.1
Etiology-related type of asthma	
nonallergic asthma	20.5
allergic asthma	79.5
Concomitant allergic diseases (n = 583)	
allergic rhinitis	63.9
dermatitis	7.0
food allergy	3.1
Time correlation of allergic rhinitis and asthma occurrence	
earlier rhinitis	60.0
at the same time	40.0
Frequency of rhinitis symptoms depending on asthma type	
allergic asthma (n = 583)	64.0 (n = 373)
nonallergic assthma (n = 150)	28.0 (n = 42)

At least one concomitant allergic disease (allergic rhinitis, food allergy, atopic dermatitis) was registered in 74.1% of the patients with allergic asthma.

Three fifths of the patients (60%) developed the symptoms of allergic rhinitis prior to asthma over the period ranging from one to 27 years. A simultaneous onset of both diseases, within the same year, was registered in two fifths (40%) of the examined subjects.

The frequency of rhinitis symptoms in the patients with allergic and non-allergic asthma was found to be 64% and 28% of the patients, respectively.

By determining a correlation coefficient, a high correlation of rhinitis and asthma was demonstrated in allergic ($r = 0.92$) and non-allergic ($r = 0.88$) asthma.

Sensitization to at least one of the standard inhalant allergens, established by the cutaneous prick test, was found in 79.5% of the subjects. Distribution of sensitization to specific allergens is shown in Figure 4.

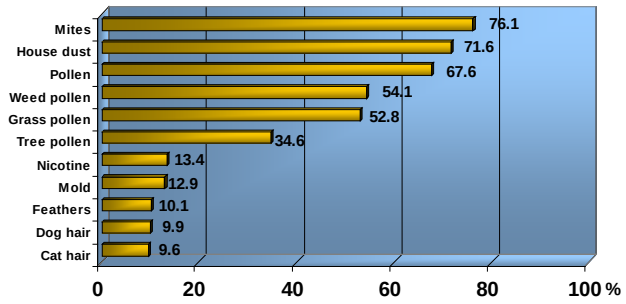

Fig. 4 – Type and frequency of hypersensitivity to standard inhalatory allergens (n = 583)

The incidence of sensitization to major inhalant allergens, classified into three classes depending on their origin (external, internal, and combined), is given in Figure 5.

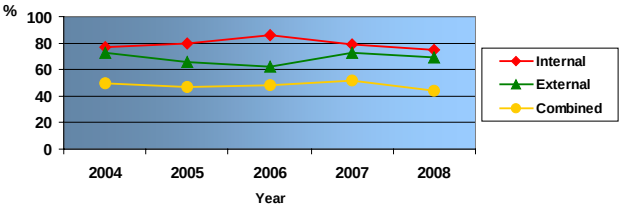

Fig. 5 – Incidence of sensitization to inhalatory allergens related to their origin

After examining exclusively the sensitization to pollens over the entire 5-year period, it was noticed that hypersensitivity to weed pollens is most common (54.1%), than to grass (52.8%) and tree (34.6%) pollens. The incidence of sensitization to pollens for each examined year is presented in Figure 6.

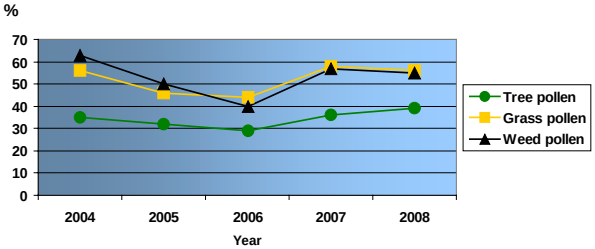

Fig. 6 – Incidence of sensitization related to the kind of pollen

Discussion

In the period from the beginning of 2004 to the end of 2008, there were 733 patients with the diagnosis of asthma registered in the Allergy Unit of the Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica. The smallest number of the patients were registered in 2006 (116), and the highest one in 2008 (183).

Asthma was significantly more often diagnosed in females (64.3%) than in males (35.7%), (female : male ratio = 1.8 : 1). This difference was statistically significant ($p < 0.05$), well correlating with the published articles, reporting the female : male ratio of asthmatic patients ranging from 1.04 to 1.9 [6, 7].

Most asthmatic patients belonged to the 20–29 year age group (39.6%), and then to the 30–39 year age group (20.5%). These results comply with the world trend. In this study, the fewest patients were older than 60 (4.5%), which is two times as few as reported in the literature [1, 8, 9].

It had long ago been clinically observed that asthma occurs more frequently in some families. In our examined group, genetic predisposition was registered in 34.9% of the subjects. Other authors report a higher percentage of atopy (44–53%) registered in the asthmatic families [1, 6].

Smoking is considered a relevant risk factor for the occurrence and a bad course and prognosis of asthma. Asthmatics who are active or ex–smokers more frequently have acute asthmatic attacks, more often require hospitalisation, respond to therapy worse, and have a worse control of the disease. Of the subjects examined in this group, 73.9% were non-smokers, 12.2% ex–smokers, and 13.9% were active smokers. Some authors report a similar structure regarding smoking habits of their asthmatic patients [1, 4, 7].

Analysing the type of asthma depending on hypersensitivity to inhalatory allergens, confirmed by the cutaneous prick test, allergic asthma was found to be much more frequent (79.5%) than non–allergic asthma (20.5%). The literature data generally suggest that patients with allergic asthma make 70–90% of all asthmatic patients [4, 10].

Allergy is a systemic disease of an organism, so allergic diseases are usually concomitant in one patient. At least one concomitant allergic disease was registered in 74.1% of the patients with allergic asthma. Food allergy was found in 3.1% of these patients, correlating to the literature data [11]. Atopic dermatitis was registered in 7.0% of these subjects. Some authors report that even up to 38.3% of the patients with allergic asthma have atopic dermatitis in their case histories [9]. The most common concomitant allergic disease is allergic rhinitis, registered in 63.9% of our patients with allergic asthma. This finding correlates with the literature data [2, 3, 7, 10]. Symptoms of allergic rhinitis usually precede the onset of asthma (in 60% of the patients), ranging in precedence from 1 to 27 years. A simultaneous onset of both diseases (in the same year) was registered in 40% of the patients.

In this study, by estimating the correlation coefficient, a high correlation was established between rhinitis and asthma in allergic ($r = 0.92$) and non-allergic ($r = 0.88$) etiology of the disease. The results obtained in our study correlate to the literature ones [7, 10].

The concomitance of upper and lower airways diseases has been known more than two millenniums, and Galen emphasized that secretion removal from the nasal pathways alleviated respiratory symptoms. Numerous contemporary studies have shown a concomitant occurrence of allergic rhinitis and asthma in the same patient. It has been observed that allergic rhinitis usually precedes the occurrence of asthma, and is therefore included among the risk factors for the disease. In addition, the presence of allergic rhinitis, particularly when untreated, aggravates the symptoms of asthma and makes its control and treatment more difficult. The concomitance and mutual influence of the two diseases are due to the anatomical proximity and histological similarity of the upper and lower airways, as well as to the similar pathophysiology and risk factors for the occurrence of both allergic rhinitis and asthma. Inflammation plays a crucial role in the pathogenesis of both diseases. The mechanisms which further explain their correlation include: obstruction of the nose and breathing on the mouth, with the nose losing its important preparatory function; aspiration of the postnasal secretion with mediators and/or cells into the lungs; resorption of the inflammatory cells and/or mediators into the systemic circulation; nasobronchial reflex [1, 3, 6, 12].

Exposure to inhalant allergens contributes to both exacerbation of the symptoms and the disease genesis itself, so the analysis of the frequency and the kind of sensitization to standard inhalatory allergens was particularly important. Hypersensitivity to at least one of standard inhalant allergens was registered in 79.5% of the patients. In most patients (77.5%), hypersensitivity to internal allergens was confirmed, while hypersensitivity to external allergens was established in 67.6% of the patients. Hypersensitivity to both allergen types was verified in 48.4% of the patients. Analysing hypersensitivity to single inhalant allergens, most patients were hypersensitive to mites (76.1%), correlating to the most internationally recognized results [1, 3, 6, 13]. In an international study, the European Community Respiratory Health Survey (ECRHS), it has been shown that rhinitis, with in fact an underlying hypersensitivity to only house dust mites, positively correlates to the occurrence of symptoms of asthma [6]. Hypersensitivity to weed pollens is most common (54.1%), then to grass pollen (52.8%), and tree pollen (34.6%). These results correlate to those obtained in the study which analyzed hypersensitivity to inhalant allergens in the population suffering from allergic respiratory diseases in the Novi Sad area, which may be due to the geographical features of Vojvodina [5]. In other countries, hypersensitivity to diverse pollens differs depending on the climatic and vegetation characteristics. Tree pollen allergy is most common in northern European countries, while hypersensitivity to grass pollens was most frequently verified in the majority of other European countries [1]. Hypersensitivity to nicotine was much more rarely verified (13.4%), as well as to mold (12.9%), and feathers (10.1%). Hypersensitivity to animal hair was registered in 16.2% of the patients, with almost even frequency of hypersensitivity to dog (9.9%) and cat hair (9.6%). Some studies report cat hair as the most common allergen in adult

population, and in children with rhinitis and/or asthma, ranging from 15–50% [3, 14]. It may be due to the fact that cat is the most common pet in these environments.

The international ECRHS study has pointed out that geographical features of a particular environment determine the presence of different external factors which affect the prevalence of atopy and asthma. Taking into account the fact that allergy is the most common inducing agent of asthma, and that most asthmatics are hyperpersensitive to at least one allergen, as well as that a recurrent exposure to that allergen may "trigger" the symptoms of asthma, current guidelines for the treatment of asthma and allergic rhinitis recommend the avoidance of the allergen as a relevant preventive measure. Although most allergens are impossible to be entirely avoided, it is generally considered that their smaller concentration and exposure may have positive effects on the control and course of asthma [3, 4, 15].

Conclusion

The number of asthmatics has been constantly increasing in recent years, predominantly in female population at the third decade of life. A high proportion of asthmatics have a concomitant rhinitis, which precedes the onset of asthma with the precedence period ranging from 1 to 27 years and a verified allergy to inhalant allergens.

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Uloga tipizacije humanog papiloma virusa i citologije u ranom otkrivanju recidiva cervikalne intraepitelne neoplazije

The role of human papillomavirus typization and cytology in early detection of relapse of cervical intraepithelial neoplasia

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Apstrakt

Uvod/Cilj. Bolesnice sa nekim od vidova lečenja intraepitelne neoplazije grlića materice imaju i do pet puta veći rizik od ostale populacije žena od recidiva bolesti. Cilj rada bio je da se ispita uloga tipizacije humanog papiloma virusa (HPV) i citologije u detekciji recidiva. **Metode.** Prospektivno kliničko istraživanje obuhvatilo je 35 bolesnica sa recidivom i 30 bez recidiva nakon lečenja cervikalne intraepitelne neoplazije. Kod svih ispitanica urađena je HPV tipizacija korišćenjem *polymerase chair reation* (PCR) metode i citološki test (konvencionalni Pap razmaz), a potom je izvršeno ocenjivanje primenjenih testova određivanjem njihove senzitivnosti, specifičnosti i pozitivne i negativne prediktivne vrednosti. **Rezultati.** Recidivi intraepitelnih neoplazija težeg gradusa javljaju se statistički značajno češće kod bolesnica koje i posle lečenja ostaju pozitivne na HPV. Bolesnice koje i posle lečenja intraepitelnih neoplazija ostaju pozitivne na HPV tipa 18 ili zajedno na HPV tipa 16 i 18, imaju češće recidive težeg gradusa, nego one koje su pozitivne samo na HPV tipa 16. Tipizacija HPV ima veću prediktivnu vrednost za dijagnostiku rezidualne bolesti kod starijih bolesnica. Senzitivnost HPV tipizacije za dijagnostiku recidiva intraepitelne neoplazije svih stadijuma je 68,57%, a za teže graduse, HSIL i MIK, 90,47%, specifičnost je 93,33%, pozitivna prediktivna vrednost 90,47%, a negativna prediktivna vrednost 93,53%. Senzitivnost citologije za dijagnostiku recidiva težeg gradusa je 80,95%. Korišćenjem HPV tipizacije zajedno sa citologijom ostvaruje se najveća senzitivnost od 95,23%. **Zaključak.** I HPV tipizacija i Pap test pružaju zadovoljavajuću senzitivnost i visoku specifičnost u otkrivanju recidiva cervikalnih intraepitelnih neoplazija posebno onih višeg gradusa. Najveća sensitivnost postiže se korišćenjem oba testa.

Ključne reči:
papiloma virus; citodijagnostika; grlić materice, neoplazme; osetljivost i specifičnost.

Abstract

Background/Aim. Female patients who underwent ceratin treatment forms of cervical intraepithelial neoplasia (CIN) are at five times greater risk for disease relapse in comparison to the rest of female population. The aim of the study was to investigate validity of human papillomavirus (HPV) typization and cytology in detection of relapse. **Methods.** The prospective clinical investigation included 35 patients with relapse and 30 ones without it after adequate treatment of cervical intraepithelial neoplasia. HPV typization using PCR methods and cytological test (conventional Pap smear) were performed in all the patients. Validation of tests applied was performed by determining their sensitivity, specificity, and positive and negative predictive value. **Results.** More severe degrees of CIN relapse occur significantly more often in patients which remain HPV positive despite of the treatment. The patients which remain positive on HPV type 18 or, on both HPV types 18 and 16, have more often CIN relapses of more severe degree in relation to those just positive on HPV type 16. HPV typization has higher predictive value for diagnosis of the rezidual disease in older patients. Sensitivity of HPV typization for diagnosis all CIN relapse degrees is 68.57%, for more severe degrees (HSIL and MIC) 90.47%, specificity is 93.33%, while positive and negative predictive values are 90.47% and 93.53%, respectively. Sensitivity of cytology for diagnosis of more severe CIN relapses is 80.95%. HPV typization used along with cytology ofters the highest sensitivity (95.23%). **Conclusion.** Both tests, HPV typization and Pap smear, offer satisfactory sensitivity and high specificity in detection of relapse, parcticularly those with more severe degree. The highest sensitivity in detection of CIN relapse is obtained by using both tests.

Key words:
papillomavirus infections; cytodiagnosis; uterine cervical neoplasms; sensitivity and specificity.

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Uvod

Procenat mladih žena koje se leče konizacijom ili nekom drugom operativnom tehnikom zbog premalignih i malignih bolesti grlića materice, u stalnom je porastu. Zbog činjenice da se radi o mladim bolesnicama koje se podvrgavaju raznim vidovima lečenja, važno je pronaći najadekvatniji i najsigurniji pristup njihovom daljem praćenju i lečenju. Najvažnija je rana detekcija eventualnih recidiva kao i detektovanje rizične grupe bolesnica za njihovu pojavu. Ovo je pogotovo važno zbog činjenice da se radi o bolesnicama koje još nisu završile svoju reproduktivnu funkciju i kojima bi svaka nova hiruška reintervencija smanjila mogućnost za obavljanje ove funkcije.

Metode koje koristimo u daljem praćenju bolesnica koje su lečene zbog intraepitelnih neoplazija grlića materice identične su onima koje se koriste pri njihovoj detekciji pre lečenja. Eksfolijativna citologija, kolposkopija, tipizacija humanog papiloma virusa (HPV tipizacija), biopsija, kiretaža cervikalnog kanala, jesu suverene dijagnostičke procedure koje se koriste za otkrivanje mogućih recidiva bolesti. Ono što iz ove cele grupe izdvaja HPV tipizaciju jeste činjenica da rezultat ove analize ne samo da pomaže u detekciji recidiva, već može da predstavlja i važan prognostički parametar koji ukazuje na povećan rizik od razvoja recidiva. Na taj način, zahvaljujući nalazu HPV tipzacije, može da se izdvoji posebna grupa bolesnica koju treba intenzivnije sagledati i pratiti nezavisno od klasičnog protokola o praćenju [1–3].

Sve bolesnice koje su imale neki od vidova lečenja intraepitelne neoplazije grlića materice i do dvadeset pet godina nakon lečenja imaju pet puta veći rizik od recidiva bolesti u odnosu na ostalu populaciju žena. Ovaj rizik daleko je veći kod bolesnica koje i posle lečenja ostanu pozitivne na HPV [1].

Procenat recidiva intraepitelnih neoplazija nakon lečenja kreće se od 8 do 19,3% [2]. Podaci iz literature o negativizaciji i eradikaciji HPV infekcije nakon lečenja, kao i podaci o procentu recidiva, razlikuju se, ali se često navodi da prosečno 39,2% bolesnica postaje negativno nakon operacije, a da su tipovi virusa koji u najmanjem procentu bivaju eradicirani lečenjem 16,18 i 33 [3].

Costa i sar. [4] iznose sličan procenat negativizacije (29,8%) na prvoj kontroli posle operacije, sa mesečnom stopom negativnih nalaza od 5,27%, tako da se na kraju taj procenat kreće do 63,5%.

Falliani i sar. [5] u jednom od radova novijeg datuma iznose podatak da je nakon 24 meseca praćenja bolesnica lečenih konizacijom laserom zbog cervikalnih intraepitelnih neoplazija (CIN) težeg gradusa (CIN II – III), procenat recidiva bio 15,4%. Negativizacija bolesnica na virus posle lečenja bila je 78,8%. Jedino su negativizacija na virus i zahvaćenost ivica konusa bili nezavisni i značajni prognostički faktori za pojavu recidiva. Ovi podaci, ujedno, pokazuju da u proseku svaka peta bolesnica lečena od CIN II–III (HSIL) promena ostaje pozitivna na HPV i posle operacije.

Cilj ovog rada bio je da se utvrdi pouzdanost HPV tipizacije, kao i citologije za detekciju recidiva intraepitelnih neoplazija grlića materice.

Metode

U Klinici za ginekologiju i akušerstvo u Nišu, u periodu od marta 2006. do marta 2009. godine sprovedena je prospektivna klinička studija. Eksperimentalnu grupu činilo je prvih 35 bolesnica koje su se javile nakon lečenja nekog od stadijuma intraepitelne neoplazije grlića materice zbog recidiva. Kontrolna grupa obuhvatala je prvih 30 bolesnica kod kojih je od lečenja prošlo više od godinu dana i koje nisu imale dijagnostikovan recidiv bolesti.

Sve bolesnice (njih 65) imale su urađen citološki i nalaz HPV tipizacije. Bolesnice eksperimentalne grupe imale su urađen i patohistološki nalaz dobijen biopsijom ili eventualnom hiruškom reintervencijom. Citološki rezultati dobijeni su primenom konvencionalne citologije. Brisevi su uzimani štapićem sa vatom i špatulom i bojeni diferencijalnom metodom bojenja prema Papanikolau. Očitavanje nalaza vršeno je na mikroskopu marke Olimpus. Klasifikacija nalaza rađena je uporedno sa Papanikolau i Bethezda klasifikacijom.

Brisevi za HPV tipizaciju uzimani su sterilnim hermetički zatvorenim epruvetama u kojima je štapić pri kraju bio obmotan vatom. Uzorak je uziman iz egzo- i endocerviksa i uz propratnu dokumentaciju odmah prosleđivan u laboratoriju za molekularnu biologiju i citogenetiku Kliničkog centra u Nišu. Uzorci su zamrzavani i skladišteni, a potom su, najdalje u roku od dva meseca, transportovani na ledu u laboratoriju za molekularnu genetiku Stomatološkog fakulteta u Beogradu na dalju analizu. U ovoj instituciji rađena je dalja izolacija i identifikacija malignih tipova HPV korišćenjem PCR metode.

Štapići sa uzorkom lomljeni su, stavljani u mikrotube i prelivani sa 400 μl sterilne, destilovane vode. Njihovim kuvanjem tokom 15 min postignuta je izolacija virusne DNK. Ostatak štapića je bio uklonjen, a u vodenom rastvoru ostala je DNK virusa. Centrifugiranjem tuba 3 min na 10 000 obrtaja/min, nečistoće sa brisa su se istaložile, a DNK je ostao u supernatantu. Urađeno je međusobno upoređivanje svih dobijenih dijagnostičkih nalaza, a rezultati su dovedeni u vezu sa mogućim poznatim faktorima rizika dobijenim anamnestičkim podacima. Svi rezultati statistički su obrađeni i tabelarno prikazani.

Rezultati

U tabela 1 prikazana je distribucija patoloških nalaza recidiva nakon lečenja intraepitelnih neoplazija grlića materice. Procentualno, broj recidiva CIN I i CIN III bio je sličan (40% : 37,14%), dok je statistički bio najmanji broj recidiva CIN II stadijuma.

Tabela 1

Distribucija patohistoloških nalaza (PH) recidiva kod bolesnica eksperimentalne grupe

PH nalaz	Bolesnice	
	n	%
CIN I	14	40,00
CIN II	5	14,28
CIN III	13	37,14
MIK	3	8,57
Ukupno	35	100,00

CIN – *cervical intraepithelial neoplasia*; MIK – mikroinvazivni karcinom

Distribucija nalaza HPV tipizacije ispitanica eksperimentalne (sa recidivom) i kontrolne grupe (bez recidiva) prikazana je u tabeli 2. Procenat HPV pozitivnih nalaza bio je statistički značajno veći kod ispitanica sa recidivom bolesti. Samo 6,66% ispitanica kontrolne grupe bile su HPV pozitivne.

U tabeli 3 prikazana je distibucija pozitivnih nalaza HPV tipizacije kod bolesnica sa recidivom niskog (LSIL) i visokog gradusa (HSIL). Procenat HPV pozitivnih nalaza bio je statistički značajno veći u grupi sa recidivom visokog gradusa (90,47% HSIL : 35,71% LSIL).

Procenat HPV pozitivnih nalaza bio je, takođe, statistički značajno češće i kod bolesnica sa recidivom CIN istog gradusa nego kod ispitanica kontrolne grupe (5 od 14; 35,71% : 2 od 30; 6,67%).

U tabeli 4 prikazana je distibucija stadijuma recidiva CIN u odnosu na tip HPV. Tabela pokazuje da ispitanice sa recidivom visokog gradusa (CIN II – III i MIK) u odnosu na one sa niskim (CIN I) gradusom imaju statistički značajno veću zastupljenost HPV tipa 18 ili udruženog HPV tipa 16 i 18. Značajnu razliku u zastupljenosti HPV tipa u okviru grupe sa visokim gradusom recidiva, nismo uspeli da dokažemo.

Distribucija HPV nalaza u grupi bolesnica sa recidivom u odnosu na godine života prikazana je u tabali 5. Najveći procenat pozitivnih nalaza HPV testa bio je u grupi starijih ispitanica (83,33%), dok je kod mlađih ispitanica procenat pozitivnih nalaza bio skoro duplo manji (44,41%). Takođe, HPV test bio je pouzdaniji za otkrivanje recidiva kod starijih bolesnica. Starije bolesnice pozitivne na HPV bile su pod većim rizikom od pojave recidiva CIN, zbog čega ih treba intenzivnije pratiti i ispitati.

Iz podataka datih u tabeli 2, korišćenjem poznatih formula za ispitivanje pouzdanosti dijagnostičkih testova, izračunati su senzitivnost i specifičnost HPV tipizacije u dijagnostici recidiva CIN. Tako, senzitivnost HPV tipizacije za detekciju svih stadijuma recidiva (CIN I, CIN II, CIN III i MIC) iznosi 68,57%. Specifičnost HPV tipizacije je visoka (93,33%). Pozitivna prediktivna vrednost ovog testa iznosi 92,30%, a negativna prediktivna vrednost 71,79% .

Tabela 2

Distribucija nalaza HPV tipizacije kod ispitanica eksperimntalne i kontrolne grupe u odnosu na prisustvo ili odsustvo recidiva bolesti

HPV nalaz	Bolesnice				Ukupno	
	sa recidivom		bez recidiva			
	n	%	n	%	n	%
Pozitivan	24	68,58	2	6,67	26	40
Negativan	11	31,42	28	93,33	39	60
Ukupno	35	100,00	30	100,00	65	100,00

HPV – humani papiloma virus

Tabela 3

Distribucija nalaza HPV tipizacije kod ispitanica eksperimentalne grupe sa HSIL (CIN II +CIN III) i MIK nalazom recidiva bolesti u odnosu na LSIL (CIN I) recidiv

HP nalaz	HPV pozitivne		HPV negativne		Ukupno	
	n	%	n	%	n	%
LSIL	5	35,71	9	64,28	14	100
HSIL i MIK	19	90,47	2	9,52	21	100
Ukupno	24	68,57	11	31,42	35	100

LSIL – *low grade squamous intraepithelial lesion*; HSIL – *high grade squamous intraepithelial lesion*; MIK – mikroinvazivni karcinom; HPV – humani papiloma virus; CIN – *cervical intraepithelial neoplasia*

Tabela 4

Distribucija nalaza recidiva u odnosu na otkriveni tip humanog papiloma virusa

Tip virusa	CIN I		CIN II		CIN III		MIK		Ukupno	
	n	%	n	%	n	%	n	%	n	%
HPV 16	14	50	4	14,28	9	32,14	1	3,57	28	100
HPV 18	0		0		1	50	1	50	2	100
HPV 16+18	0		1	20	3	60	1	20	5	100
Ukupno	14	40	5	14,28	13	37,14	3	8,57	35	100

HPV – humani papiloma virus ; CIN – *cervical intraepithelial neoplasia*; MIK – mikroinvazivni karcinom

Tabela 5

Distribucija HPV nalaza u odnosu na starosti ispitanica eksperimentalne grupe

Starost bolesnica	HPV pozitivan		HPV negativan		Ukupno	
(godine)	n	%	n	%	n	%
20–29	4	44,44	5	55,55	9	100
30–39	9	64,28	5	35,71	14	100
40–49	10	83,33	2	16,66	12	100
Ukupno	23	65,71	12	34,28	35	100

HPV – humani papiloma virus

U tabeli 6 posebno su prikazani parametri prednosti HPV tipizacije za recidive visokog gradusa (CIN II, III i MIK), koji su značajniji, jer zahtevaju hirušku reintervenciju. Izračunata senzitivnost HPV tipizacije za otkrivanje recidiva višeg gradusa je veća i iznosi 90,47%, specifičnost je 93,33%, pozitivna prediktivna vrednost 90,47%, a negativna prediktivna vrednost 93,53%.

vih bolesnica došlo je do negativizacije na HPV. U kontrolnoj grupi bilo je samo 4% detektovanih recidiva. U eksperimentalnoj grupi (27% bolesnica) sa perzistentnom HPV infekcijom otkriven je recidiv CIN kod 50% slučajeva. Bolesnice eksperimentalne grupe sa perzistentnom HPV infekcijom u većem procentu bile su starije od bolesnica kontrolne grupe. U odnosu na podatak o težini intraeptilne neoplazije

Tabela 6

Pouzdanost HPV tipizacije za dijagnostikovanje težih HSIL i MIK stadijuma recidiva bolesti

HPV test	HSIL i MIK		Bez recidiva		Ukupno	
	n	%	n	%	n	%
Patološki nalaz	19	90,47	2	9,52	21	100
Normalan nalaz	2	6,66	28	93,33	30	100
Ukupno	21	40	30	60	51	100

HPV – humani papiloma virus; HSIL – ***high grade squamous intraepithelial lesion*****;**
MIK – mikroinvazivni karcinom

Distribucija citoloških nalaza kod bolesnica sa i bez recidiva bolesti prikazana je u tabeli 7 . Senzitivnost citologije za detekciju recidiva, slična je senzitivnosti HPV tipizacije i iznosi 68,57%. Specifičnost citologije je veća od HPV tipizacije i iznosi 96,66%.

Distribucija citoloških nalaza u odnosu na stadijum recidiva (tabela 7), pokazuje veću senzitivnost citologije za detekciju recidiva višeg gradusa (CIN II – III i MIK) – 80,95%, nego za niže graduse (CIN I) – 50%. Korišćenjem HPV tipizacije uporedo sa citologijom povećava se senzitivnost za detekciju recidiva na 95,23%.

zbog koje je rađena konizacija, bolesnice eksperimentalne grupe bile su češće operisane zbog težeg stadijuma. Otkrivenih recidiva kod pozitivnih ivica konizata bilo je 83%. Ako je uz podatak o pozitivnim ivicama, HPV tipizacija bila pozitivna, onda je kod 100% bolesnica otkriven recidiv. Na taj način, pokazano je da HPV tipizacija snižava za 17% nepotrebno urađene hiruške reintervencije [7].

Distribucija HPV pozitivnih bolesnica u odnosu na stadijum recidiva intraepitelne neoplazije u našoj studiji pokazuje da je statistički značajno veći procenat bolesnica sa intraepitelnim neoplazijama težeg gradusa, nego onih sa bla-

Tabela 7

Distribucija citoloških nalaza kod ispitanica bez i sa recidivom bolesti u zavisnosti od stadijuma recidiva

Bolesnice	Nalaz Pap testa				Ukupno	
	patološki		normalan			
	n	%	n	%	n	%
Sa recidivom	24	96	11	27,5	35	53,84
Bez recidivom	1	4	29	72,5	30	46,16
Ukupno sa recidivom	25	100	40	100	65	100
LSIL	7	50	7	50	14	100
HSIL i MIK	17	80,95	4	19,05	21	100
Ukupno	24	68,58	11	31,42	35	100

Pap – Papanikolau; LSIL – ***low grade squamous intraepithelial lesions;*** **HSIL –** ***high grade squamous intraepithelial lesion*****; MIK – mikroinvazivni karcinom**

Diskusija

Procentualna zastupljenost bolesnica sa recidivom CIN koje su imale pozitivan nalaz HPV tipizacije prikazana je u tabeli 2. Iz tabele se vidi da je 68,57% bolesnica sa recidivom bilo HPV pozitivno, što se pokazalo statistički značajno. Kod 31,42% bolesnica nalaz HPV tipizacije bio je lažno negativan. Bolesnice kontrolne grupe (bez recidiva) imale su 6,66% lažno pozitivan nalaz HPV tipizacije. Procenat HPV pozitivnih recidiva, iznet u radu holandskih autora, sličan je i iznosi 62,5%, dok je procenat lažno pozitivnih nalaza bio veći (55%) [6].

Veći procenat HPV pozitivnih nalaza kod bolesnica sa recidivom CIN, nego kod bolesnica kontrolne grupe, nalaze i Bodner i sar. [7]. Tri meseca nakon konizacije kod 73% njihožim (CIN I), bio pozitivan na HPV (90,47% : 35,71%). Sa druge strane, pokazano je da je veći procenat ovih blažih lezija bio pozitivan sa HPV nego kod bolesnica kontrolne grupe (35,71% : 6,5%). Na osnovu ovih podataka vidi se da je pozitivnost na onkogene HPV tipa 16 i 18 u visokom procentu povezana sa pojavom recidiva.

Zbirne vrednosti HPV tipizacije u našoj studiji, za sve stadijume recidiva, niže su zato što je rađena PCR metoda na dva najonkogenija tipa HPV 16 i 18, koja su u malom procentu prisutna kod blagih (CIN I) intraepitelnih lezija. Na taj način, ova grupa recidiva najnižeg stadijuma snizila je ukupnu pouzdanost HPV tipizacije (senzitivnost 68,57%, specifičnost 93,33%). Patogeneza i evolucija ovih blagih promena (CIN I) pokazuje u najvećem procentu spontanu regresiju i ne zahteva nikakvu dodatnu hirušku reintervenciju. Smatra

se da ove promene imaju i drugačiju etiopatogenezu i da ne predstavljaju stadijum nastanka promena ka težem gradusu (CIN II, III) i karcinomu. Iz tih razloga, posebno smo izračunali pouzdanost otkrivanja onkogenih virusa za teže stadijume recidiva koji zahtevaju reintervenciju. Dobijeni rezultati pokazuju visoku senzitivnost HPV tipizacije za otkrivanje težih recidiva koja iznosi 90,47%, specifičnost je 93,33%, pozitivna prediktivna vrednost (pozitivan nalaz kod recidiva) 90,47%, a negativna prediktivna vrednost (negativan nalaz kod bolesnica bez recidiva) 93,53%. U ovom istraživanju dobili smo više vrednosti za specifičnost od onih koje nalazimo u literaturi, takođe zato što smo koristili PCR i dokazivali isključivo visoko onkogene tipove HPV koji su u znatno manjem procentu prisutni kod zdravih ispitanica. Na taj način, dobili smo manji procenat lažno pozitivnih nalaza i veću specifičnost HPV testa.

U podacima iz literature pouzdanost HPV tipizacije najčešće se određuje upoređivanjem sa validnošću citologije. Značaj citologije u postoperativnom praćenju bolesnica posle urađene konizacije mnogi autori opisuju i podržavaju kao jednu od najkorisnijih dijagnostičkih metoda u detekciji rezidualne bolesti. Tako, Buxton i Luesley [8] navode da bolesnice koje su imale postoperativno normalan citološki Pap nalaz nisu imale rezidualnu bolest, dok je kod 57% sa abnormalnim Pap razmazom otkrivena rezidualna bolest. U radovima novijeg datuma, sa druge strane, više se naglašavana uloga HPV tipizacije nego citologije. Zaključak je da HPV tipizacija kod operisanih bolesnica ima statistički značajno veću senzitivnost nego citologiju, a statistički zanemarljivo nižu specifičnost za detekciju recidiva. Takođe, u istim radovima na osnovu dobijenih podataka ističe se značaj tipizacije za trijažu bolesnica sa graničnim citološkim nalazom nakon operacije – ASCUS nalazom [9].

Komparativni prikaz pouzdanosti tipizacije humanog papiloma virusa i eksfolijativne citologije u dijagnostici recidiva radili su Alonso i sar. [10]. Podaci koje su dobili pokazali su da HPV tipizacija ima veću senzitivnost nego citologija (97,2% : 83,3%), a manju specifičnost (81,4% : 92,2%). Kombinacijom i citologije i HPV tipizacije u detekciji recidiva senzitivnost se povećeva na 100%, negativna prediktivna vrednost je, takođe, 100%, dok specifičnost ostaje nešto niža, prihvatljivih 76,6%.

Upoređivanje senzitivnosti HPV tipizacije i citologije u utvrđivanju recidiva CIN uradili su Lee i sar. [11] koristeći *hybrid capture* II test (HC II) dokazali su skoro identičnu vrednost senzitivnosti tipizacije HPV i citologije. Senzitivnost tipizacije iznosila je 89,9%, a citologije 86,2%. U odnosu na stadijum intraepitelne neoplazije senzitivnost HPV tipizacije iznosila je 88,1%, za najblaže stadijume (CIN I) do 92,5% za intraepitelne neoplazije težeg (CIN III) stadijuma. U istom radu prilikom određivanja vrste (genotipa) virusa i njihove udruženosti sa neoplazijama višeg gradusa, sem visokog procenta HPV 16 i 18 utvrdili su i veliki procenat virusa tipa 52 i 58. Iz tih razloga, ovi autori naglašavaju da bi se i ove vrste virusa možda mogle uključiti u HPV vakcinaciju [11].

Grupa autora iz Italije, Venturoli i sar. [12], takođe su upoređivali pouzdanost citologije i HPV tipizacije za detekciju CIN. Za razliku od podataka u prethodnom radu, njihovi podaci za senzitivnost HPV tipizacije još su bolji nego za citologiju i iznose 91,7%, dok je specifičnost 95,4%. Veća senzitivnost verovatno je posledica toga što su ovi autori za detekciju virusa koristili PCR – ELISA test, a ne *hybrid capture* II test, što je naročito bitno za diferencijaciju LSIL i HSIL promena. Statističkom analizom svih dobijenih parametara ipak je ustanovljena direktna korelacija HPV DNA pozitivnosti sa težinom dijagnoze, dobijenom citološkim nalazom ($p < 0,005$).

Senzitivnost i specifičnost HPV tipizacije u detekciji intraepitelnih neoplazija težeg gradusa pokazali su i drugi autori [13]. Tako npr. od 60,4% HPV pozitivnih bolesnika CIN II – III nađen je kod 18%. Izračunata vrednost senzitivnosti bila je 89,5% (41 bolesnica od 57 HPV pozitivnih na biopsiji imala je nalaz intraepitelnih neoplazija težeg gradusa). Specifičnost HPV tipizacije prema podacima iz ovog rada imala je vrednost od 45,8%, što je najmanji procenat od svih prethodno objavljenih radova. Negativna prediktivna vrednost bila je visoka i iznosila je 95,3%. Na kraju ovog istraživanja zaključeno je da postoji 7,2 puta veći rizik od dobijanja nalaza CIN II – III kod bolesnika sa pozitivnim vrednostima HPV tipizacije [13].

Ukupna pouzdanost citologije za detekciju recidiva svih stadijuma, koja je dobijena u našem iztraživanju, prikazana je u tabeli 7. Senzitivnost citologije za sve stadijume recidiva je niska i iznosi 68,57%, dok je specifičnost visoka (96,66%). Pozitivna prediktivna vrednost je 96%, a negativna 72,5%. Senzitivnosti citologije je niža vrednosti zato što citologija ima visok procenat lažno negativnih nalaza kod bolesnika sa blažim stadijumom (CIN I) recidiva. Korišćenjem HPV tipizacije uporedo sa citologijom smanjuje se broj lažno negativnih nalaza za 4, odnosno za 11,42%, što povećava senzitivnost na 80%.

U tabeli 7 date su izračunate vrednosti pouzdanosti citologije za detekciju svih stadijuma recidiva pojedinačno. Podaci za teže stadijume recidiva slični su onima koje su dobili Alonso i sar. [10]. Senzitivnost citologije je 80,95% (50% za CIN I), a upotrebom HPV tipizacije senzitivnost se povećava na 95,23%, što su najveće vrednosti za senzitivnost (veće i od onih dobijenih kolposkopijom).

Pozitivnu prediktivnu vrednost za detekciju recidiva CIN različitih stadijuma upotrebom HPV tipizacije i citologije, ispitivali su Almog i sar. [14]. Ukupan procenat patoloških citoloških nalaza tri meseca posle konizacije bio je 22,4%. Procenat LSIL razmaza bio je kod 55%, a HSIL razmaza 45%. Biopsijom grlića materice rezidualna bolest nađena kod 19,1%, tj, kod 85% bolesnica sa patološkim citološkim nalazom. Recidiv LSIL je nađen je kod 52,94%, a HSIL kod 47%. Pozitivna prediktivna vrednost citologije za detekciju LSIL recidiva niža je od HPV tipizacije i iznosi 54,5%, dok je HPV vrednost kod ovih bolesnika bila u 100 niskih i graničnih vrednosti pozitivnosti. Kod HSIL recidiva citologija ima pozitivnu prediktivnu vrednost 89%, dok HPV tipizacija pokazuje 100% prisutnost visokih vrednosti prisustva virusa. Na osnovu ovih podataka autori su došli do zakjučka da HPV tipizacija ima bolju prediktivnu vrednost za LSIL recidive od citologije. Ovakvi podaci slažu se sa rezultatima naše studije

u kojoj je zahvaljujući HPV tipizaciji snižen procenat lažno negativnih citoloških nalaza za 11,42% [14].

Prevalenciju HPV pozitivnosti kod bolesnika sa citološki normalnim nalazom ispitivali su Gupta i sar. [15]. Procenat HPV pozitivnih nalaza sa normalnim Pap nalazom u ovom radu iznosio je 16,6%. Kod 67% bolesnica urađen je tip virusa 16 i 18. Pozitivnost HPV i u ovoj grupi bolesnika bila je veća kod starijih bolesnica, kod većeg pariteta i lošijeg socioekonomskog statusa [15].

U radu Vergutsa i sar. [16] dobijene su veće vrednosti za senzitivnost HPV tipizacije. Ovi autori u prospektivnoj studiji upoređivali su ulogu HPV tipizacije, statusa ivica i citologije u praćenju bolesnica operisanih konizacijom nožem zbog promena CIN II – III. Bolesnice su praćene na šest meseci tokom dve godine. Ukupan procenat recidiva bio je 8%. Bolesnice sa recidivom bile su statistički značajno starije od bolesnica kontrolne grupe (51,5 : 39,8 godina). Senzitivnost HPV tipizacije bila je najveća u prognozi rezidualne bolesti i iznosila je 100%. Senzitivnost citologije bila je 66,7%, dok je senzitivnost pozitivnih ivica konusa bila 33,33%. Specifičnost citologije bila je najveća (90,9%), specifičnost ivica konizata bila je nešto niža (81,8%), dok je HPV tipizacija imala najnižu vrednost specifičnosti (77,3%). Kontrolna grupa imala je pozitivan nalaz HPV tipizacije kod 22,7% i Pap patološki nalaz kod 9%. Niže vrednosti specifičnosti HPV tipizacije povećavale su se udruženom primenom ovog testa sa citologijom. Tako, ako je patološki nalaz testa HPV tipizacije povezan sa patološkim citološkim nalazom, recidiv intraepitelne neoplazije otkriva se kod više od 50%, dok je učestalost recidiva samo 15%, ako je citološki nalaz normalan, a nalaz HPV tipizacije patološki [16].

Predmet ispitivanja u mnogim radovima, sem pozitivnosti na HPV virus, bila je i distribucija perzistencije pojedinih tipova HPV. Nagaia i sar. [17] u grupi perzsitentnih HPV infekcija sa recidivom našli su HPV tipa 16 kod 50%, 18 kod 26, 8%, dok su ostali tipovi bili zastupljeni kod 32,1% bolesnica.

Podaci o zastupljenosti pojedinih tipova virusa u rekurentnoj bolesti dobijeni u radu Terra i sar. [18] slični su prethodnim. Najzastupljeniji tip virusa bio je tip 16, ali je procenat pojave ovog tipa još veći nego prema rezultatima prethodnih autora i iznosio je 69,7%, tipa 18 bio je 33,3% dok je udruženost oba tipa virusa bila pristuna kod 15,1% bolesnica. Konkomitantna infekcija HPV tipa 16 bila je prisutna kod 15,1%, dok je udruženost infekcije ovih tipova sa HPV tipa 18 bila 33,3%. Isti autori korišćenjem metilacio-specifične PCR reakcije analizirali su promoter hipermetilaciju 8 gena (p16, RARbeta,GSTP1, MGMT, p14, TIMP3, E-cad i DAPk). Recidiv se javio samo u slučaju hipermetilacije kod tri i više od pomenutih osam gena. Na taj način aberantnost promotera metilacije može biti još jedan koristan biomarker recidivantnih intraepitelnih neoplazija [18].

Podaci o distribuciji pojedinih tipova HPV u odnosu na stadijum recidiva CIN dobijeni u našem istraživanju kazuju da je najveći procenat ispitanica bio pozitivan na HPV tipa 16 (80%). Ovakvi podaci su očekivani s obzirom na to da se radilo o recidivima skvamoznih neoplazija. Bolesnice koje su bile pozitivne na HPV tipa 18 (njih 5,7%) ili koje su bile pozitivne na oba virusa HPV 16 i 18 (njih 14,28%), imale su statistički značajno teži gradus recidiva. U odnosu na podatke iz prethodnog rada procenat HPV 18 je manji, dok je procenat konkomitantne HPV 16 i 18 infekcije skoro identičan (15,1%). Što se tiče povezanosti težeg stadijuma recidiva i udružene infekcije sa HPV 16 i 18, naši gore izneti podaci razlikuju se od podataka Venturolija i sar. [19]. Ovi autori nalaze značajnu povezanost stadijuma recidiva i udružene HPV infekcije.

Oni su ispitivali, takođe, pozitivnost određenih tipova HPV kod bolesnica sa recidivom. Upoređivan je preoperativni i postoperativni HPV status dve godine nakon operacije. Nađeno je da je kod bolesnica sa recidivom bolesti bila veća zastupljenost HPV 16 i 18 (82,4%), nego virusa iz grupe dva (HPV 31, 33, 35, 45, 52 i 58) (66,7%), dok je zastupljenost treće grupe (HPV 39, 51, 56, 59, 68, 26, 53, 66, 73 i 82) bila još manja (14,3%) [19].

Slične podatke o zastupljenosti pojedinih tipova HPV kod recidiva dobili su i Sigurdsson i sar. [20]. Ova grupa autora posebno je ispitivala zastupljenost pojedinih tipova virusa za različite stadijume recidiva bolesti. Ukupno 95% recidiva CIN III i 92% karcinoma bilo je pozitivno na virus. Kod promena CIN II i III najčešće je bio zastupljan HPV tipa 16, zatim 33, 31, 52, 35, 18, 58, 56, 39, 45. Kod karcinoma bio je zastupljen, takođe, najviše tip 16, dok je zastupljenost ostalih tipova bila drugačija i iznosila je redom: 18, 33, 45, 31, 39, 52, 35, 51, 56. Iz ovoga sledi da distribucija HPV tipova kod bolesnica sa recidivom zavisi od gradusa bolesti. Sem ovog faktora, u toku ispitivanja ustanovljena je povezanost tipova HPV infekcije i recidiva sa starosnim dobom bolesnica i histološkim tipom tumora (tip 18 bio je češći kod adenokarcinoma). Udruženost više vrsta HPV tipova kod iste bolesnice nije se pokazala značajnom za češću pojavu recidiva ili pojavu težih stadijuma recidiva. Na osnovu ovakve distribucije tipova virusa u raznim stadijumima bolesti i njihove zastupljenosti u postojećim HPV vakcinama isti autori zaključuju da vakcine štite u 60% slučajeva od karcinoma, a u 40% slučajeva od intraepitelnih neoplazija grlića materice [20].

Rezultati našeg istraživanja, za distibuciju HPV pozitivnih i negativnih nalaza, u odnosu na starost ispitanica eksperimentalne grupe pokazuju da su starije bolesnice sa recidivom bile HPV pozitivne u statistički značajno većem procentu. Takođe, zaključili smo da je nalaz HPV tipizacije kod starijih bolesnica značajniji faktor u predviđanju recidiva bolesti. Ovakvi podaci ističu značaj perzistencije HPV pozitivnosti za razvoj recidiva, što znači da starije bolesnice koje nisu uspele da svojim imunitetom negativiziraju HPV i imaju rizik od recidiva.

I na kraju, bolesnice koje i posle godinu dana od operacije ostanu pozitivne na onkogene tipove HPV 16 i 18, imaju veći rizik od pojave recidiva koji je po nekim autorima i 18 puta veći od onog u grupi negativnih na ove tipove virusa [21].

Kasnijom analizom patološkog nalaza bolesnica sa recidivom bolesti kod kojih je urađena reintrevencija, utvrđeno je najveće slaganje u stadijumu za recidive visokog (CIN III) gradusa (84,61%), dok su recidivi manjeg gradusa (CIN II –

I) pri reintervenciji bili zastupljeni kod 50%, bolesnica, što sugeriše da bi, poštujući specifičnosti svakog slučaja recidiva ponaosob, možda za ove stadijume trebalo zauzeti ekspektativniji stav.

Zaključak

Recidivi intraepitelnih neoplazija težeg gradusa javljaju se statistički značajno češće kod bolesnica koje i posle lečenja ostaju pozitivne na HPV.

Bolesnice koje i posle lečenja intraepitelnih neoplazija ostaju pozitivne na HPV tipa 18 ili, zajedno, na HPV tip 16 i 18, imaju češće recidive težeg gradusa nego one koje su pozitivne samo na HPV tipa 16.

Tipizacija HPV ima veću prediktivnu vrednost za dijagnostiku rezidualne bolesti kod starijih bolesnica.

Senzitivnost HPV tipizacije za dijagnostiku recidiva intraepitelne neoplazije svih stadijuma je 68,57% a za teže graduse (HSIL i MIK) 90,47%; specifičnost iznosi 93,33%, pozitivna prediktivna vrednost 90,47%, a negativna prediktivna vrednost 93,53%.

Senzitivnost citologije za dijagnostiku recidiva težeg gradusa je 80,95%. Korišćenjem HPV tipizacije zajedno sa citologijom ostvaruje se najveća senzitivnost od 95,23%.

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Hormonski status bolesnika sa uznapredovalim karcinomom prostate lečenih androgenim blokadama

Hormonal status in patients with advanced prostatic cancer on the therapy with androgen blockade

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Apstrakt

Uvod/Cilj. Hormonska supresiona terapija koristi se u lečenju muškaraca sa uznapredovalim karcinomom prostate u cilju poboljšanja šanse za duže preživljavanje. Cilj ove studije bio je da se ispita uticaj androgenih blokada na vrednosti testosterona i luteinizirajućeg hormona (LH) kod bolesnika sa lokalno uznapredovalim i metastatskim karcinomom prostate. **Metode**. Grupa od 45 bolesnika sa karcinomom prostate bila je podeljena na 3 podgrupe na osnovu primenjenog terapijskog protokola (15 bolesnika na monoterapiji agonistima gonadotropnog rilizing hormona (LH-RH) – grupa I, 15 bolesnika na totalnoj androgenoj blokadi (grupa II) i 15 bolesnika na monoterapiji antiandrogenom (grupa III). Kontrolnu grupu činilo je 15 bolesnika sa benignom hiperplazijom prostate. Svim bolesnicima praćene su vrednosti testosterona, LH i prostata-specifičnog antigena (PSA) neposredno pre, kao i tri, odnosno šest meseci nakon uvođenja odgovarajućeg protokola. **Rezultati.** Kod bolesnika grupe I, II i III, vrednost testosterona posle tri meseca opala je za 95,58%, 95,72%, odnosno 67%. U odnosu na početnu vrednost razlika je bila statistički visokoznačajna ($p < 0,01$). Između vrednosti posle tri i posle šest meseci nije ustanovljena statistički značajna razlika. Vrednosti testosterona bile su značajno više kod bolesnika grupe III, a poređenjem vrednosti između grupa III i I, kao i grupa III i II utvrđena je statistički visokoznačajna razlika i nakon tri meseca, kao i nakon šest meseci terapije ($p < 0,01$). Između grupa I i II u 3. i 6. od utvrđenja terapije mesecu utvrđena je razlika u vrednostima testosterona, ali bez statističke značajnosti. Sve tri vrste primenjenih terapijskih protokola u lečenju karcinoma prostate statistički značajno snižavale su vrednosti LH u odnosu na početne vrednosti. **Zaključak.** Totalna androgena blokada i primena LH-RH agonista značajnije snižavaju nivo testosterona (pad na kastracione vrednosti) u odnosu na monoterapiju antiandrogenima, kada vrednosti testosterona ostaju iznad kastracionog nivoa. Takav terapijski pristup ima prednost, jer obara vrednosti testosterona na nivoe koji omogućavaju bolji odgovor na terapiju. Pri primeni totalne androgene blokade i monoterapije LH-RH agonistima, postoji razlika u nivoima testosterona koja nije statistički značajna. Registrovane su niže početne vrednosti LH kod bolesnika sa karcinomom prostate, što može biti dodatni laboratorijski marker za dijagnozu ovog karcinoma.

Ključne reči:
prostata, neoplazme; androgeni, antagonisti; lh; testosteron; prostata, specifični antigen.

Abstract

Background/Aim. Hormone suppression therapy is used in men with advanced prostate cancer improving chances of longer survival. The aim of this study was to investigate the influence of androgen blockades on testosterone and luteinizing hormone (LH) values in patients with locally advanced and metastatic prostatic cancer. **Methods.** The study included a total of 60 patients out of which 45 with prostatic cancer divided into 3 subgroups based on the type of the applied treatment protocol: 15 patients on monotherapy with luteinizing-releasing hormone (LH-RH) agonists (group I), 15 patients on total androgen blockade (group II) and 15 patients on monotherapy with antiandrogen (group III)). The control group consisted of 15 patients with benign prostatic hyperplasia. In all the patients, values of testosteron, LH and prostate-specific antigen (PSA) were monitored initially, as well as 3 and 6 months after the treatment protocol introduction. **Results.** In the patients of the groups I, II and III, values of testosterone decreased after three months by 95.58%, 95.72%, and 67%, respectively. The difference was significant ($p < 0.01$). Between the values after three and six months there was no significant difference in these groups of participants. Testosterone values were significantly higher in the patients of the group III in both analyses. Comparing the values between the groups III and I, as well as those of the groups III and II, a significant difference was found after three and six months of the therapy ($p < 0.01$). There was a difference in testosterone values between the groups I and II after 3 and 6 months, but not significant. All types of the applied treatment protocols in

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the therapy of prostatic cancer significantly decreased the values of LH compared to the basal ones. **Conclusion.** Total androgen blockade and LH-RH agonists are more effective in lowering testosterone values (to castration values) compared to the antiandrogen monotherapy, where testosterone values stay above the castration level. This therapy approach has advantages, since it decreases testosterone values providing better therapy response. There is a difference in testosterone values, but not significant, when total androgen blockade and monotherapy with LH-RH agonists are administered. Registered lower basal values of LH in all patients with prostatic cancer open the possibility to introduce LH as a new additional, significant marker in diagnosis of this neoplasm.

Key words:
prostatic neoplasms; androgen antagonists; luteinizing hormone; testosterone; prostate-specific antigen.

Uvod

Savremena terapija karcinoma prostate sprovodi se po određenim indikacijama. Za svaki stadijum bolesti koriste se određene metode lečenja (praćenje, kurativni tretman i hormonska terapija).

Androgena zavisnost prostate i semenih kesica poznata je već nekoliko vekova. Pošto karcinom prostate potiče od adultnog epitela prostate, još su Huggins i Hodges [1] 1941. godine izneli pretpostavku da je taj karcinom zavisan od hormonskog dejstva androgena.

Endokrina terapija deluje adjuvantno [2–4] i primenjuje se sa ciljem da se ćelije karcinoma prostate liše stimulatornih efekata androgena. Ovo se postiže hirurškom ili farmakološkom kastracijom. Farmakološka blokada ostvaruje se primenom agonista ganodotropnog rilizing hormona (LH-RH) [5, 6] i/ili antiandrogenima. Antiandrogeni su jedinjenja koja inhibiraju dejstvo androgena u ćelijama karcinoma prostate tako što blokiraju androgene receptore i sprečavaju vezivanje androgenih hormona za njih. Pošto se hirurškom ili farmakološkom kastracijom eliminišu testikularni androgeni, dejstvo ekstratestikularnih androgena suprimira se davanjem različitih antiandrogena [5–7]. Spajanjem ova dva modaliteta terapije, postiže se maksimalna ili totalna androgena blokada (TAB) [8, 9]. Intermitentna androgena blokada i dalje ostaje eksperimentalni pristup, za čije potvrđivanje su neophodna dalja istraživanja.

Odgovor na endokrinu terapiju karcinoma prostate može biti procenjen određivanjem nivoa prostata specifičnog antigena (PSA), testosterona i luteinizirajućeg hormona (LH). Ove vrednosti su u korelaciji sa prognozom bolesti.

Cilj rada bio je da se ispita uticaj androgenih blokada na vrednosti PSA, testosterona i LH kod bolesnika sa lokalno uznapredovalim i metastatskim karcinomom prostate.

Metode

Ova prospektivna studija obuhvatila je 60 ispitanika starijih od 50 godina, sa karcinomom prostate, odnosno benignom hiperplazijom prostate klinički ili ambulantno lečenih u Klinici za urologiju Kliničkog centra Vojvodine u Novom Sadu. Ispitanici sa karcinomom prostate imali su dijagnostikovan lokalno uznapredovali ili metastatski karcinom (T3-4, No-N1, Mo; T3-4, N1, M1). Svi ispitanici pristali su da dobrovoljno učestvuju u navedenom istraživanju. Nakon detaljnog objašnjenja procedure, potpisali su pristanak za učešće u ovom ispitivanju. Studiju je odobrio Etički komitet Medicinskog fakulteta u Novom Sadu.

Ispitanici su bili podeljeni u četiri grupe od po 15 ispitanika. Prvu grupu činili su bolesnici sa karcinomom prostate, prosečne starosti 67,2 god, koji su bili podvrgnuti monoterapiji LH-RH agonistima (goserelin-acetat 3,6 mg supkutano na svakih 28 dana tokom šest meseci). Druga grupa ispitanika sa karcinomom prostate, prosečne starosti 67,33 god, bila je podvrgnuta totalnoj androgenoj blokadi (hirurška kastracija i antiandrogen – supkapsularna bilateralna orhiektomija u spinalnoj anesteziji, a zatim ciproteron-acetat *per os* u dozi od 2 × 50 mg dnevno tokom šest meseci). Treća grupa obuhvatila je ispitanike prosečne starosti 67,27 god, sa uznapredovalim karcinomom prostate, kojima je uključena monoterapija antiandrogenima (ciproteron-acetat 2 × 100 mg dnevno tokom šest meseci). Ispitanicima prve tri grupe dijagnoza je bila potvrđena transrektalnom biopsijom prostate. Metastaze u kostima dijagnostikovane su scintigrafijom skeleta. Ispitanici kontrolne grupe, prosečne starosti 64,67 god, sa dijagnostikovanom benignom hiperplazijom prostate, nisu bile podvrgnuti nijednom terapijskom protokolu.

Svim ispitanicima uzeti su anamnestički podaci, obavljen je klinički pregled, digitorektalni pregled, transrektalni ultrazvuk (UZ) prostate, određene su vrednosti PSA, testosterona i LH. Nakon tri i šest meseci od započete terapije, svim ispitanicima su kontrolisane vrednosti PSA, testosterona i LH. Sve analize vršene su u laboratoriji Specijalističke poliklinike Kliničkog centra Vojvodine u Novom Sadu, automatizovanom metodom elektrohemiluminescencije na aparatu Elecsys 2010.

U statističkoj obradi podataka primenjeni su Studentov *t*-test i multivarijantna analiza varijanse (MANOVA), a značajnim su smatrane razlike srednjih vrednosti na nivou $p < 0,05$.

Rezultati

Izmerene vrednosti PSA, testosterona i LH za sve grupe ispitanika pre, kao i tri, odnosno šest meseci nakon primenjenog terapijskog protokola predstavljene su u tabeli 1.

Kod bolesnika grupe I (terapija LH-RH agonistima) i II (na totalnoj androgenoj blokadi) vrednost testosterona posle tri meseca opala je za 95,58%, odnosno 95,72% u odnosu na početnu, a razlika je bila statistički visokoznačajna ($p < 0,01$). Između vrednosti posle tri i šest meseci nije bilo razlike u ovim grupama ispitanika. Kod bolesnika grupe III (monoterapija antiandrogenom) vrednost testosterona posle tri meseca opala je za 67% u odnosu na početnu, a razlika između ove dve vrednosti, takođe, bila je statistički veoma značajna ($p < 0,01$).

Tabela 1

Srednje vrednosti prostata specifičnog antigena (PSA), testosterona i luteinizirajućeg hormona (LH) ispitanika pre, kao i tri, odnosno šest meseci nakon primenjenog terapijskog protokola

Grupa bolesnika (terapija)	Parametar	Vreme merenja posle uvođenja terapije		
		Bazalne vrednosti ($\bar{x} \pm SD$)	Posle tri meseca ($\bar{x} \pm SD$)	Posle šest meseci ($\bar{x} \pm SD$)
I (LH-RH agonist)	Testosteron (nmol/L)	12,07 ± 3,29	0,53 ± 0,24*,†	0,55 ± 0,35*
	LH (U/L)	6,31 ± 2,66	3,13 ± 0,83*	3,20 ± 0,85*
	PSA (ng/mL)	96,03 ± 54,88	5,51 ± 5,79*	5,87 ± 5,77*
II (totalna androgena blokada)	Testosteron (nmol/L)	12,93 ± 3,31	0,55 ± 0,27*,†	0,52 ± 0,30*
	LH (U/L)	6,67 ± 2,37	4,21 ± 0,88*	4,09 ± 0,94*,‡
	PSA (ng/mL)	97,21 ± 56,46	5,12 ± 4,56*	5,48 ± 3,43*
III (antiandrogena)	Testosteron (nmol/L)	13,54 ± 3,58	4,47 ± 1,73*	4,48 ± 1,76*
	LH (U/L)	6,54 ± 2,22	4,09 ± 1,02	3,85 ± 0,82*,‡
	PSA (ng/mL)	97,26 ± 58,86	15,04 ± 17,54*	15,05 ± 17,46*
IV (kontrola – bez terapije)	Testosteron (nmol/L)	14,24 ± 3,64	14,06 ± 3,12	14,29 ± 3,48
	LH (U/L)	6,72 ± 7,11	6,59 ± 6,78	6,47 ± 7,02
	PSA (ng/mL)	3,07 ± 1,36	3,04 ± 1,23	3,15 ± 1,20

LH – luteinizirajući hormon; LH-RH – gonodotropni rilizing hormon; PSA – prostata specifični antigen
***$p < 0,01$ *vs* bazalne vrednosti; †$p < 0,01$ *vs* odgovarajuća vrednost grupe III; ‡$p < 0,01$ *vs* odgovarajuća vrednost grupe I**

Poređenjem vrednosti testosterona između grupa III i I, kao i III i II, utvrđena je statistički visokoznačajna razlika nakon tri meseca terapije. Ova razlika održavala se i nakon šest meseci terapije ($p < 0,01$). Između grupa I i II posle tri i šest meseci postojala je razlika u vrednostima testosterona, ali ona nije bila statistički značajna.

Vrednosti LH u svim grupama bolesnika sa terapijskim protokolom bile su statistički značajno niže nakon tri meseca lečenja ($p < 0,01$). Statistički značajna razlika ($t = 3,03$; $p < 0,05$) nađena je između vrednosti LH nakon šest meseci terapije kod bolesnika grupa I i II, kao i između srednjih vrednosti LH kod bolesnika grupa III i I nakon tri meseca od početka terapije. Statistički značajna razlika ($t = 2,143$; $p < 0,05$) između srednjih vrednosti LH kod bolesnika grupa III i I zabeležena je i nakon šest meseci terapije. Nivo LH bio je viši kod bolesnika grupe III i posle tri i posle šest meseci. Iako je uočen niži početni nivo LH kod svih bolesnika sa karcinomom prostate u odnosu na kontrolnu grupu razlika nije bila statistički značajna.

Kod bolesnika sa karcinomom prostate, u sve tri ispitivane terapijske grupe, registrovan je statistički značajni pad vrednosti PSA nakon tri, odnosno šest meseci terapije.

Kod bolesnika kontrolne grupe srednje vrednosti testosterona, LH i PSA održavale su se u posmatranom šestomesečnom periodu u okviru referentnih vrednosti za životno doba.

Diskusija

Cilj endokrine terapije karcinoma prostate je da snizi nivo testosterona na kastracioni nivo čime dolazi do apoptoze i usporavanja rasta androgeno zavisnih karcinoma prostate. Ocena efekata endokrine terapije postiže se praćenjem nivoa testosterona, LH i PSA.

U prvoj grupi bolesnika, kod kojih je ispitivan endokrini efekat i efikasnost LH-RH agonista, zabeleženo je statistički značajno sniženje nivoa testosterona (na kastracioni nivo) i LH u trećem mesecu u odnosu na početni nivo. Razlike između njihovih vrednosti u trećem i šestom mesecu nisu bile statistički značajne, što znači da se kastracioni nivo testosterona održavao sve vreme primene LH-RH agonista. Sarosdy i sar. [10] sproveli su multicentričnu studiju u grupi koja je obuhvatila 59 bolesnika, ispitujući uticaj monoterapije LH-RH agonistima (goserelin-acetat 3,6 mg) na nivo testosterona i LH u serumu bolesnika sa lokalno uznapredovalim ili metastatskim karcinomom prostate (T3, T4, ili M1). Merenja su vršena prvog dana, u 4, 8, 12. i 24. nedelji nakon davanja goserelin-acetata. Rezultati studije pokazali su da srednja vrednost testosterona pada ispod 0,5 nmol/L u 4. nedelji i ostaje u nivou kastracionih vrednosti u toku dalje terapije. Svih 59 bolesnika zadržalo je kastracioni nivo testosterona u 3. mesecu. U 6. mesecu tri bolesnika (5%) imala su beznačajno povećanje nivoa testosterona, ali još uvek u okviru kastracionih nivoa, što je u skladu sa našim rezultatima. U našem istraživanju jedan bolesnik (6%) imao je u 6. mesecu minimalni porast nivoa testosterona, ali u okviru kastracionih vrednosti. Saradosy i sar. [10] kod šest bolesnika (10%) u 6. mesecu ustanovili su prolazan porast testosterona iznad kastracionog nivoa, ali koncentracije van ovog vremenskog perioda bile su u granicama kastracionog nivoa. Debruyne i sar. [11] pokazali su da je klinički ishod za bolesnike sa testosteronskim skokom isti kao i za one koji su imali testosteron u granicama kastracionog nivoa. U našem istraživanju nije bilo ovakvih odstupanja. Pad vrednosti testosterona na kastracioni nivo potvrđuje u svojoj studiji i Schally [12]. Oefelein i Cornum [13] ispitivali su efekat LH-RH agoniste koji se daje kao depo preparat jednom na tri meseca na serumski nivo testos-

terona. Oni su merili nivo testosterona na svakih 28 dana, tri meseca nakon poslednje aplikacije LH-RH agoniste. Iz njihovih rezultata vidi se da lekovi ove grupe održavaju kastracioni nivo u proseku šest meseci nakon poslednje aplikacije depo preparata. Iz ovoga se može zaključiti da LH-RH agonisti snižavaju nivo testosterona na kastracioni (ispod 0,5 nmol/L) tri nedelje nakon početka terapije i održavaju ga u nastavku lečenja. Parmar i sar. [14] u svom istraživanju dobili su statistički značajno sniženje nivoa LH u odnosu na početne vrednosti koje se održavalo tokom dalje primene terapije, što je saglasno sa našim rezultatima. Ovo sniženje nivoa testosterona na kastracioni nivo može se objasniti time što dugotrajna primena LH-RH agonista dovodi do inhibicije hipofizno-gonadalne osovine. Ovo predstavlja mehanizam na osnovu kojeg LH-RH agonisti usporavaju rast karcinoma prostate. Nekoliko nezavisnih autora, npr. Oefelein i Cornum [13], u svojim studijama imali su bolesnike kod kojih nije postignut kastracioni nivo testosterona (5% od ukupno 38 bolesnika). U našem istraživanju kod svih bolesnika ove grupe nivo testosterona pao je na kastracioni.

Hirurška ili farmokološka kastracija snižavaju nivo cirkulišućeg testosterona u proseku za 95%, ali intraprostatični androgeni su i dalje prisutni zahvaljujući konverziji nadbubrežnih androgena u dihidrotestosteron u ćelijama prostate [15]. Dejstvo nadbubrežnih androgena blokira se dodavanjem antiandrogena (ciproteron-acetat) hirurškoj ili farmakološkoj kastraciji čime se postiže koncept totalne ili maksimalne androgene blokade (MAB). Drugu grupu ispitanika predstavljali su bolesnici podvrgnuti MAB (hirurška kastracija i antiandrogen-ciproteron acetat). Uključivanje antiandrogena značajno je jer postoje studije koje ukazuju na to da oni u sklopu ne snižavaju značajnije nivo testosterona, u odnosu na monoterapiju (hirurška ili farmakološka kastracija). U slučajevima monoterapije perifernim (nesteroidnim) antiandrogenima, nivo testosterona u serumu se čak i povećava 1–2 puta u odnosu na inicijalni nivo [15]. To povećanje objašnjava se blokadom odgovarajućih receptora u hipotalamusu tako da androgeni (testosteron) ne mogu ispoljavati svoje negativno "*feed-back*" dejstvo. Ukoliko se rezultati grupe sa TAB-om uporede sa prvom grupom (farmakološka kastracija) vidi se da nivo testosterona u obe grupe u trećem mesecu pada na kastracioni i da se održava na tom nivou i u šestom mesecu. Statistički značajna razlika u nivou testosterona u trećem i šestom mesecu ne postoji. LH-RH agonisti (farmakološka kastracija) blokiraju LH receptore i snižavaju nivo testosterona, ali je blokada nekompletna jer se male količine androgena još uvek stvaraju u nadbubregu. Efekat je jednak hirurškoj kastraciji. Značajna statistička razlika između ove dve grupe postoji kod LH u trećem mesecu. Ovo se može objasniti gubitkom inhibitornog uticaja testosterona na hipotalamus i nemogućnošću antiandrogena da snizi nivo LH u istoj meri kao i LH-RH agonisti. Naše istraživanje ukazuje na to da ova razlika u nivou LH između grupa nije značajna za klinički rad ukoliko se u obzir uzmu klinički odgovor i preživljavanje, što potvrđuju Parmar i sar. [16] u svojoj studiji. Oefelein [17] je pratio 32 bolesnika sa uznapredovalim karcinomom prostate, od kojih je sedam bolesnika bilo na totalnoj androgenoj blokadi (kastracija i antiandrogen), dok su ostali bili podvrgnuti farmakološkoj kastraciji (LH-RH agonisti). On je u svojim rezultatima naveo da se nivoi testosterona održavaju u šestom mesecu od početka terapije na kastracionom nivou u obe grupe, bez statistički značajne razlike u prosečnim nivoima testosterona između grupa [17]. Rađene su mnogobrojne studije koje su poredile kastraciju (farmakološku, hiruršku) sa TAB. Neki autori su našli da dodatak antiandrogena ne pojačava efekat hirurške kastracije, odnosno da je uticaj TAB beznačajan. Crawford i sar. [18] u velikoj studiji u SAD koja je obuhvatila 1 400 bolesnika utvrdili su da orhiektomija kombinovana sa antiandrogenom nije klinički superiornija u odnosu na orhiektomiju i placebo. To su pokazali i Iversen i sar. [19] u metaanalizi 22 randomizirana ispitivanja, sa ukupno 5 710 bolesnika. Maksimalna androgena blokada nije dala statistički značajno veće petogodišnje preživljavanje u odnosu na samu hiruršku ili farmakološku kastraciju. Prosečna dužina praćenja bila je 40 meseci tokom kojih je ukupan mortalitet bio 56,3% u grupi sa maksimalnom androgenom blokadom, a 58,4% posle kastracije. Ova razlika nije bila statistički značajna. Petogodišnje preživljavanje bilo je 26,2% u grupi bolesnika lečenih maksimalnom androgenom blokadom, a 22,8% u grupi lečenih kastracijom. Ni ova razlika od 3,4% nije bila statistički značajna [20]. Iako pojedine studije pokazuju izvesno preimućstvo maksimalne androgene blokade, kao što to navode Labrie i sar. [21], može se reći da prema poslednjim metaanalizama i sitstematskim pregledima petogodišnjih preživljavanja, maksimalna androgena blokada daje manju prednost u preživljavanju (manju od 5%) kada se poredi sa monoterapijom (hirurška, farmakološka) [22].

U terapiju za treću grupu uveden je antiandrogen (steroidni), ciproteron acetat. Nivo testosterona opao je 67% u odnosu na početni. Varenhorst i sar. [23] u svojoj studiji su u kojoj je ispitivana monoterapija ciproteron-acetatom našli da se nivo testosterona snižava u proseku 70% u odnosu na preterapijski. Ovi rezultati poklapaju se sa našim nalazima. Moffat [24] je uporedio nivo testosterona kod bolesnika na terapiji ciproteron-acetatom i goserelinom, uz praćenje petogodišnjeg preživljavanja. Nivoi testosterons pali su na prosečno za 68% u odnosu na početne (što se poklapa sa našim rezultatima), međutim, kod bolesnika na ciproteron-acetatu preživljavanje je bio znatno lošije u odnosu na druge oblike terapije [24]. Thorpe i sar. [25] poredili su monoterapiju farmakološkom kastracijom, monoterapiju ciproteron-acetatom i totalnu androgenu blokadu tri grupe, ukupno 525 bolesnika. Bolesnici su praćeni klinički i biohemijski svaki drugi mesec, godinu dana, potom na svakih šest meseci, tokom 48 meseci. Značajan pad nivoa testosterona i LH utvrđen je u grupi koja je bila podvrgnuta farmakološkoj kastraciji što je saglasno sa našim rezultatima. Ovoj studiji se zamera što nisu objavljeni podaci o preživljavanju bolesnika.

Nivoi LH u grupama bolesnika sa karcinomom prostate pre terapije bili su niži u odnosu na nivoe u kontrolnoj grupi, ali bez statistički značajne razlike. Hilz i sar. [26] sproveli su studiju u kojoj su mereni nivoi LH i testosterona kod bolesnika sa uznapredovalim karcinomom prostate i bolesnika nakon radikalne prostatektomije i poređeni sa kontrolnom grupom (benigna hiperplazije prostate). Njihovi rezultati poka-

zuju da postoji značajna redukcija nivoa LH kod bolesnika sa uznapredovalim karcinomom prostate u odnosu na bolesnike sa benignom hiperplazijom prostate, što odgovara i našim rezultatima, s tim što u našem istraživanju nema statistički značajne razlike. Redukcija nivoa LH kod bolesnika sa uznapredovalim karcinomom prostate u odnosu na zdrave osobe potvrđena je i u studiji Hammonda i sar. [27] Phadke i sar. [28] navode da postoji sniženje LH kod uznapredovalog karcinoma u odnosu na bolesnike sa benignom hiperplazijom prostate, što odgovara rezultatima naše prethodne studije [29]. Niži nivo LH može se objasniti direktnim uticajem karcinomskih ćelija na serumski LH (produkcija materija koje snižavaju LH u serumu od strane karcinoma) ili indirektno, kao posledica dediferencijacije kojom se gubi normalna (stimulatorna) funkcija zdrave prostate na homeostazu LH. Otkriće LH receptora na ćelijama prostate može ukazivati da je prostata jedan od ciljnih organa LH koja učestvuje u njegovoj homeostazi.

Sve tri vrste primenjenih terapijskih protokola u lečenju karcinoma prostate statistički su značajno snižavale vrednosti PSA u odnosu na početne vrednosti. Iako PSA nije specifičan marker za karcinom prostate, dinamika njegove promene u toku androgene blokade, predstavlja značajan pokazatelj terapijskog efekta.

Zaključak

Totalna androgena blokada i primena LH-RH agonista značajnije snižavaju nivo testosterona na kastracione vrednosti nego monoterapija antiandrogenima, kada vrednosti testosterona ostaju iznad kastracionog nivoa. Ovakav terapijski pristup ima prednost jer snižava vrednosti testosterona na nivoe koji omogućavaju bolji odgovor na terapiju. Pri primeni totalne androgene blokade i monoterapije LH-RH agonistima, postoji razlika u nivoima testosterona, ali ona nije statistički značajna.

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Significance of magnetic resonance imaging in differential diagnosis of nontraumatic brachial plexopathy

Značaj magnetne rezonance za diferencijalnu dijagnozu netraumatskih brahijalnih pleksopatija

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Abstract

Background/Aim. Nontraumatic brachial plexopathies may be caused by primary or secondary tumors, radiation or inflammation. The aim of this study was to present the significance of MRI in revealing the cause of nontraumatic brachial plexopathy. **Methods.** A two-year retrospective study included 22 patients with nontraumatic brachial plexopathy. In all the patients typical clinical findings were confirmed by upper limb neurophysiological studies. In all of them MRI of brachial plexus was performed by 1.5 T scanner in T1 and T1 FS sequence with and without contrast, as well as in T2 and T2 FS sequences. **Results.** Seven (32%) patients had brachial plexopathy with signs of inflammatory process, 5 (23%) patients had secondary tumors, in 4 (18%) patients multifocal motor neuropathy was established and in the same number (18%) of the patients postradiation fibrosis was found. Two patients (9%) had primary neurogenic tumors. **Conclusion.** According to the results of this study MRI is a method which may determine localization and cause of brachial plexopathy. MRI can detect focal nerve lesions when other methods fail to find them. Thus, MRI has a direct impact on further diagnostic and therapeutical procedures.

Key words:
brachial plexus neuropathies; diagnosis; magnetic resonance imaging; diagnosis, differential.

Apstrakt

Uvod/Cilj. Netraumatske brahijalne pleksopatije mogu izazvati primarni ili sekundarni tumori, zračenje i upala. Cilj ove studije bio je da se prikaže značaj magnetne rezonance (MR) kao sofisticirane dijagnostičke metode u otkrivanju uzroka netraumatskih brahijalnih pleksopatija u našoj sredini. **Metode.** Dvogodišnja retrospektivna studija uključila je 22 bolesnika sa netraumatskom brahijalnom pleksopatijom. Kod svih ispitanika tipičan klinički nalaz oštećenja brahijalnog pleksusa potvrđen je elektromioneurografskim pregledom. Svim bolesnicima urađen je potom MR pregled brahijalnog pleksusa u T1 i T2 sekvenci, T1 FS sekvenci sa kontrastom i T2FS sekvenci, aparatom jačine 1,5 T. **Rezultati.** Kod sedam (32%) bolesnika MR pregled pokazao je znake jasne inflamacije brahijalnog pleksusa, pet (23%) bolesnika imalo je sekundarne tumore, kod četiri (18%) utvrđena je multifokalna motorna neuropatija, kod četiri (18%) postiradijaciona fibroza i kod 2 (9%) bolesnika primarni neurogeni tumori. **Zaključak.** Primena MR pregleda, a posebno T2 sekvence sa kontrastom, omogućava lokalizaciju i identifikaciju promena brahijalnog pleksusa i sužava spektar mogućih uzoraka brahijalne pleksopatije. Poseban značaj MR pregled ima za otkrivanje fokalnih lezija brahijalnog pleksusa koje se ne mogu otkriti drugim metodama. Na ovaj način MR direktno utiče na izbor daljih postupaka u dijagnostici i lečenju bolesnika sa oštećenjem brahijalnog pleksusa.

Ključne reči:
neuropatije, brahijalni pleksus; dijagnoza; magnetna rezonanca, snimanje; dijagnoza, diferencijalna.

Introduction

The brachial plexus is formed from the anterior primary rami from the fifth cervical (C5) to the first thoracic spinal segment (T1), with or without minor branches from the fourth cervical (C4) and the second thoracic (T2) rami [1]. The brachial plexus is a complex structure which carries motor, sensory and autonomic fibers that supply the upper limb [1].

Brachial plexopathies develop when lesions occur anywhere along the course of the brachial plexus [2]. These lesions are often due to trauma [3]. Nontraumatic brachial plexopathies may be caused by primary or secondary tumors, radiation or inflammation (Parsonage-Turner syndrome, multifocal motor

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neuropathy, post infectious and post vaccination plexitis, chronic inflammatory demyelinating polyneuropathy, connective tissue diseases etc.) [4].

A diagnosis of brachial plexopathy is based on clinical evaluation and electromyography (EMG) [2]. EMG may help clarify whether a lesion is central or peripheral, in distinguishing between radiculopathy and plexopathy, as well as in revealing extensiveness and level of lesion [2]. However, for precise determination of the localization and characterization of the cause, imaging technique is necessary [2]. Magnetic resonance imaging (MRI) is the method of choice in evaluation of brachial plexus pathology because of its multiplanar capabilities and exquisite soft-tissue contrast [5]. Normal anatomy of the brachial plexus and surrounding structures is well demonstrated on T1-weighted images, while T2-weighted, fat-suppressed or STIR sequences are important as they provide more specific tissue characterization of the plexus itself [6]. Contrast may be useful for more precise characterization of the cause of plexopathy [7].

The aim of this research was to demonstrate the significance of MRI in revealing a cause of nontraumatic brachial plexopathy.

Methods

This retrospective study included patients with brachial plexopathy hospitalized in the Institute of Neurology from January 1st 2008 until December 31st 2009. The clinical diagnosis of brachial plexopathy was based on a specific pattern of muscle weakness, sensory loss and loss of muscle reflexes. The diagnosis was made by the neurologist specialized in peripheral nervous system disorders and it was confirmed by EMG. Patients with a history of trauma of brachial plexus were excluded from the study, thus 22 patients fulfilled inclusion criteria. Four patients exhibited bilateral asymmetrical weakness of muscles innervated by radial, ulnar and/or median nerve. They clinically appeared as multifocal motor neuropathy (MMN). EMG revealed denervation in muscles innervated by aforementioned nerves, but conduction blocks were not observed, thus diagnosis of MMN was not confirmed.

The investigated group consisted of 16 males and 6 females. The mean age of patients was 47.3 ± 11.9 (range 27 to 71) years.

MRI of cervical spine and brachial plexus was performed in all the patients using a Siemens Avanto 1.5 T unit. The following sequences were applied: an axial turbo spin-echo T1 sequence (FoV 280 mm, slice thickness 3.0 mm, TR 561 ms, TE 11 ms, flip angle 150 degree, acquisition number 1, base resolution 320), an axial turbo spin-echo T2 sequence (FoV 280 mm, slice thickness 3.0 mm, TR 3600 ms, TE 127 ms, flip angle 170 degree, acquisition number 1, base resolution 512), a coronal fat-saturated (FS) turbo spin-echo T1 sequence (FoV 350 mm, slice thickness 2.5 mm, TR 550 ms, TE 11 ms, flip angle 150 degree, acquisition number 1, base resolution 256), and a coronal fat-saturated (FS) turbo spin-echo T2 sequence (FoV 350 mm, slice thickness 2.5 mm, TR 7500 ms, TE 157 ms, flip angle 170 degree, acquisition number 1, base resolution 384). The T1 sequences were also made after application of paramagnetic contrast.

Results

MRI imaging in the patients with the admission diagnosis of brachial plexopathy showed signs of inflammation in 7 (32%) of the patients. Secondary tumor was observed in 5 (23%) of the patients and signs of MMN were revealed in 4 (18%) of the patients. The same number of patients was diagnosed with postradiation fibrosis of the brachial plexus (18%), while the remaining two (9%) patients had a primary tumor on MRI imaging (Figure 1).

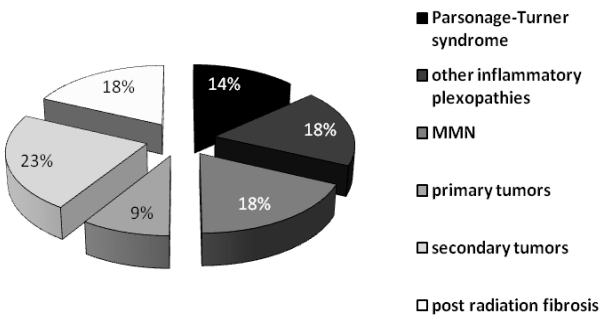

Fig. 1 – Causes of nontraumatic brachial plexopathy in the 22 investigated patients
MMN – multifocal motor neuropathy

MRI in 7 (32%) of the patients with inflammatory plexopathy depicted diffuse plexus enlargement with homogenously high signal on T2 sequences and T1 sequences after contrast application (Figure 2). In 3 patients the disease

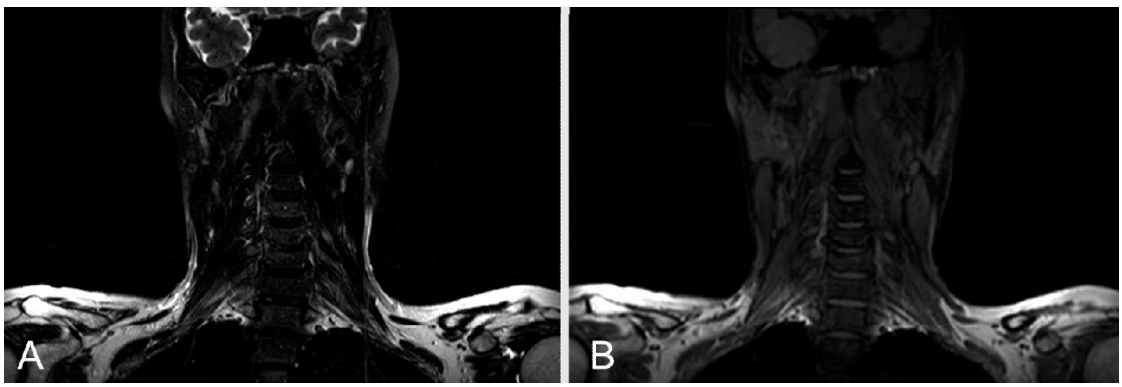

Fig. 2 – Brachial plexitis (A – coronal T2 FS sequence and B – coronal T1 FS sequence with contrast, show enhanced signal of nerve roots)

started with intensive pain in one shoulder accompanied with muscle weakness and wasting in the same arm after a few days. On the basis of a characteristic clinical presentation, the diagnosis of acute idiopathic brachial neuritis (Parsonage-Turner syndrome) was made. The remaining 4 patients did not feel shoulder pain and the disease progressed more slowly. Additional investigation revealed positive hepatitis C virus (HCV) antibodies in two of four patients.

In all four (18%) patients with clinically suspected MMN, MRI showed focal high signal lesions on T2 sequences and T1 sequences after contrast application along the structures of brachial plexus and ventral rami of cervical roots (Figure 3).

ment after gadolinium contrast application (Figure 4). Additional investigations revealed breast cancer in two female patients, lung carcinoma in two patients and chronic lymphocytic leukaemia in one patient.

Postradiation fibrosis of the brachial plexus was found in 4 (18%) females as a diffuse thickening and enhancement of the plexus structures without visible focal masses, with soft tissue changes of the low signal intensity on both T1 and T2 sequences. Postradiation fibrosis was due to radiation therapy of breast cancer in all the patients.

In two (9%) patients MRI examination revealed a primary neoplasm as a lesion isointense to muscle on T1 and hyperintense on T2 images (Figure 5). Subsequent

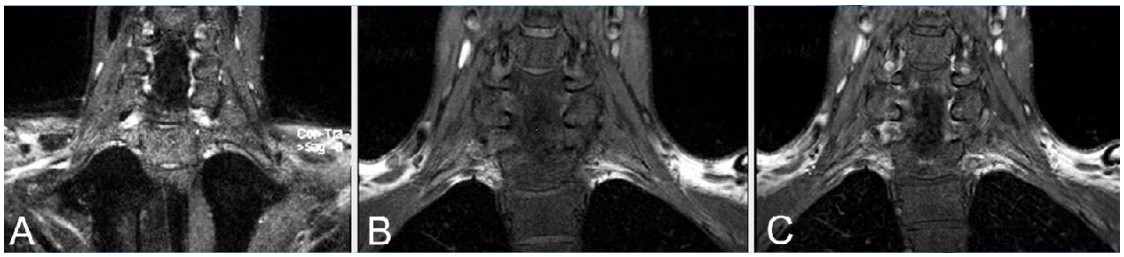

Fig. 3 – Multifocal motor neuropathy (A – coronal T2 FS sequence; B – coronal T1 sequence without contrast and C – coronal T1 FS sequence with contrast, show focal high signal intensity lesions)

In 5 (23%) of the patients MRI depicted secondary tumors as masses of high signal intensity on T2 sequences and low signal intensity on T1 sequences with signal enhance-

histopathological findings confirmed the diagnosis of neurofibromatosis in one patient and Schwannoma in the other.

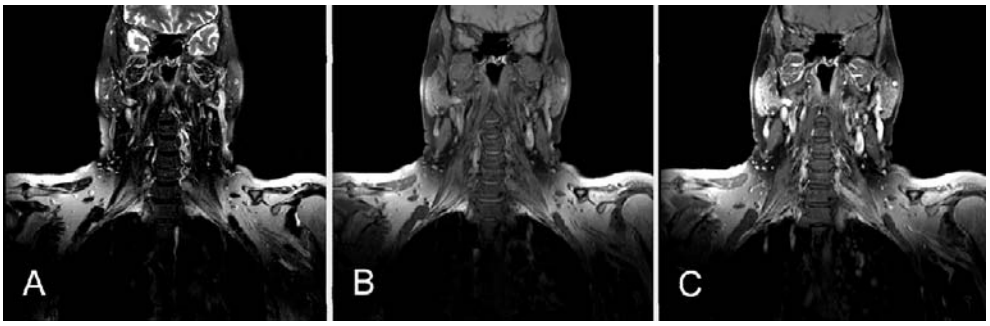

Fig. 4 – Secondary deposits (A – coronal T2 FS sequence B – coronal T1 sequence without contrast and C – coronal T1 FS sequence with contrast, show nerve roots enlargement in the left plexus)

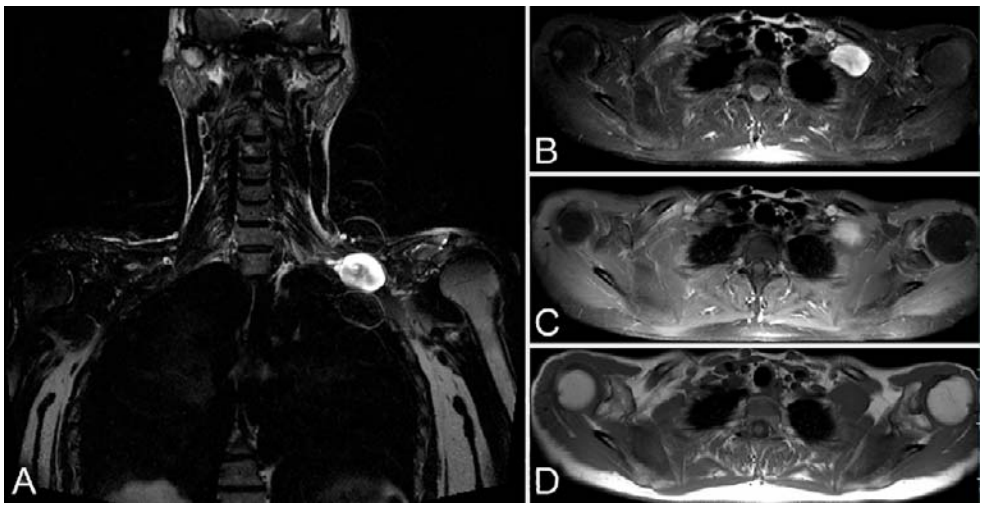

Fig. 5 – Schwannoma in the left supraclavicular fossa (A – coronal T2 FS sequence; B – axial T2 FS sequence; C – axial T1 sequence with contrast and D – axial T1 sequence without contrast, show nerve roots compression by tumor)

Discussion

Our study shows that MRI can significantly contribute to causal diagnosis of brachial plexopathy. The most common plexopathies in our study were inflammatory (32%) and plexopathies caused by secondary tumors (23%). One previous study from Ireland showed a similar distribution of the most frequent causes of brachial plexopathy [1]. According to Wittenberg et al [8], with the exception of patients with plexitis, the most common causes of brachial plexopathies are postradiation fibrosis and secondary tumors.

Among the patients with inflammatory plexopathies, 3 were diagnosed with acute idiopathic brachial neuritis (Parsonage-Turner syndrome) on the basis of characteristic clinical presentation. Parsonage-Turner syndrome is the most common form of nontraumatic brachial plexopathy [8]. The cause of brachial plexus neuritis usually remains undetected, but 25% of patients have a preceding infection (usually respiratory) and in 15% of patients plexitis follows different vaccinations [9]. Additional investigation revealed positive HCV antibodies in two patients with signs of diffuse brachial plexus inflammation, but we cannot state with certainty that this infection contributed to the onset of plexitis. MRI is not able to reveal the cause of the inflammatory plexopathy and further investigations are needed, including virological, immunological and cerebrospinal fluid analyses, as well as repeated EMG examinations [10]. However, MRI is of major importance in revealing extensiveness of the inflammatory process which largely determines prognosis of disease [10]. MRI helps in distinguishing between plexitis and radiculopathy because MRI findings may be positive only a few days after the onset of disease while EMG still does not show any abnormalities [6]. Therefore, MRI imaging enables early and accurate diagnosis and unnecessary surgical treatment can be avoided [6].

In all patients with clinically suspected MMN, MRI showed focal lesions along the structures of brachial plexus and ventral rami of cervical roots. MMN is an immune-mediated demyelinating polyneuropathy characterized by progressive asymmetric weakness and atrophy of the limbs muscles in the distribution of peripheral nerves [4, 11]. Diagnostic criterion for MMN is the presence of conduction blocks on electrodiagnostic studies [11]. However, conduction blocks may be localized in proximal nerve segments and, so, difficult to be detected by EMG as was the case in our patients. These patients may be misdiagnosed with amyotrophic lateral sclerosis (ALS). In these cases, the significance of MRI is crucial [11]. MRI also may distinguish MMN from other inflammatory plexopathies – in MMN patients MRI shows focal high signal lesions on T2 sequences, while in other inflammatory plexopathies MRI usually depicts diffuse and homogenous signal enhancement [9].

In 23% of the patients MRI revealed a secondary tumor of brachial plexus. In the region of the neck and axilla secondary tumors are more common than primary [4]. Brachial plexopathy caused by metastatic disease or by *per continuitatem* tumor spread is most often seen in patients with breast and lung carcinoma, lymphoma, leukaemia or multiple myeloma [4, 8, 12]. Secondary deposits are usually isointense with primary malignancy [9]. MRI imaging is important in determining further therapeutic approach (surgical, radiation or chemotherapy) as early as possible with regard to the level of plexus infiltration [12].

Post radiation fibrosis of the brachial plexus was found in 18% of patients due to radiation therapy of breast carcinoma. Radiation plexitis is a subacute or chronic plexopathy with an incidence less than 1% [11]. This damage may occur 6 months to 20 years after completion of radiation (generally after 10–20 months) and it is more likely to occur after doses in excess of 60 Gy [11, 13]. Chronic radiation plexitis is usually presented as postradiation fibrosis [6] and it is most frequently associated with breast cancer radiation therapy [14], as was the case in our patients. Similar clinical presentation of recurrent tumor, radiation plexopathy and postradiation fibrosis can be frequently overcome with MRI examination [14]. Recurrent tumor appears as a nonuniform, asymmetric, diffuse or focal enlargement with high signal intensity on T2 sequence and with postcontrast enhancement on T1 sequence [2]. Radiation plexopathy is diffuse, uniform, symmetric plexus swelling with high signal intensity on T2 sequence [10]. Postradiation fibrosis appears as symmetric hipointensity on both T1 and T2 sequences [10].

In 9% of patients MRI examination revealed a primary neoplasm of brachial plexopathy. The most common primary neurogenic tumors of the plexus are neuroma, neurofibroma and Schwannoma, while malignant peripheral nerve sheath tumors are less frequent [10, 15, 16]. Neurofibromas and Schwannomas are isointense to muscle on T1 sequence with intense signal enhancement after contrast administration and they are hyperintense on T2 images [4]. Central areas with low signal intensity (the so-called target sign) are more often seen in neurofibroma than in Schwannoma [9]. Histopathological examination is usually necessary to determine the exact type of tumor [17]. Nevertheless, the importance of MRI examination in the diagnosis of primary tumors of the brachial plexus, as well as consequential decision about further diagnostic and therapeutic procedures is unambiguous [9].

Conclusion

MRI examination, especially T2 sequence with fat saturation and T1 sequence with contrast, enables localization and identification of brachial plexus lesions and narrows the spectrum of possible causes of brachial plexopathies. The exceptional significance of MRI examination is in the detection of focal lesions of the brachial plexus that cannot be detected by other methods. Therefore MRI directly influences the choice of further diagnostic procedures and treatment of patients with brachial plexopathy.

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Heritabilnost bipolarnog poremećaja raspoloženja – studija familija

Heritability of bipolar affective disorder – family study

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Apstrakt

Uvod/Cilj. Bipolarni poremećaj raspoloženja je mentalni poremećaj sa poligenim tipom nasleđivanja. Za procenu veličine doprinosa genetičke varijabilnosti (varijanse) fenotipskoj varijansi koristi se heritabilnost – odnos genetičke i sredinske varijanse. Rezultati novijih studija pokazuju tendenciju opadanja vrednosti heritabilnosti bipolarnog poremećaja raspoloženja, što ukazuje na ovaj poremećaj kao na kompleksnu ponašajnu pražnu karakteristiku. Cilj ovog rada bio je da se proceni doprinos genetičke varijanse fenotipskoj varijansi bipolarnog poremećaja raspoloženja, odnosno da se proceni heritabilnosti ovog poremećaja. **Metode.** Anketiranjem 80 bolesnika koji su prešli prag za bipolarni poremećaj raspoloženja, dobijene su funkcionalne informacije o članovima njihovih familija koji se nalaze u prvom stepenu srodstva sa njima (očevi, majke i rođena braća i sestre). Pomoću „Apleta za izračunavanje heritabilnosti pražnih karakteristika (oboljenja)“ koji koristi regresionu analizu, procenjena je heritabilnost bipolarnog poremećaja raspoloženja i njena statistička značajnost (χ^2 test). **Rezultati.** Heritabilnost, odnosno odnos genetičke i sredinske varijanse bipolarnog poremećaja raspoloženja je 0,2 i statistički je značajno različita od nule ($p < 0{,}001$). **Zaključak.** Procenjen doprinos genetičke varijanse fenotipskoj varijansi bipolarnog poremećaja je nizak i iznosi 20%, dok doprinos sredinske varijanse iznosi 80%. Ovaj rezultat predstavlja doprinos shvatanju bipolarnog poremećaja raspoloženja kao kompleksne ponašajne pražne karakteristike.

Ključne reči:
psihoze, manično-depresione; nasledne osobine, zastupljenost; porodica; upitnici.

Abstract

Background/Aim. Bipolar affective disorder is mental disorder with polygenic type of heredity. Heritability – relation between genetic and environmental variance is used to estimate the level of influence of genetic variance to phenotype variance. Study results show decreasing trend in the value of heritability of bipolar affective disorder, thus indicating that this disorder is a complex behavioral threshold characteristic. Therefore, the aim of this study was to estimate the contribution of genetic variance to phenotype variance of bipolar affective disorder, i.e. to estimate heritability of this disorder. **Methods.** By the use of a questionnaire, 80 patients with over crossed threshold for bipolar affective disorder were asked for functional information about the members of their families belonging to the first degree of relation (fathers, mothers and full–sibs). By using ”Applet for calculating heritability for threshold traits (disease)“, and regression analysis, heritability of bipolar affective disorder as well as its statistical significance, were estimated (χ^2 test). **Results.** Heritability and relationship of genetic and environmental variance of bipolar affective disorder is 0.2 with statistically significant difference from zero ($p < 0.001$). **Conclusion.** The estimated contribution of genetic variance to phenotype variance of bipolar affective disorder is low being 20%, while the contribution of environmental variance is 80%. This result contributes to the understanding of bipolar affective disorder as a complex behavioral threshold trait.

Key words:
bipolar disorder; quantitative trait, heritable; family; questionnaires.

Uvod

Bipolarni poremećaj raspoloženja je mentalni poremećaj i poremećaj ponašanja čijim fenotipom dominira poremećaj emocija kao mentalne funkcije, odnosno poremećaj raspoloženja kao posebnog stanja emocija [1–3].

Bipolarni poremećaj raspoloženja eksprimira se u dve alternativne fenotipske klase: kao klasa jedinki koje nisu prešle prag (*threshold*) za bipolarni poremećaj raspoloženja, i kao klasa jedinki koje su prešle prag za bipolarni poremećaj raspoloženja [4].

Tip nasleđivanja bipolarnog poremećaja raspoloženja je poligen [5] što znači da fenotipskoj varijabilnosti (varijansi)

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ovog poremećaja doprinose genetička varijansa i sredinska varijansa [4]. Za procenu veličine doprinosa genetičke varijanse fenotipskoj varijansi u kvantitativnoj genetici koristi se statistička konstrukcija koja se zove heritabilnost [6].

Heritabilnost, po definiciji, jeste odnos genetičke varijanse i sredinske varijanse, $h^2 = S^2_G / S^2_E$, gde je S^2_G genetička varijansa, odnosno varijansa koja je posledica poligenog tipa nasleđivanja, a S^2_E sredinska varijansa [6, 7].

Heritabilnost bipolarnog poremećaja raspoloženja procenjivana je u različitim studijama, na različitim uzorcima jedinki koje su pripadale različitim populacijama sa različitih lokaliteta, na primer: na populaciji koju su činila 104 para blizanaca od kojih je bar jedan proband prešao prag za bipolarni poremećaj raspoloženja, od toga 37 parova bili su monozigotni i 67 parova dizigotni blizanci, sa teritorije Engleske [8] (studija blizanaca); na populaciji koju su činila 303 para blizanaca od kojih je bar jedan proband prešao prag za bipolarni poremećaj raspoloženja, od toga 108 parova bili su monozigotni i 195 parova dizigotni blizanci koji su bili istog pola kao monozigotni, sa teritorije Norveške [9] (studija blizanaca, takođe); na uzorku koji je činilo 40 487 jedinki: bioloških roditelja i dece, rođenih braće i sestara, polubraće i polusestara, adoptivnih roditelja i dece, od kojih su određeni probandi prešli prag za bipolarni poremećaj raspoloženja, iz preko 2 miliona familija – podaci su uzimani iz multigeneracijskog Registra švedskog zdravstvenog osiguranja od 1973. do 2004. godine (epidemiološka studija) [10]; na različitim uzorcima jedinki koje su prešle prag za bipolarni poremećaj raspoloženja, sa različitih lokaliteta, čija je genetička osnova ovog poremećaja, nakon studija familija, studija blizanaca i studija adopcije potvrđena u studijama vezanog nasleđivanja (*linkage* studijama) i molekularno-genetičkim istraživanjima [11] – revijalna studija.

Studije familija, ipak, pokazale su se kao najpodesnija metoda za istraživanje genetičke osnove bipolarnog poremećaja raspoloženja [12, 13].

Rezultati pomenutih studija pokazuju tendenciju opadanja vrednosti heritabilnosti bipolarnog poremećaja raspoloženja, na primer: 0,85 [8]; 0,77 [9]; 0,64 [10]; 0,89–0,62 [11] što, uz poligeni tip nasleđivanja ovog poremećaja i dve alternativne fenotipske klase u kojima se on eksprimira, ukazuje na bipolarni poremećaj raspoloženja kao na kompleksnu ponašajnu pražnu karakteristiku, odnosno adaptivnu karakteristiku. Naime, ponašanje je izrazito adaptivna karakteristika jedinke i, kao takvo, veoma podložno sredinskim promenama [4] (vrednost heritabilnosti je niska). Stoga, cilj ovog rada bio je da se proceni doprinos genetičke varijanse fenotipskoj varijansi bipolarnog poremećaja raspoloženja, odnosno da se proceni heritabilnosti ovog poremećaja. Smatramo da je bitno naglasiti da, prema našim saznanjima, heritabilnost bipolarnog poremećaja raspoloženja nije nikada procenjivana na uzorku jedinki koje pripadaju populaciji sa nekog našeg lokaliteta.

Metode

Ovo istraživanje obavljeno je u Centru za psihofarmakoterapiju Instituta za mentalno zdravlje u Beogradu u periodu od 10.9.2004. do 29.3.2005. godine. Početni uzorak činilo je 80 bolesnika koji su prešli prag za bipolarni poremećaj raspoloženja, od toga 24 muškarca i 56 žena. Dijagnoza bipolarnog poremećaja raspoloženja postavljena je po MKB – 10 klasifikaciji mentalnih poremećaja i poremećaja ponašanja – Dijagnostički kriterijumi za istraživanje [14]. Bolesnici su imali između 20 i 63 godine, a prosečno 35,92 godine. Bolesnici su, u navedenom vremenskom periodu, lečeni u dnevnoj bolnici Instituta za mentalno zdravlje ili su dolazili na svoje redovne kontrolne preglede u ovu ustanovu. Početni uzorak bio je izabran metodom slučajnog izbora.

U istraživanju korišćena je anketa koja je, osim demografskog podatka o srodstvu članova familije sa bolesnicima, sadržala još dva pitanja koja su se odnosila na funkcionalne informacije o njihovim familijama. Funkcionalne informacije ukazuju na „medicinske probleme, emocionalno funkcionisanje i ponašanje različitih članova familije“ [15].

Anketa je predvidela funkcionalne informacije o članovima familija bolesnika koji se nalaze u prvom stepenu srodstva sa njima (očevi, majke, rođena braća i sestre) – ciljni uzorak. Takođe, anketa je predvidela funkcionalne informacije o članovima familija bolesnika koje se odnose na podkategoriju „Bipolarni poremećaj raspoloženja“ koja se nalazi u MKB – 10 klasifikacije mentalnih poremećaja i poremećaja ponašanja – Klinički opisi i dijagnostička uputstva [1].

U cilju povećanja pouzdanosti informacija, odgovore na anketna pitanja davali su bolesnici i još po jedan do dva člana njihovih familija: brat, sestra, otac, majka, supružnik i drugi. Osim toga, pregledana je medicinska dokumentacija bolesnika koja se odnosi na prisustvo bipolarnog poremećaja raspoloženja u njihovim familijama. Na kraju, konsultovani su lekari kod kojih se/su se leče/lečili bolesnici i članovi njihovih familija koji su prešli prag za bipolarni poremećaj raspoloženja.

Heritabilnost bipolarnog poremećaja raspoloženja procenjena je pomoću „Apleta za izračunavanje heritabilnosti pražnih karakteristika (oboljenja)“ [16, 17]. Procena heritabilnosti pomoću navedenog apleta bazira se na frekvencijama bipolarnog poremećaja raspoloženja, i to: na frekvenciji bipolarnog poremećaja raspoloženja u opštoj populaciji i na frekvenciji bipolarnog poremećaja raspoloženja u ciljnom uzorku. Za izračunavanje heritabilnosti aplet koristi formulu koja proističe iz metode procene ove statističke konstrukcije pomoću eksperimenata veštačke selekcije – $h^2 = R / S$, gde je R selekcioni odgovor, a S selekcioni diferencijal. Naime, aplet tretira ciljni uzorak kao veštački selekcionisani uzorak zato što je frekvencija bipolarnog poremećaja raspoloženja u njemu statistički značajno veća nego frekvencija ovog poremećaja u opštoj populaciji. Navedena formula dobijena je statističkom metodom, regresionom analizom, koju aplet koristi. Za testiranje statističke značajnosti heritabilnosti aplet koristi χ^2 test.

Ovo istraživanje sprovedeno je u skladu sa etičkim standardima Komiteta za eksperimente na ljudima i, takođe, uz saglasnost Etičkog odbora Instituta za mentalno zdravlje u Beogradu.

Rezultati

Frekvencija bipolarnog poremećaja raspoloženja u uzorku opšte populacije koja je poznata iz sumiranih epidemioloških studija [2, 18, 19] i frekvencija ovog poremećaja u cilj-

nom uzorku – uzorku koji čine članovi familija bolesnika koji su prešli prag za bipolarni poremećaj raspoloženja i koji se nalaze u prvom stepenu srodstva sa njima (očevi, majke, rođena braća i sestre), a koja je izračunata pomoću apleta navedenog u metodama prikazane su u tabeli 1.

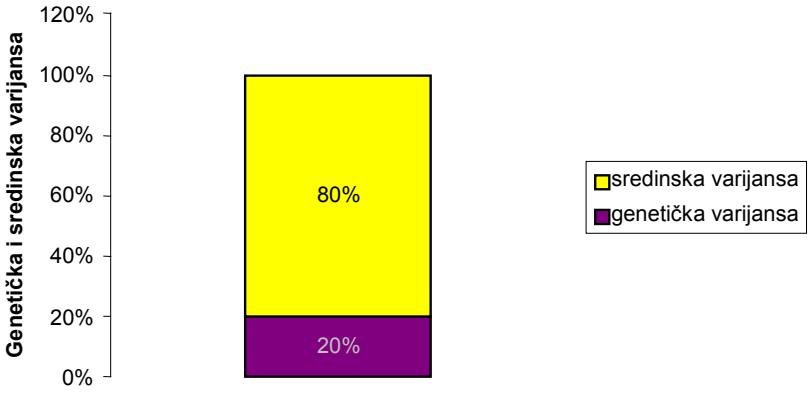
Drugim rečima, naša studija pokazala je da je doprinos genetičke varijanse fenotipskoj varijansi kod bipolarnog poremećaja raspoloženja 0,2 (20%), a doprinos srediske varijanse ovoj varijansi 0,8 (80%) (slika 1).

Tabela 1

Eksperimentalni uzorci i frekvencije bipolarnog poremećaja raspoloženja

Uzorak	N_U	N_Z	N_B	f_B
Opšta populacija	1 000	975	25	0,0250
Bolesnici – startni uzorak	80	–	–	
Roditelji	160	147	13	–
Braća i sestre	66	61	5	–
Roditelji, braća i sestre – ciljni uzorak	226	208	18	0,0796

N = 306 (startni uzorak + ciljni uzorak)
N_U – ukupan broj jedinki; N_Z – broj jedinki koje nisu prešle *threshold* za bipolarni poremećaj raspoloženja;
N_B – broj jedinki koje su prešle *threshold* za bipolarni poremećaj raspoloženja;
f_B – frekvencija bipolarnog poremećaja raspoloženja



Sl. 1 – Odnos genetičke i sredinske varijanse kod bipolarnog poremećaja raspoloženja

Uzimajući u obzir različite podatke o vrednosti prevalencije bipolarnog poremećaja raspoloženja iz navedenih sumiranih epidemioloških studija, kako evropskih tako i vanevropskih, i zaključke o ovim podacima koji se tiču različitih dijagnostičkih kriterijuma (ICD – 10, DSM – 4), i različitih metoda (upitnika), te pouzdanosti postavljanja dijagnoze ovog poremećaja [2, 18–20], smatrali smo podatak o njegovoj prevalenciji u vrednosti od 2,5% (25 od 1 000) iz druge studije [18], uz princip „istorijske kontrole", validnim za našu studiju (naša populacija, ICD – 10 dijagnostički kriterijumi koji se koriste kod nas, anketa koja je u skladu sa ICD – 10 dijagnostičkim kriterijumima).

Frekvencija u ciljnom uzorku bila je 3 puta veća nego u opštoj populaciji – 0,0796 : 0,0250 = 3,1840. Zato se ciljni uzorak tretira kao veštački selekcionisani uzorak. Zbog apletske simulacije eksperimenta veštačke selekcije, iz ukupnog uzorka (306 jedinki) koji čine: startni uzorak – bolesnici (80 jedinki) i ciljni uzorak – očevi, majke, rođena braća i sestre bolesnika (226 jedinki), izostavljaju se bolesnici. Iz istog razloga u ciljni uzorak nisu ušla deca bolesnika koja se sa njima, takođe, nalaze u prvom stepenu srodstva.

Heritabilnost bipolarnog poremećaja raspoloženja koja je izračunata pomoću apleta navedenog u metodama, iznosi 0,2 i statistički je značajno različita od 0 ($p < 0,001$).

Diskusija

Iako je heritabilnost bipolarnog poremećaja raspoloženja procenjivana u različitim studijama – od studija familija, preko studija blizanaca, studija adopcije, *linkage* studija, do molekularno-genetičkih istraživanja, najpodesniji alat za istraživanje genetičkih determinacija i veličine sredinskih uticaja na razviće ovog poremećaja i dalje su upravo studije familija [12].

Moglo bi se reći da se rezultat naše studije – heritabilnost, odnosno odnos genetičke varijanse i sredinske varijanse koji iznosi 0,2, razlikuje od rezultata drugih studija u kojima je procenjivana statistička konstrukcija ovog poremećaja. Naime, heritabilnost bipolarnog poremećaja raspoloženja u drugim studijama iznosi preko 0,6, na primer: 0,64 (epidemiološka studija) [10]; 0,77; 0,85 (studije blizanaca) [8, 9]; 0,62–0,89 (studije familija, studije blizanaca, studije adopcije, *linkage* studije i molekularno-genetička istraživanja) [11].

U vezi sa neskladom između rezultata naše studije i rezultata drugih studija u procenjivanju heritabilnosti bipolarnog poremećaja raspoloženja bitno je ukazati na relativnost same heritabilnosti. Naime, heritabilnost je odraz kompleksnosti populacije koja je činila uzorak koji se, prema nekim autorima, mora manje ili više uprosečiti u zavisnosti od manje ili veće kompleksnosti i trenutnih sredinskih faktora koji

su na njega delovali [21]. Takođe, različitim metodama za procenu heritabilnosti dobijaju se različiti rezultati [21].

Međutim, treba istaći da je rezultat naše studije u skladu sa trendom opadanja vrednosti heritabilnosti bipolarnog poremećaja raspoloženja u drugim studijama, na primer: 0,85 [8]; 0,77 [9]; 0,64 [10]; 0,89–0,62 [11]. Takođe, Donelly [22] ističe da su vrednosti heritabilnosti bipolarnog poremećaja raspoloženja veoma precenjene.

Za objašnjenje tendencije opadanja vrednosti heritabilnosti smatramo najadekvatnijim shvatanje bipolarnog poremećaja raspoloženja kao kompleksne ponašajne pražne karakteristike. Naime, ponašanje podrazumeva svaku aktivnost jedinke čiji su ciljevi vijabilitet i fertilitet što znači da je ponašanje izrazito adaptivna karakteristika jedinke. Zbog toga je ono veoma podložno sredinskim promenama, a to znači da ima nisku vrednost heritabilnosti [4]. Nadovezujući se na ovu činjenicu, Ballas [23] ističe da su jedinke koje su prešli prag za bipolarni poremećaj raspoloženja u fazi manije produktivnije i kreativnije nego jedinke koje nisu prešli prag za ovaj poremećaj. Produktivniji i kreativniji muškarci uspešniji su u takozvanoj kompeticiji između muškaraca, kao jednom od dva mehanizma seksualne selekcije kod čoveka [21].

U svetlu shvatanja bipolarnog poremećaja raspoloženja kao kompleksne ponašajne pražne karakteristike, rezultat naše studije odgovara rezultatima drugih studija u kojima je procenjivana heritabilnosti ponašajnih karakteristika. Tako su, na primer, rezultati 105 studija pokazali da je prosečna vrednost heritabilnosti različitih ponašajnih karakteristika kod životinja 0,3 [24, 25]. Isti rezultati dobijeni su i prilikom procenjivanja heritabilnosti različitih ponašajnih karakteristika kod ljudi [26].

Iako je procenjena heritabilnost bipolarnog poremećaja raspoloženja, dobijena u ovoj studiji, niska, njena statistička značajnost sugeriše da je taj genetički dobrinos sasvim dovoljan da opstane u populaciji uprkos dejstvu prirodne selekcije.

Zaključak

Procenjen doprinos genetičke varijanse fenotipskoj varijansi bipolarnog poremećaja raspoloženja, odnosno heritabilnost ovog poremećaja je niska (0,2). Naša studija predstavlja skroman doprinos shvatanju bipolarnog poremećaja raspoloženja kao kompleksne ponašajne pražne karakteristike, odnosno adaptivne karakteristike, što doprinosi razumevanju razloga opstanka ovog poremećaja u populaciji.

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Optical metrology analysis of the lower jaw deformations

Analiza deformacija donje vilice optičkom metrologijom

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Abstract

Background/Aim. New optical stereometric methods based on both contact and noncontact mechanisms for displacement measurement have become common methods in biomechanical behavior research of biomaterials, bone and soft tissue. The aim of this study was to register and measure possible deformations of the lower jaw (mandible) with the intact dental arch using optical metrology method. **Methods.** The system for full field measurement of deformations (strains) comprised of two digital cameras for a synchronized stereoview of the specimen, and the Aramis software. **Results.** The maximum mandibular bone strains were measured in the regions of the lower first premolar and the lower second molar. In the action force of 500 N simulated in the region of the first lower premolar the intensity of deformation was 86 μm. The value of maximum strain in the bone around the molars was 24 μm for the force of 500 N acting on the second lower molar. When it comes to premolars, 3–5 times stronger deformation was observed in the region of the first lower premolar, compared to the deformation values of the second lower premolar area. **Conclusion.** Under loading of the applied forces the measured strains were in the elastic deformation area, meanning that the dependence of force and deformity is linear. The highest values of strain measurements obtained by the optical method were found in the jaw bone tissue around the loading teeth, and the bony regions of the triangle and mental region. According to the obtained results from the Aramis processing software it can be concluded that this method is applicable in a variety of biomedical research.

Key words:
mandible; numerical analysis, computer-assisted; optical devices; orthodontics.

Apstrakt

Uvod/Cilj. Nove optičke stereometrijske metode koje se zasnivaju na kontaktnim i nekontaktnim mehanizmima za merenje zapremine postaju uobičajene metode u istraživanju biomehaničkog ponašanja biomaterijala, koštanog i mekog tkiva. Cilj ove studije bio je da se optičkom metodom merenja registruju i izmere eventualne deformacije koštanog fundamenta donje vilice sa intaktnim zubnim nizom i da se, ujedno, prikažu mogućnosti primene optičke metrologije u istraživanjima u stomatologiji. **Metode.** Sistem za merenje deformacija ispitivane donje vilice sa intaktnim zubnim lukom obuhvatio je dve digitalne kamere koje obezbeđuju stereosinhronizovani prikaz primerka, i softver Aramis. **Rezultati.** Najveće deformacije koštanog tkiva donje vilice izmerene su u regionu donjeg prvog premolara i donjeg drugog molara. Pri delovanju sila od 500 N za region prvog donjeg premolara veličina deformacije bila je 86 μm. Vrednost maksimalne srednje deformacije u koštanom sistemu oko molara iznosila je 24 μm pri delovanju sile od 500 N na drugi donji molar. Kada su u pitanju premolari, 3–5 puta jače deformacije uočene su u regionu prvog donjeg premolara, nego u predelu drugog donjeg premolara. **Zaključak.** Prilikom delovanja primenjenih sila deformacije se nalaze u elastičnom deformacionom polju, a međusobna zavisnost sile i deformacije ima linearan karakter. Najveće vrednosti deformacija dobijene optičkom metodom merenja registruju se u koštanom tkivu donje vilice koja je u neposrednom kontaktu sa zubima koji se opterećuju, kao i u koštanim regionima zakutnjačkog trougla i bradnog (mentalnog) otvora. Na osnovu analize rezultata dobijenih primenom softvera Aramis može se reći da postoje mogućnosti primene ove metode u različitim biomedicinskim istraživanjima.

Ključne reči:
mandibula; numerička analiza, kompjuterski asistirana; pribor, optički; ortodoncija.

Introduction

Biomechanical simulation of mastication force analysis on dried human skull has a wide application in planning and manufacture of prosthetic restorations, maxillofacial-skull surgery and orthopedics, analysing the manner of healing process, estimating the bone-implant interaction and reconstruction of bone segments after tumor elimination [1]. The said analysis can help in understanding the mechanism of the response of human tissues and its functional adaptation. The works of Wolf's (1892) and Frost (1990) describe functional adaptation of bone tissue and the relationship between the

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amount of bone microenvironment stress and consequent biological responses [2]. From the biomechanical view, stress is generated during occlusal function, and in the case of existence of dental-oseal connection (junction) transmittes over the teeth through periodontium, mandibular bone, maxilla and skull. Stresses and strains are induced as a consequence of occlusal thrill (pulsation) in all of the above mentioned structures of the orofacial system. The newly created stresses and strains will cause an adequate response in terms of functional adaptation and morphology adjustment [3]. Methods used so far for analyzing and measuring stress and strain involve application of computer-simulated force on virtual models of the skull. Finite element method (FEM) and photoelastic analysis (PA) are indirect methods for measuring stress and deformation of bone tissue. In recent years a number of optical methods based on direct strain measurement of the tested object have appeared and been widely used for verification of the virtual models and results. Non-invasive optical methods for direct measurement of stereometric strain biomaterials, bone and soft tissue are binocular stereovision, laser-speckle interferometry, photorefractive holographic interferometry technique and optical metrology. The above methods register deformations with extremel occurance and find a broad application in biomedical sciences [4–6].

The aim of this study was to register and mesure any deformation of the lower jaw bone fundament with intact dental arch (complete dentate), and also to show the possibilities of optical metrology in dentistry research by the use of selection (defining) the lower jaw model with intact dental arch; positioning of experimental models in the standard press and occlusal loading simulation; deformation measuring with digital cameras, and analysis of the results.

Methods

A model of the lower jaw with intact dental arch was used in the experiment. The lower jaw with intact dental arch was borrowed from the Laboratory for Anthropology, Institute of Anatomy, School of Medicine, University of Belgrade. According to the data from the laboratory archive, a mandible donor was a man, in late fourties from Serbian population. The lower jaw was inspected visually and evaluated, because it was necessary for experimental model to be without evident traumatic and pathological damages and to have all teeth present. Afterwards, the lower jaw was immersed and left in the physiological (saline) solution (0.9% NaCl) for 48 h in order to reach the volume and elasticity as *in vivo* studies [7].

After drying at 27°C in a drying chamber, the lower jaw with teeth was lacquered with a fast-setting acrylic lacquer white spray of high density (manufactured by Motip). The prepared model was placed in a tensile testing machine (standard press system) to measure deformation. The lower jaw was positioned on a horizontal plate of the tensile testing machine, fixed in specially constructed grooves. The forces applied in the experiment were within the range from 100 N to 500 N (1 N = 0.10 kg). Literature data suggest that maximal force in humans measured in the molar region is 500–700 N, and in the region of incisors 100–200 N [8]. According to Martinović [9], the value of masticatory force in patients with intact dental arch is 200 N. The study adopted intensity of the applied force up to 500 N, since forces greater than 500 N caused fractures of the loaded teeth and the system was unable to register further deformation.

Precise and controlled loading was measured using a gnathodynamometer (Siemens, Germany) horizontal extension. Direction of the applied load was axial to second premolar and first molar with maximal distribution in centric supporting contacts. In such a way, actual loading was simulated in places where it normally receives dental contacts in the position of central occlusion. The position of central occlusion in the human dentition is very common and only during swallowing is repeated 800 times per day [10]. The largest masticatory forces in natural and artificial teeth were observed in the second premolar and first molar area, which is the area of the tooth called "stable area" or "mastication center" (key of occlusion).

Precise measurment of strain in this research was conducted using the equimpment manufactured by GOM. The system consists of two digital cameras and software Aramis. Mobile cameras at a specific time interval make a photo of the distance between the reference points before the load in the calibration phase and later, during the action force.

Before experimental deformation measuring of the experimental models, calibration had to be performed. Calibration procedure was necessary for calibrating system and setting measuring parameters, ensuring dimensional consistence and obtaining precise results. For three-dimensional (3D) strain measuring, two cameras were positioned manually and adjusted in accordance with the measuring volume of the calibration object. The choice of the measuring volume dimensions directly depends on the measuring object dimensions. By choosing the measuring volume the distance between the sensor and the measuring object is determined. The basic elements of the camera system and the measuring volume are shown in Figure 1.

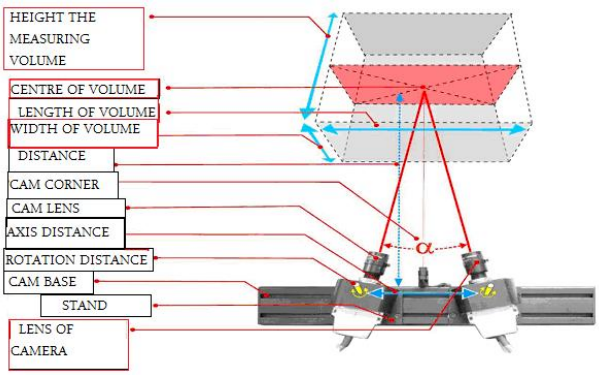

Fig. 1 – The basic elements of a camera system and measuring volume

Calibration objects may be calibration panels or calibration crosses of different dimensions. The project was defined in each new measuring and images were shown in various phases of applying the force. The software process-

ing of the successfully measured data enables 3D presentation, presentation of the results, statistic data, diagrams, reports. The optical measuring system (digital image correlation – DIC) can measure the parts and constructions of different dimensions (from 1 mm to 2 000 mm) by the same sensor and display deformation with 0.01%–100% preciseness [11].

The Aramis software used in the study is based on the principle of objective raster (fine-ground) procedure. It serves for measuring 3D changes of shape and distribution of deformation on the surface of statically and dynamically loaded objects. With high accuracy and contactlessly, Aramis determines the shape of the filmed object, its dimensions, field of three-dimensional movements, vector of distorted field and the features of the biomaterial [12]. Unlike tensiometer or extensometer that give only separate measuring values and measure deformities by elastic strips at the places where deformities are expected, Aramis defines deformity distribution over the whole analyzed area enabling better understanding of the material behaviour, constructions and the real measuring objects used in medicine and dentistry. In this study, Aramis separated the superficial layer of the tested bone 2 mm thick and showed distorted fields over the whole surface of the filmed bone, which meant that only the part of the bone spoted by the camera was analyzed by Aramis.

Aramis analyses, estimates and presents the report of material deformation. Also, the graphic presentation of the measured results gives an optimal understanding of the tested object behaviour, especially suitable for three-dimensional deformation measuring under statical and dynamical forces, in order to analyze deformations of real components. The surface of mandible with intact dental arch had to be prepared by putting a fine layer of the dispersive color with expressive contrast (Figure 2). The fine reference points of this spray occupied certain mutual distances that were changed under the loading and registered by the cameras.

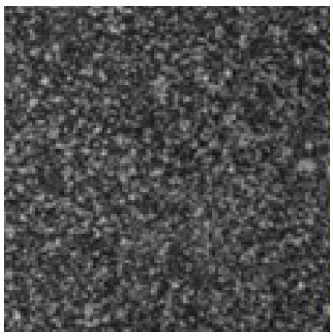

Fig. 2 – The layer with finely dispersive color over the experimental model

Results

Figure 3 shows a deformation field (major strain field) when the load is directed on the central fissure of the lower first premolar with the force intensity, F1 = 500 N. The strain values within the section line are shown in Figure 3. This line connects the points of reference applied to the observed object, the lower jaw bone foundation, and changes its length with the changing in the intensity and the force attack point. Vertical line is placed by the software under the force attack point acting on the lower first premolar. The scale next to Figure 3 allows registration of quantitative changes of the lower jaw, and is expressed in percents. According to the formula for deformation $\varepsilon = (L_0\text{-}L_1) / L_0 \times 100$, where L_0 and L_1 are the length before and after the load force respectively, the intensity of deformation expressed in the percents for the case of the lower first premolars loading, is visible in the scale next to Figure 3 presented in different colors.

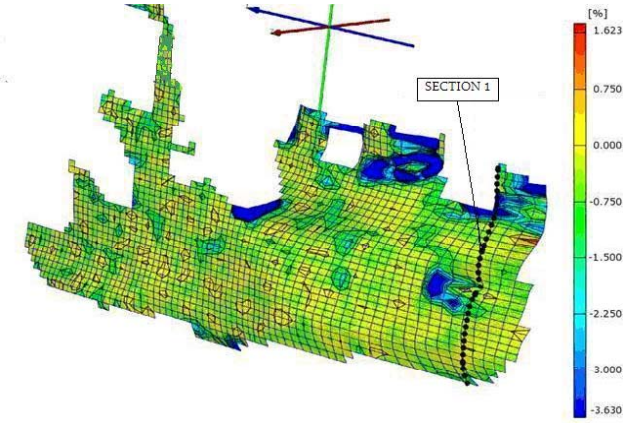

Fig. 3 – Percentage size of the lower jaw deformations at the load of the lower first premolar

In the second part of the experiment, the force was directed to a second premolar. Intensities of the forces that were applied were in the range of 100 to 500 N in the crop of 100 N. Deformation field is illustrated in Figure 4 and is located in the region of elasticity field. The intensity of deformation and strain field within the display was monitored by the scale on the right side next to Figure 4. Software set-section line (section 2) below the precipitating force whose attack (offensive) point is on the central fissure of the lower second premolar.

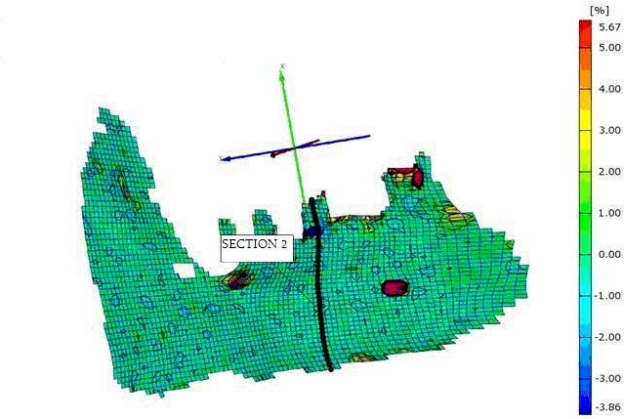

Fig. 4 – Percentage size of the lower jaw deformations at the load of the lower second premolar

The force attack point in the third part of the experiment was localized to the part of realization of the central contacts on the lower first molar so that the sagittal line section (section 3) was below the lower first molar. The acting forces were vertical and the intensity of forces was 100 to 500 N as in the first and second part of the experiment. The most intensive changes were present in the part of strain fields just below the force attack point. When the force intensity was 500 N, the percentage value of deformation and shortening of the length of the line section (Δ L) had the maximum value as shown in Figure 5.

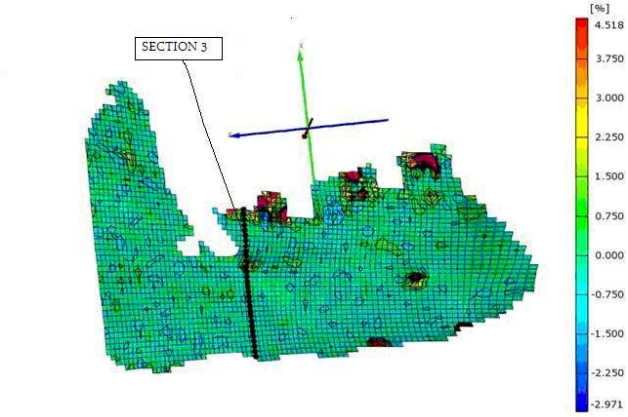

Fig. 5 – Percentage size of the lower jaw deformations at the load of the lower first molar

The force attack point in the last fourth part of the experiment was localized in the central fissure of the lower second molar. Elastic deformation field shows the most intensive deformation in the region of the retromolar triangle, as well as in the bone tissue that is in direct contact with the tooth. Figure 6 shows the distribution of deformation changes in the elastic strain field (major strain field), and the scale beside the figure describes intensity of deformation in the percentage.

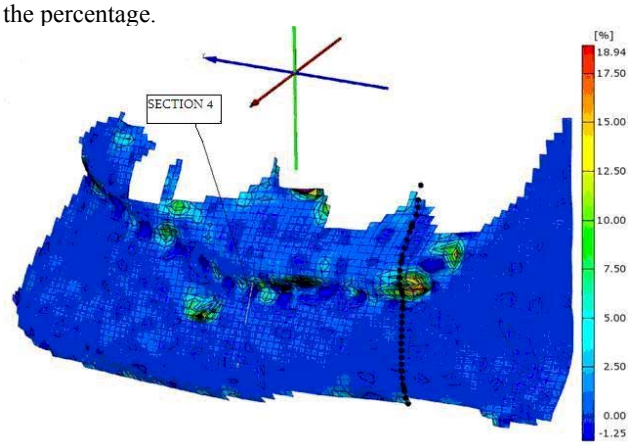

Fig. 6 Percentage size of the lower jaw deformations at the load of the lower second molar

Discussion

The results of previous studies showed a good correlation between the mathematical modeling finite element analysis (FEA) and *in vitro* measurements [12, 13]. In this study, distribution and intensity of deformation of the lower jaw bone with intact dental arch was conducted using the method of optical strain measurement. Reproducibility of optical measurements is very high, with a coefficient of variation of 0.5% [14]. The above fact enables high accuracy verification of virtual models that measure volume changes of bone tissue in the function of the load. The advantage of 3D optical measurement is registration of bone lamellas microdisplacements in direct access to the monitored segments within shortened intervals for obtaining accurate results, without scanning. Disadvantages of this method are that the process of measuring requires a slightly longer time period for registering movement of reference points. The negative side of the optical strain measurement is neutralized by fixing the body of mandible on the horizontal plate of standard presses (tensile testing machine) allowing unlimited time for makin photo of the loaded lower jaw (mandible). This paper presents only one possible application of optical method in dentistry. The study was not performed by modeling of soft tissue and periodontal tissues, but knowing the thickness and quality of these tissues, it is considered that it could affect only the intensity of deformation, that would be slightly lower, but not to the direction of strain distribution [15]. Within the obtained experimental results it can be noticed that under the same force action, deformations in the region of the first and second lower premolars are more expressed than deformations in the region of the molars. The reason for this strain distribution lies in the presence of the fewer number and lesser volume of the roots of lower premolars in relation to the massive and two-roots molars. The maximum mean strain that occurs in bone tissue around the lower premolars is 86 micrometers for the force of 500 N, compared with the maximal mean strain of 24 μm in the bone tissue around the molars (periodontium) at the action force of 500 N on the second lower molar. As for the premolars, stronger deformations are observed in the region of the first lower premolar, than in the region of the second lower premolars (3–5 times higher intensity of deformation) and that is explained by the presence of bone aperture (mental foramen) and poor bone density. Deformations in the region of the first lower molar are less expressed compared with the deformations obtained below the lower second molar. This data is consistent with the anatomical structure (morphology) of the lower jaw that possesses arms in the distal segments (medial crus and lateral crus of the retromolar triangle) that concentrates deformations more than the alveolar bone below the lower first molar. Regularity in the concentration of deformation is expressed as high deformation accumulation in the bone lamellas of alveolar the bone around tooth exposed to the masticatory forces, and in the region of mental foramen. The findings are consistent with other research data where maximum compressive strain was observed in the region of root apexis and bifurcations, therefore in the vicinity of the dental roots showed in the lower jaw of pigs [16, 17]. In all four experimental parts where occlusal loading of premolars and molars teeth was performed, Aramis software registered a marked accumulation of deformation in the region of the mental foramen. Also, a significant strain concentration was present in bone tissue of the lower jaw angle (*angulus mandibulae*) and in the anatomical structure of the

retromolar triangle. The results of the research confirmed the findings of strain distributions obtained by as FEA on the scanned model of the lower jaw, where no presence of specific bone paths (trajectories) in the region of the lower jaw body and branches (*corpus* and *ramus mandibulae*) was registered [18].

Analyzing the results of the research, it may be speculated that the biomedical aspects of optical strain measurement method may find a broad application in dental science. It is particularly important in: simulation or reproduction of clinical situations that exist in the oral cavity and more frequent application of biomechanics in clinical practice; better understanding of the distribution of masticatory forces (vertical and inclined) through the bony foundation; monitoring the deformation of the jaw, which was subjected to the action of forces and predicting the intensity and direction of subsequent resorption and remodeling; verification of indirect methods (FEM, PA), thanks to precise data obtained by Aramis software analysis.

Conclusion

Skeletal deformities of the lower jaw with intact dental arch using the optical method of measurement were recorded and analyzed. After evaluating the obtained results the following conclusions may be drawn: when loading the lower jaw with intact dental arch, the distribution of strain through the lower jaw bone system is uniform; the highest values of strain measurements are found in the jaw bone around the loading teeth, and the bony regions of the retromolar triangle and mental region; higher values of strain were observed with the anterior load shifting; the applied force and deformation are mutually linearly dependent, and deformations are in the elastic deformation field; based on the evaluation results it may also be noted that further research using optical methods of strain measurement should focus on monitoring the deformation of bone under the loaded dental restorations, as well as testing qualitative and quantitative characteristics of dental materials.

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Selective intraarterial radionuclide therapy with Yttrium-90 (Y-90) microspheres for hepatic neuroendocrine metastases: initial experience at a single center

Selektivno intraararterijsko radionuklidno lečenje primenom mikrosfera sa itrijumom-90 kod neuroendokrinih metastaza u jetri: prvo iskustvo u jednom centru

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Abstract

Background/Aim. Selective intraarterial radionuclide therapy (SIRT) with Yttrium-90 (Y-90) microspheres is also known as radioembolization and delivers high doses of radiation to hepatic tumors with minimum healthy liver exposure. The aim of this study was to present our preliminary experience in the role of liver directed radiotherapy with Y-90 microspheres for the treatment of unresectable hepatic metastases from neuroendocrine tumors (NET). **Methods.** The results of SIRT in 10 patients (5 males, 5 females; mean age 48.7 years; age range 24–73 years) with metastatic liver disease from NETs during the period from April 2008 through August 2010 were reviewed. All patients had meticulous pre- and post-imaging studies as a part of their work-up procedure, as well as serologic tests of liver function to determine the extent of liver function damage. The patients who were eligible for SIRT had pretreatment visceral angiography to define and occlude non-target arteries. **Results.** The mean ± SD administered SIR-Spheres® activity was 1.49 ± 0.42 GBq (range 0.72–2.21 GBq) in all the patients. These treatments delivered a dose of 99.73 ± 66.36 Gy (range 49–420.8 Gy) to the target tumors. The estimated dose to the lungs and normal liver was 4.45 ± 1.95 Gy (range 2.4–8.5 Gy) and 26.73 ± 14.19 Gy (range 5–58.9 Gy), respectively. Overall response rate of 90% and patient tolerance was satisfactory for most patients. **Conclusion.** From our limited experience, we can conclude that SIRT with Y-90 microspheres is a safe and efficacious treatment option for patients with liver metastasis of NET without any serious side effects.

Key words:
neuroendocrine tumors; liver; neoplasm metastasis; injections, intra-arterial; yttrium; prognosis.

Apstrakt

Uvod/Cilj. Selektivno intraarterijsko radionuklidno lečenje (*selective intraarterial radionuclide therapy* – SIRT) primenom mikrosfera sa itrijumom-90 (Y-90) poznato je kao radioembolizacija kojom se hepatički tumori zrače visokom dozom radijacije uz minimalno izlaganje zdravog dela jetre. Cilj ove studije bio je prikazivanje našeg prvog saznanja o ulozi SIRT, primenom mikrosfera Y-90, kod neoperabilnih hepatičkih metastaza sa poreklom od neuroendokrinih tumora (NET). **Metode.** Prikazani su rezultati SIRT kod 10 bolesnika (pet muškaraca i pet žena, srednje starosti 48,7 godina, u rasponu od 24 do 73 godine) sa metastatskim oboljenjem jetre izazvanim NET u periodu od aprila 2008. do avgusta 2010. Svi bolesnici prošli su kroz detaljno ispitivanje pre i posle snimanja, što je deo procedure, kao i kroz serološka ispitivanja funkcije jetre kako bi se utvrdio stepen njenog funkcijskog oštećenja. Bolesnicima koji su bili određeni za SIRT urađena je visceralna angiografija pre početka lečenja da bi se definisale i okludirale neciljne arterije. **Rezultati.** Prosečna aktivnost datih mikrosfera (SIR-Spheres®) iznosila je 1,49 ± 0,42 GBq (opseg 0,72–2,21 GBq) kod svih bolesnika. Ovim tretmanom ciljni tumori ozračeni su dozom od 99,73 ± 66,36 Gy (opseg 49–420,8 Gy). Procenjena doza za pluća i zdravu jetru bila je 4,45 ± 1,95 Gy (opseg 2,4–8,5 Gy) i 26,73 ± 14,19 Gy (opseg 5–58,9 Gy), respektivno. Ukupni terapijski odgovor od 90% i podnošljivost tretmana bili su zadovoljavajući kod većine bolesnika. **Zaključak.** Prema našem ograničenom iskustvu možemo zaključiti da SIRT primenom mikrosfera sa Y-90 predstavlja sigurni i efikasni način lečenja bolesnika sa metastazama u jetri nastalih zbog NET i to bez ozbiljnijih neželjenih dejstava.

Ključne reči:
neuroendokrini tumori; jetra; neoplazme, metastaze; injekcije, intra-arterijske; itrijum; prognoza.

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Introduction

One of the most important prognostic factors that dramatically affects survival of a patient with neuroendocrine tumor (NET) is the presence of liver metastasis. It has been shown that patients with liver metastasis have a worse survival rate compared to those without liver involvement in all types or digestive NETs [1, 2]. Liver metastasis has a negative impact on survival with a 10–20% 10-year survival compared to 90–100% without liver metastases [3]. Unfortunately, the majority of patients with NETs (up to 60–75%) already present with liver metastases. In particular, patients with non-functioning tumor (without hormonal symptoms) mostly present with liver disease in up to 50% of the cases [1]. Besides that, patients with liver metastases present an overall poor prognosis compared to those without liver metastases for all NETs regardless of the primary [3].

Although surgical resection remains the gold standard in the treatment of liver metastases achieving a survival rate of 60–80% at 5 years with low mortality (0–5%) and acceptable morbidity (close up to 30%), only a limited number of patients can meet the minimum requirements for curative surgical procedures [4–6]. Another possible therapy option for carefully selected patients with diffuse unresectable liver metastases or who suffer from severe hormonal disturbances refractory to medical therapy is liver transplantation. However, a long-term cure from the disease by transplantation will be an exceptional event even in this highly selected subgroup [7].

Local ablative techniques such as radiofrequency ablation (RFA) [8, 9], selective hepatic transcatheter arterial embolization (TAE) [10, 11] or chemoembolization (TACE) with hepatic artery occlusion [12, 13], can be employed in the treatment of unresectable liver metastases from NETs regardless of the origin of the primary tumor. Nonetheless, these ablative therapies as well as some others such as laser therapy, ethanol injection or cryotherapy are applicable to a small proportion of patients with few tumors [14–16].

Symptoms related with hormonal hypersecretion are frequent in functional tumors with liver metastases. Somatostatin analogues (with or without interferon) are often effective and helpful in controlling these symptoms [17]. However, medical therapies with somatostatin analogues and/or interferon have weak antiproliferative effects [18]. Systemic chemotherapy with streptozotocin achieves modest response rates of limited duration and is better for pancreatic NETs compared with metastatic carcinoid tumors [19]. A randomized study using doxorubicin with fluorouracil or streptozocin with fluorouracil followed by dacarbazine in the patients at disease progression of metastatic carcinoids demonstrated response rate of 8.2% with significant treatment related toxicity [20]. On the other hand, peptide receptor related radionuclide therapy may be used in the treatment of metastatic NETs, with 90 Y-DOTATOC and 177 Lu-DOTATOC revealing particular promise [21, 22].

Radioembolization with Yttrium-90 (Y-90) labeled microspheres has shown promise for the treatment of patients with nonsurgically resectable primary and metastatic liver metastatic disease [23–26]. It is known that hepatic tumors receive 80–100% of blood supply from the hepatic artery [27]. Since liver tumors are fed mainly by arterial rather than portal venous blood, a selective intraarterial radionuclide therapy (SIRT) via hepatic arterial administration of Y-90 microspheres may deliver high radiation doses to tumor tissue with minimal effect to the surrounding normal liver parenchyma. In SIRT, Y-90 microspheres are used to both embolize and irradiate tumors in the liver by delivering the microspheres through the hepatic artery [26]. In this study, we presented our initial experience with early follow-up results of SIRT with Y-90 microspheres for hepatic neuroendocrine metastases.

Methods

We retrospectively evaluated the data from 10 patients (male/female 5/5, mean ± SD age 48.7 ± 16.63 years; age range 24–73 years) between April 2008 and August 2010, who had SIRT with Y-90 microspheres (SIR-Spheres® Sirtex Medical, Lane Cove, Australia) for biopsy–proven progressive unresectable hepatic NET metastases.

All patients were neither suitable nor responsive to other local treatment options and showed inadequate response to systemic chemotherapy. Prior to the treatment, all patients were discussed in an interdisciplinary tumor board composed of medical oncologist, interventional radiologist, radiation oncologist, surgeon and an expert in nuclear medicine. All patients had to give a formal written informed consent after explanation of the whole treatment steps, alternative therapeutic options and possible complications.

The pretreatment evaluation included a medical history compatible with time course of the disease, chemotherapy, somatostatin analogues and/or interferon, laboratory tests and comorbid disease. All patients had FDG-PET scan at least 4 weeks prior to SIRT at least 4 weeks before to determine the extent of disease. Besides that, In-111 OctreoScan whole body scan results were evaluated if available. Other imaging studies, such as chest radiography, computed tomography (CT) scan of chest and abdomen, abdominal ultrasound and a bone scan are also done in determination of disease extent.

Adequate coagulation parameters and sufficient pulmonary function to undergo arterial catheterization, and adequate liver function [total biluribine (TB) level less than 2 mg/dL and alanine transaminase (ALT) or aspartate transaminase (AST) levels less than five times of upper limit of normal] were required in all patients. Patients who had previous external beam radiation therapy to the liver, ascites or were in clinical liver failure, markedly abnormal synthetic and excretory liver function tests (LFTs), complete portal vein thrombosis, life threatening major extra hepatic metastases, and those with expected survival < 3 months were not considered for SIRT.

All patients with sufficient lab results had pretreatment meticulous visceral angiography of the abdominal aorta, the mesenteric artery and the celiac trunk including the common hepatic artery. Moreover, a selective catheterization of the

right and left hepatic artery was done to identify and occlude non-target arteries with extrahepatic communication while ruling out any high grade stenosis or occlusion which would be contraindication for SIRT. Subsequently prophylactic embolization of extrahepatic vessels such as the right gastric, gastroduodenal or falciform artery was performed (Figure 1). Noncorrectable flow to gastrointestinal tract was an exclusionary criteria for SIRT. Technical details for performing mesenteric angiography prior to SIRT have been described in the literature [28].

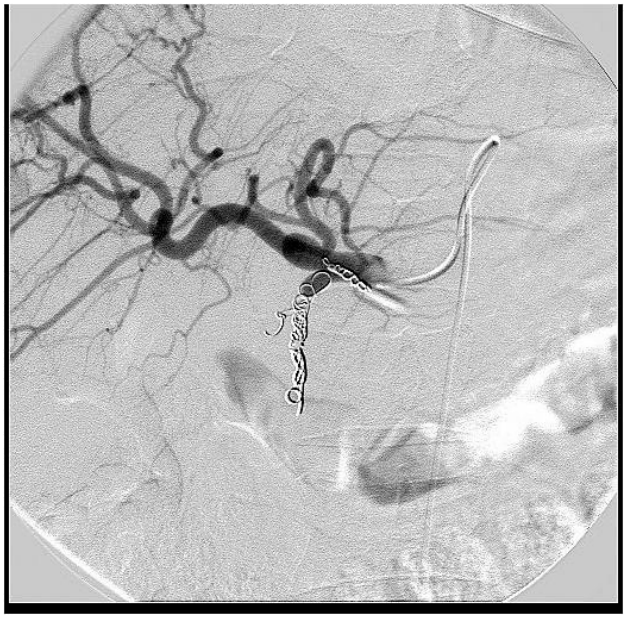

Fig. 1– Embolic coiling of the right gastric artery to eliminate pathways for visceral shunting in a 44-year-old male patient with hepatic metastatic medullary thyroid cancer lesions

Following initial angiographic evaluation with/without necessary prophylactic embolization of non-target extra hepatic vessels, 150 MBq (4mCi) Technetium-99m Macro-Aggregated Albumin (Tc-99m MAA) was injected *via* the hepatic artery catheter or implanted port to assess the fraction that passes through the liver to the lungs and the relative distribution of MAA (and hence SIR-Spheres®) between tumor and normal liver. Anterior and posterior scintigraphic images of the abdomen and thorax, and right lateral images of the abdomen were obtained to rule out any unexpected delivery of the activity (based on aberrant gastrointestinal flow) and to estimate the percentage of injected activity shunting from the liver into the lungs. Regions of interest are drawn around the whole of lung fields and the whole of liver field (Figure 2A). Additionally, a Single Photon Emission Computed Tomography (SPECT) (64 projections, 360º, 25 seconds *per* projection) study was done (Millenium, GE) in 6 patients (Figure 2B). Lung shunt fraction (LSF) was determined using the following formula [29]: all embolization procedures were preferentially performed on the day of the MAA scan before the administration of Y-90 radiomicrospheres.

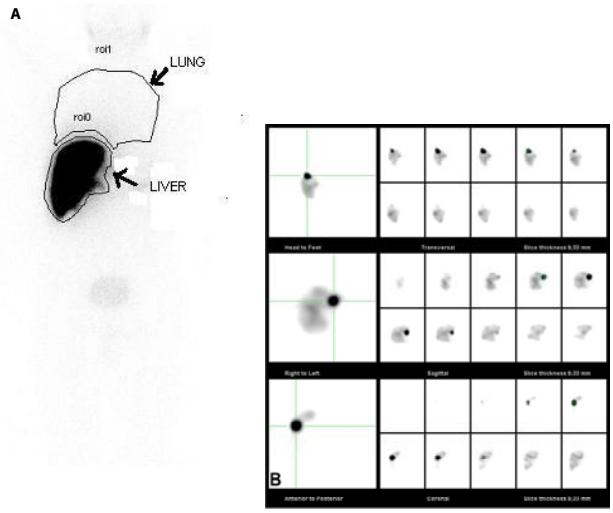

Fig. 2 – Regions of interest were drawn around the whole of lung fields and the whole of liver field on planar (A) Tc-99m MAA perfusion scan; SPECT (B) images were also obtained for simulation of Y-90 radiomicrospheres distribution before SIRT (note the faint gastric and salivary uptake due to free Tc-99m-pertechnetate)

$$LSF = \frac{\sqrt{Counts_{\text{Lung (Anterior)}} \times Counts_{\text{Lung (Posterior)}}}}{\sqrt{Counts_{\text{Lung + Liver (Anterior)}} \times Counts_{\text{Lung + Liver (Posterior}}}}$$

Since the particle size of the 99mTc-MAA is quite comparable to that of the microspheres, the gamma scintigraphy provided valuable information concerning the predicted distribution of the therapeutic dose and allows the quantification of hepato-pulmonary shunts [23, 26]. If the LSF is between 10–15% and 15–20% then a SIR-Spheres® dose reduction of 20% and 40% was done respectively. Although it was not the case in our patients, LSF more than 20% would be an absolute contraindication (with a dose to the lungs > 30 Gy) for SIRT. Additionally, there was no need for reduction in shunting <10%.

Selective intraarterial radionuclide therapy procedure was done in 2 to 3 weeks following the completion of the above-mentioned procedures. Treatment of one side of the liver was done for patients with disease limited to one lobe on CT and/or FDG PET-CT scan. Treatment of both sides of the liver was done by selective administration of Y-90 radiomicrospheres into the right and left hepatic vascular bed sequentially in the same session. A specially designed plexiglas delivery box provided by Sirtex Medical (Wilmington, MA) was used for SIRT procedure (Figure 3). The body surface area (BSA) method was used to calculate the prescribed SIR-Spheres® activity according to the following formula [26, 29]:

$$\text{Activity (GBq)} = \frac{(\text{BSA} - 0.2) + \text{Tumor Volume}}{\text{Liver Volume}}$$

Immediate medication consisting of antiemetics and analgesics were prescribed for all patients. Additionally, light

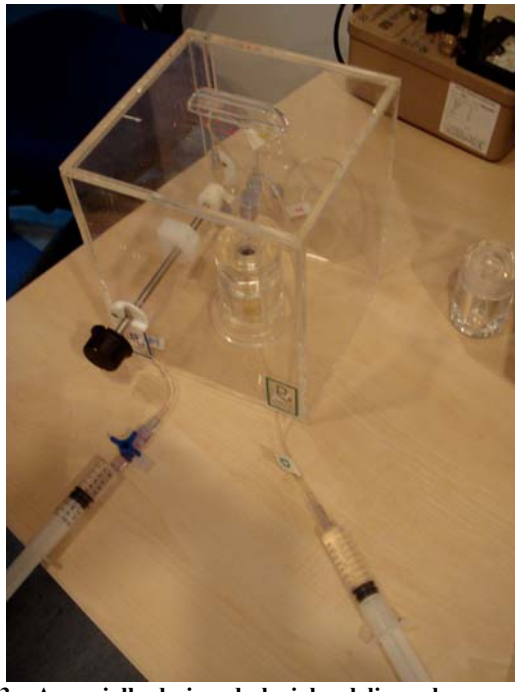

Fig. 3 – A specially designed plexiglas delivery box provided by Sirtex Medical (Lane Cove, Australia) which is used for injecting Y-90 radiomicrospheres

diet and sufficient hydration before and after SIRT were maintained. The patients were discharged after one night stay, and received a preventive gastric antisecretory treatment (proton pump inhibitors) for 1 month and low dose corticosteroids for one week to overcome flu-like reaction.

Since Y-90 is a pure beta emitter, Bremsstrahlung imaging is the only method for post treatment localization study of radiomicrospheres. According to the patients' clinical stability, post-therapeutic Bremsstrahlung imaging was performed to confirm and document the distribution of SIR-Spheres® in all patients between 2 to 24 hours after SIRT (Figure 4).

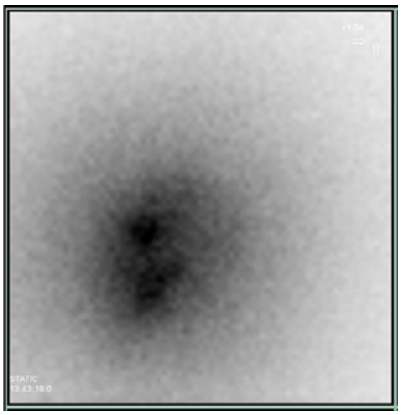

Fig. 4 – Bremsstrahlung imaging was performed to confirm and document the distribution of SIR-Spheres® after SIRT

All patients continued on low-dose steroids and proton-pump inhibitors for 1 week and 4 weeks after SIRT, respectively. Complete blood count, liver function tests, and routine biochemical tests were obtained at 24th hour after SIRT, and then at every 4 weeks for the next 3 months. Tumor response was assessed using RECIST criteria on CT. Moreover, FDG PET scan was performed at 10 to 12 weeks after SIRT for the evaluation of metabolic response and to differentiate viable components of the tumor from necrotic tissue.

The quantitative data was analyzed using the Wilcoxon Signed Ranks Test. Quantifiable data was given as mean ± standard deviation (SD) (if no otherwise specified). A statistically significant difference was considered when p values < 0.05. All calculations were performed using SPSS for Windows, Version 9.01.

Results

Sixteen SIRT procedures were carried out in 10 patients with metastatic hepatic disease from NETs during a 24-month period between April 2008 and May 2010. Out of the 10 patients, the primary NET site was the bronchus in 3 patients, the stomach in 1 patient, the medullary thyroid in 1 patient, the pancreas in 2 patients, the kolon/rectum in 2 patients and of unknown origin in 1 patient. The primary NETs were classified as carcinoid in 8 patients, medullary thyroid cancer in 1 patient and well differentiated unknown NET in one patient (Table 1).

Nine out of 10 patients had multifocal metastases in both lobes of the liver. Only one patient had multifocal disease in the right lobe of the liver. Of the 10 patients, 6 patients had whole liver treatments, and the other 4 patients had unilobar treatments initially. While 6 out of the 10 patients had single SIRT procedure, 2 patients had SIRT twice and 2 patients had SIRT for three times (Table 1). Whole liver treatments were administered either *via* common hepatic artery for bilobar disease or by the administration of 2 separate doses into the right and left hepatic arteries (after adjusting the dose according to the volume of the right and left hepatic lobes).

Because of the differences in the FDG avidity of NETs, we used a combined approach for the calculation of tumor volumes and normal livers. For the calculation of SIR-Spheres® activity to be administered, the total volume of the all FDG avid hepatic metastases, and also normal liver volumes were initially computed from the contoured and thresholded region by counting the number of voxels in the three-dimensional region and automatically multiplying by the known volume of a voxel using MIM® Software (MIM-vista Corp., USA). Secondly, we reviewed the CT scans and calculate the volumes of non FDG avid tumor sites with positive radiologic findings for hepatic metastases using the same software. Calculated mean ± SD liver involvement was 31.1 ± 10.43% (range 10–45%).

None of the patients showed marked pulmonary activity on Tc-99m MAA scan. The mean ± SD pulmonary shunt was 6.04 ± 2.47% (range 3.4–10.03%). There was no scintigraphically detectable extrahepatic uptake on planar Tc-99m MAA scan. Furthermore, we did not observe any additional extrahepatic uptake on SPECT in the patients (4/10) where available.

Table 1

Clinical characteristics, Y-90 activities, estimated tumor, normal liver and lung doses of the patients treated with Y-90 radiomicrospheres.

Case no:	Sex:	Age (years)	H. Dx	Tumor site	Site of extrahepatic metastases on FDG PET	Liver tumor involvement (%)	Cumulative delivered SIR-spheres® activity (GBq)	Estimated Y-90 tumor dose (Gy)	Side(s) of liver treated	Cumulative estimated lung dose (Gy)	Cumulative estimated normal liver dose (Gy)	Follow-up (month)	RECIST response on CT
1*	M	44	MTC	T	Bilateral cervical LAP	10	3.6	128	RL	5	52	28	CR
								137	LL				
2	F	33	C	B	Solitary lumbar vertebra	30	1.4	420.8	WL	6.2	15	26	CR
3†	M	24	C	B	Multiple bone and soft tissue	45	2	64.2	WL	3.1	31.8	18	PR
4	F	57	C	P	None	33	4.65	49	RL	15.5	65.2	20	SD
								57	LL				
								52.3	RL				
5	F	39	C	G	Celiac and paracaval LAP	33	1.48	70	WL	7.4	41.2	17	PD
6	M	73	C	U	None	35	2	66.8	RL	6.1	58.9	12	PR
7*	F	73	C	P	None	35	2.8	84.1	WL	8.7	42.1	12	PR
								146.2	WL				
8*	M	52	C	CR	None	25	4.2	283.6	RL	13.5	80.6	12	PR
								103.8	LL				
								172.7	WL				
9	F	57	U	CR	None	40	1.1	77.4	LL	3.3	34.4	8	PR
10	M	35	C	B	Mediastinal LAP and lung nodule	20	0.7	69.4	RL	2.4	5	4	CR

CR – complete response; PD – progressive disease; SD – stable disease; PR: partial response
***patients treated with multiple SIR-Spheres® procedures; †patient died from extensive systemic metastatic disease 18th month after SIRT**
C – carcinoid; U – unkown; T – thyroid; B – broncus; P – pancreas; G – gastric; CR – colorectal; MTC – medullary thyroid cancer; H.Dx – histopathological diagnosis; Tm – tumor
WL – whole liver; RL – right lobe; LL – left lobe;

The mean ± SD administered SIR-Spheres® activity was 1.49 ± 0.42 GBq (range 0.72–2.21 GBq) in all patients. The 4 patients with bilobar disease were treated with whole liver administration of SIR-Spheres® with the activity of 1.62 ± 0.26 GBq (range 1.40–2.20 GBq) at a single session. The other 6 patients had unilobar treatment initially with the activity of 1.40 ± 0.52 GBq (range 0.7–2.02 GBq) initially. Three out of these 6 patients with unilobar injection had opposite lobe treatment in the follow-up. These treatments delivered a dose of 99.73 ± 66.36 Gy (range 49–420.8 Gy) to the target tumors. The estimated dose to the lungs and normal liver 4.45 ± 1.95 Gy (2.4–8.5 Gy) and 26.73 ± 14.19 Gy (range 5–58.9 Gy) respectively (Table 1)[29].

We experienced difficulty in the administration of SIR-Spheres® in 2 patients because of vessel spasm during the procedure. Both of the 2 patients were planned to have whole liver treatment (Cases 8 and 10). But both of them had left hepatic vessel spasm, shifting to treatment procedure from whole liver to only right lobe therapy. They had similar celiac axis with no definitive anatomic variation. The first patient had retreatment to the opposite lobe 4 weeks later. The second one is still on follow-up and scheduled for the left lobe treatment.

In our study group, there was no patient with portal vein thrombosis. The mean ± SD follow-up time for SIR-Spheres® therapy was 6 ± 2.8 months (range 3–28 months). Although, almost all patients reported some degree of mild-to-moderate abdominal pain, nausea, lethargy, anorexia, and fever from 1 week to 1 month after the treatment, no one needed intravenous narcotics and antiemetics. Liver function tests during the follow-up period were stable in all patients. However, we experienced mild to moderate increase in TB, ALT or AST, gamma glutamyl transferase (GGT) and serum alkaline phosphates (ALP) levels in 70% of all patients. Among these parameters, post treatment AST, GGT and ALP levels [39 (27.7–55.2)% and 69 (55.5–141.7)%; 137.5 (116.5–238.7)% respectively] were significantly higher than baseline values [26 (21–33)%, 36 (27.7–120.7)% and 116 (102.5–219.2)% respectively] ($p \leq 0.05$). On the other hand, the increase in ALT ($p = 0.080$) and TB levels ($p = 0.104$) after SIRT compared to baseline levels were not statistically significant. The patient with medullary thyroid cancer developed transient elevation of serum amylase which resolved in 48 hours without any medication. There was no case with radiation pneumonitis or radiation induced liver disease (RILD).

Of the 10 patients, 3 showed complete response (CR), 5 showed partial response (PR) and one showed stable disease (SD) of the target lesions according to RECIST criteria (Figures 5a and 5b). One patient showed progressive disease (PD) on follow-up. One of the patients with PR for hepatic tumors died of extensive bone, soft tissue and solid organ metastases 18 month after SIRT (Table 1). The data representing the extent of hepatic involvement, administered activities, estimated tumor doses with tumor responses are presented in Table 1.

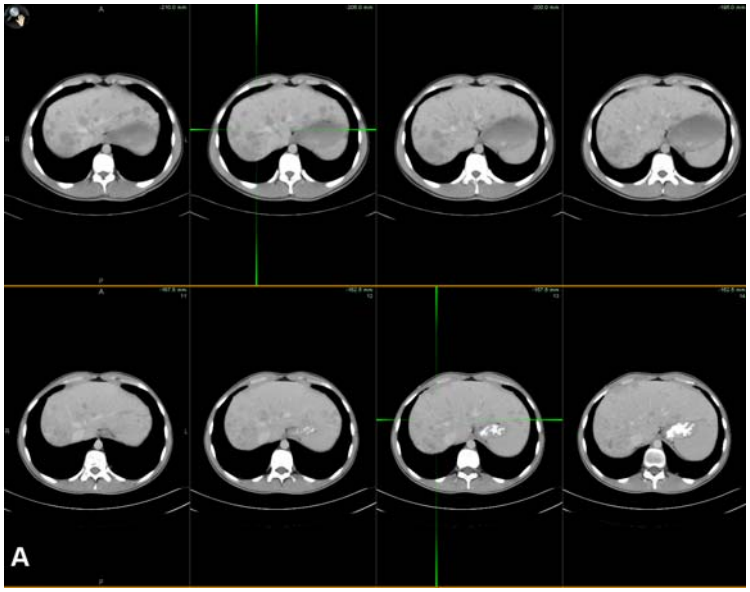

Fig. 5a – Abdominal CT scan before (top raw) and 3 months (bottom raw) after SIRT, showed partial response in a 21-year-old male patient with multiple metastatic liver lesions from carcinoid tumor (case N^{o} 3)

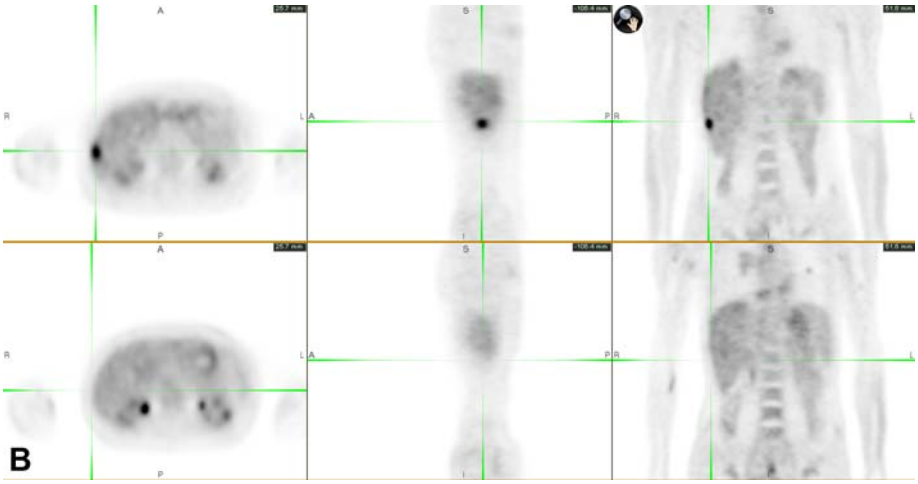

Fig. 5b – Corresponding transaxial (1st column), sagittal (2nd column) and coronal (3rd column) slices of the pretreatment (top raw) and posttreatment (bottom raw) FDG PET studies showed noticeable metabolic response in metastatic sites in the same patient

Discussion

Neuroendocrine tumors are heterogeneous group of neoplasms with endocrine metabolism and special histological structure. Despite the lack of private residential areas, 90% of NETs is derived from the gastroenteropancreatic system. They are generally low grade malignancies and tend to grow slowly. Although metastases from NETs are rare in tumors less than 2 cm, presence of liver metastases is one of the worst prognostic factors. Resection and successful local treatments can be related with long term survival [30], but the treatments are seldom curative, and the 5-year survival rate for patients who have unresected liver metastasis is between 25% and 50% [31]. Therefore, if metastases are limited to the liver and the surgical resection is not possible, other local interventional treatment options should be considered, even in the presence of extrahepatic metastases. Although, TAE, TACE or RFA may be one of the local treatment options, each of them has its own restrictions in the appropriate patient selection [32], and also in application which limits their effectiveness as a single therapy [3].

SIRT, a form of intraarterial brachytherapy, is a technique in which glass (TheraSphere®) or resin (SIR-Spheres®) particles are labeled with Y-90. The radioisotope Y-90 is a pure β emitter with no primary gamma radiation. SIR-Sphere, a permanent implant, is not metabolized or excreted and it stays permanently in the liver in the form of biocompatible particles, measuring approximately 20 to 60 microns. The mean energy of the particles is 0.9367 MeV, has a mean tissue penetration of 2.5 mm, and has a maximum penetration of 10 mm. In therapeutic use, requiring the isotope to decay to infinity, 94% of the radiation is delivered in 11 days [33]. Administration of radiomicrospheres is performed *via* a catheter placed in the hepatic artery delivering the spheres to the capillary bed where they are fixed and decay with the physical half-life of Y-90 (64 h). Malignant liver tumors are fed mainly by hepatic arterial system rather than portal venous blood. Therefore, the dominant arterial flow of

malignant tissue allows the delivery of high doses of radiation to tumors while keeping the exposure of the healthy liver at minimum with selective microsphere distribution.

At our institution, 48 SIRT procedures were carried out in 38 patients during a 28-month period between April 2008 and August 2010. Of the 38 patients, 10 patients suffering from extensive hepatic metastatic disease from NET were included in this study. Although, there are some physical differences in quality, size and particle number, Tc-99m MAA is the current standard for the evaluation of hepatic arterial flow. Since all aberrant and non-target vessels were coiled prior to Y-90 therapy in all patients, we did not observe any extra hepatic activity on Tc-99m MAA scan. Moreover, we observed great concordance with pre-SIRT Tc-99m MAA scan and post-therapeutic Bremsstrahlung imaging. We performed Bremsstrahlung imaging as soon as possible, but no later than 24 hours after the radioembolization as recommended in the literature [29]. Although we did not observe any hormonal crisis, we experienced vasospasm during Y-90 radiomicrosphere injection in 2 patients. As reported before, vessel spasm is not unusual during the Y-90 infusion. Therefore, it may be crucial to use microcatheter injections as recommended, particularly if the vessels are small in caliber or demonstrate significant tortuosity [34]. Moreover, it may be helpful to use short acting somatostatin analogs for symptomatic patients presenting with carcinoid related symptoms before local treatment procedures. Most of our patients, except cases N° 1 and 10, were on sandostatin treatment during the SIRT procedure.

As RECIST are commonly used to evaluate the success of a treatment, we used CT scan data to document therapy response [29]. Of the 10 patients, 5 had both hepatic and extrahepatic metastases on pre-SIRT FDG PET scan (Table 1). One out of 10 patients showed PD for hepatic metastases despite SIRT. Of the 10 patients, 5 showed PR after SIRT. One patient with PR for hepatic metastases showed new bone and soft tissue metastatic sites on follow-up (case N° 3) and died of systemic spread of metastatic disease 18 months after SIRT while on streptozotocin and sandostatin treatment. The other 3 patients showed CR for hepatic metastases. The patient with medullary thyroid cancer (case N° 1) had bilateral cervical lymph node metastases, and had lymph node dissection after SIRT. The patient with bronchial carcinoid tumor (case N° 2) had external radiotherapy for bone metastases concomitantly with SIRT. Fortunately, the bone and hepatic metastases completely resolved on follow-up in this patient. The other patient with bronchial carcinoid tumor (case N° 10) showed CR to SIRT at the beginning. However, we observed new mediastinal lymph nodes and solitary lung nodule, showing markedly increased FDG uptake, very suspicious for recurrence on the recent FDG PET-CT in the same patient 4 months after completing the therapy.

The tolerability of SIRT for all patients was very good. We observed minor side effects (fatigue, nausea, transient elevation in serum liver enzymes and abdominal pain) which resolved in 1 week. No severe side effect, treatment related mortality, radiation hepatitis or veno-occlusive liver failure was seen in our patient population. The common side effect was a slight increase in serum ALP and GGT levels. These results are concordant with the procedure guidelines and previously published reports [26].

The response rates in this preliminary study are consistent with previously published data on large series of patients [35, 36]. According to the CT data, we observed 90% response rate (CR – 30%; SD – 10%; PR – 50%). PD was observed in 10% (1/10) of all patients on CT scan. However, patients with PD or SD on CT, have either stable or decreased FDG uptake values on FDG PET study. Therefore, from our limited experience we may conclude that FDG-PET-CT and quantitative FDG data such as SUVmax, SUV mean, and Tissue Lesion Glycolysis (TLG) may have a crucial role in the evaluation of response to SIRT in future.

The results of this study should be considered preliminary and exploratory by nature in this study involving a limited number of patients. Moreover, not having serum tumor markers such as Chromogranine A, Neurokinin-A or HIAA, and In-111 OctreoScan in all patients, are the main drawbacks of this study. We still need to improve our practice and knowledge regarding radiation dosimetry and fractionation, including more than one application of microspheres, imaging and follow-up guidelines and long-term results.

Conclusion

From our limited experience, SIRT with Y-90 radiomicrospheres for liver metastases from NETs seems to be safe and efficacious with limited toxicity. However, there is a need to combine Y-90 radiomicrospheres treatment with systemic therapeutics for the patients with extra hepatic metastases to control the disease.

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Cochlear implant – speech and language development in deaf and hard of hearing children following implantation

Kohlearni implantat – razvoj govora i jezika kod gluve i nagluve dece posle implantacije

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Abstract

Bacground/Aim. Almost 200 cochlear implantations were done in the four centers (two in Belgrade, per one in Novi Sad and Niš) in Serbia from 2002 to 2009. Less than 10% of implantees were postlingually deaf adults. The vast majority, i.e. 90% were pre- and perilingually profoundly deaf children. The aim of this study was to assess the influence of improved auditory perception due to cochlear implantation on comprehension of abstract words in children as compared with hearing impaired children with conventional hearing aids and normal hearing children. **Methods.** Thirty children were enrolled in this study: 20 hearing impaired and 10 normal hearing. The vocabulary test was used. **Results.** The overall results for the whole test (100 words) showed a significant difference in favor of the normal hearing as compared with hearing impaired children. The normal hearing children successfully described or defined 77.93% of a total of 100 words. Success rate for the cochlear implanted children was 26.87% and for the hearing impaired children with conventional hearing aids 20.32%. **Conclusion.** Testing for abstract words showed a statistically significant difference between the cochlear implanted and the hearing impaired children with hearing aids (Mann-Whitney *U*-test, $p = 0.019$) implying considerable advantage of cochlear implants over hearing aids regarding successful speech development in prelingually deaf children.

Key words:
cochlear implants; deafness; hearing loss; language tests; cochlear implantation; child; child preschool.

Apstrakt

Uvod/Cilj. U periodu od 2002. do 2009. godine u Srbiji je urađeno oko 200 kohlearnih implantacija u četiri klinička centra (Beograd – KC Srbije i KBC Zvezdara, Novi Sad, Niš). Oko 10% bile su odrasle osobe sa razvijenim govorom, a oko 90% deca i to u prelingvalnoj, perilingvalnoj i ranoj postlingvalnoj fazi. Cilj ovog istraživanja bio je da se ispita u kojoj meri poboljšanje auditivne percepcije govora pomoću kohlearnog implanta utiče na razvoj razumevanja apstraktnih pojmova kod gluve i nagluve dece u odnosu na decu sa konvencionalnim slušnim aparatima i čujuću decu. **Metode.** Istraživanje je urađeno na uzorku od 30 dece: 20 gluve i nagluve i 10 čujuće. Instrument je bio test rečnik. **Rezultati.** Računajući sve pojmove (100 reči) rezultati pokazuju i dalje značajnu razliku između čujuće i gluve dece. Deca koja čuju imenovala su, opisala ili definisala svih 100 reči sa 77,93% uspešnosti. Deca sa kohlearnim implantima postigla su 26,87%, a deca sa konvencionalnim slušnim aparatima 20,32% uspešnosti na svim pojmovima. **Zaključak.** Ispitivanje razlike u poznavanju apstraktnih pojmova pokazalo je statistički značajnu razliku (Mann-Whitney *U*-test, $p = 0.019$) između dece sa kohlearnim implantima i dece sa konvencionalnim slušnim aparatima, ukazujući na to da kohlearni implant ima značajnu prednost u odnosu na konvencionalne slušne aparate u povećanju uspešnosti razvoja govora kod gluve i veoma teško nagluve dece.

Ključne reči:
kohlea, implantat; gluvoća; sluh, gubitak; jezici, testovi; kohlearna implantacija; deca; deca, predškolska.

Introduction

More than 200 cochlear implantations were performed from 2002 to 2009 in Serbia (four cochlear implant centers – Clinical Center of Serbia and Clinical Hospital Center "Zvezdara" in Belgrade, Clinical Center of Vojvodina in Novi Sad and Clinical Center Niš). Less than 10% of patients were postlingually deaf adults while more than 90% were children with prelingual, perilingual or early postlingual deafness. Auditory deprivation and lacking of speech stimuli

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leads to severe consequences in speech and language development. If early intervention during „critical period“ for speech development is missed stigma of the deaf will remain permanently and a person will be handicapped [1–3]. Stigma of the deaf is characterized by poor speech comprehension and language communication. Cochlear implantation tends to improve auditory perception in severely to profoundly deaf children. Auditory skills of hearing impaired children are specific and variable due to different etiologic factors [4]. Listening progress improves communication capacity and overall quality of life of hearing impaired children. Numerous studies have shown considerable progress following cochlear implantation [5]. Communication skills could be severely affected by poor speech perception in case of prelingual or perilingual hearing loss [6, 7]. Studies regarding short-term auditory memory for short words have shown a rapid increase in cochlear implanted children shortly after the implantation [8]. Auditory perception is not the only variable inducing speech and language development [7–9]. A whole lot of factors such as intelligence, rehabilitation, education, social and psychological issues could affect speech and language of hearing impaired children. Severe to profound deafness affects not only listening and speech but changes the whole deaf personality [10].

The aim of the study was to assess the impact of improved auditory perception on speech and language development in cochlear implanted children. Abstract words are hard to understand and acquire for severely to profoundly deaf children. Therefore, we decided to evaluate communication outcome in deaf children with cochlear implants and hearing aids in comparison with their hearing peers.

Methods

The overall sample in the study consisted of 30 children aged 4 to 7 years, divided into three groups: E1 – 10 deaf children with cochlear implants, E2 – 10 deaf children with hearing aids and C – 10 hearing children of the same age. All of the deaf children in this study had a severe to profound congenital hearing loss. They were enrolled in speech and hearing rehabilitation in Audiology Rehabiltation Department of Institute for ENT&HNS of Clinical Center of Serbia, Belgrade.

There is a lack of valid instruments for speech and language evaluation in children. „Vocabulary test“ [1] is an instrument for language evaluation in children aged 3–7 years. It has 100 items divided into five groups of 20 words each, according to age, for 3-, 4-, 5-, 6- and 7-year old children. The test for 3-year-old children has 9 common and 1 abstract word. The number of abstract words gradually rises so that the test for 7-year-old children consists of 5 common and 15 abstract words. It represents vocabulary and understanding abstract words, such as death, life, punishment, satisfaction, etc. The test results were evaluated as follows: absent (0), recognizes the word (1), describes the word (2) and defines the word (3).

The data were statistically analysed and displayed in tables and graphs. Mann-Whitney-*U*-test for small independent samples was used.

Results

Table 1 shows overall results of the vocabulary test for all three groups. The hearing children had much better achievements than hearing impaired children in both E1 and E2 groups. We would like to point out superior results of cochlear implanted children as compared to children with hearing aids.

Table 2 shows the list of the least recognized words for all three groups. The hearing children did not recognize the word *purpose*, except one kid. The list of the abstract words that were not recognized by cochlear implanted children consisted of 25 items, whereas the list of words that the children with hearing aids did not recogize was consderably longer (36 items).

Table 1

Overall results of the vocabulary test

Group	All words				Abstract words			
	AS	%	+	–	AS	%	+	–
Control (C)	2.33	77.93	2.76	1.34	2.07	69.01	2.59	0.68
E1	0.80	26.87	1.18	0.16	0.32	10.57	0.62	0.06
E2	0.60	20.32	0.95	0.17	0.11	3.69	0.32	0

C – hearing children; E1 – cochlear implanted children; E2 – children with hearing aids; AS – average score; (+) – best score in the group; (-) – worst score in the group

Table 2

The least recognized abstract words

Group	Abstract words	Points
Control (C)	purpose	3
E1	compassion, belief, purpose, respect, defeat, choice, progress, crime, construction, trust, need, truth, punishment, knowledge, success, hope, fortune, courage, battle, future, peace, strength, satisfaction, work, freedom	0
E2	life, story, work, happiness, friend, death, satisfaction, strength, peace, laughter, pain, future, battle, courage, fortune, hope, success, wedding, knowledge, punishment, truth,Wednesday, need, trust, construction, crime, progress, choice, guilt, defeat, respect, purpose, husband, poison, belief, compassion	0

C – hearing children; E1 – cochlear implanted children; E2 – children with hearing aids

Table 3 shows a comparison of the overall results for all three groups of the children. The overall achievement of cochlear implanted children (E1) was superior to the results of the children with hearing aids (E2) but the difference was not statistically significant.

Table 4 shows a comparison of the results for abstract words for all tree groups. Ability to describe and define abstract words was highest in rehabilitation of congenitally deaf children. The superior achievement of the children with cochlear implants regarding abstract words understanding is clearly emphasized in Table 4.

The hearing children were superior to both experimental groups with cochlear implant (E1) and hearing aids (E2), in defining both common and abstract words. Such results were expected. Congenital or early acquired prelingual hearing loss interferes with speech and language development affecting phonological, semantic and

Table 3

Comparison of overall results

Group	Average range	Mann-Whitney *U*-test	*p*
Control (C)	15.50	1.00	0.000*
E1	5.60		
C	15.50	0.00	0.000*
E2	5.50		
E1	12.35	31.50	0.165
E2	8.65		

C – hearing children; E1 – cochlear implanted children; E2 – children with hearing aids; *significant

Table 4

Comparisson of the results for abstract words

Group	Average range	Mann-Whitney *U*-test	*p*
Control (C)	15.50	0.00	0.000*
E1	5.50		
C	15.50	0.00	0.000*
E2	5.50		
E1	13.55	19.50	0.019*
E2	7.45		

C – hearing children; E1 – cochlear implanted children; E2 – children with hearing aids; *significant

Discussion

Analysis of the results revealed that regarding abstract words all of the children achieved best results for words *mother, love* and *friendship*. All of them found those words familiar and easy to define. Quantitative analysis showed that the deaf kids had a limited vocabulary and poor grammar, but nevertheless they understood the meaning (example, from the group E1– S.P. "Mother – mom is mine, loves you, gives milk" and from the control group of hearing kids - N.M. "Mother – everyone has got a mother, that is the most important...").

The hearing children from the control group described all of the words, yet they did not reach the maximal score of 141 (3 points for each word), but only 97 (69%). Among 47 abstract words the word *purpose* was the least recognized. Only one kid described it and got 3 points.

The cochlear implanted children (E1) did not recognize 25 out of 47 abstract words (*compassion, belief, purpose, respect, defeat, choice, progress, crime, construction, trust, need, truth, punishment, knowledge, success, hope, fortune, courage, battle, future, peace, strength, satisfaction, work, freedom*) while the children with hearing aids (E2) did not recognize 36 words *(life, story, work, happiness, friend, death, satisfaction, strength, peace, laughter, pain, future, battle, courage, fortune, hope, success, wedding, knowledge, punishment, truth Wednesday, need, trust, construction, crime, progress, choice, guilt, defeat, respect, purpose, husband, poison, belief, compassion*) (Table 2).

every other aspect of speech [11]. Early intervention with hearing aids or cochlear implant could reduce the consequences of deprivation but never fully eliminate them so that backlog compared to the hearing peers is always obvious.

The difference in overall achievement for both children with cochlear implants and hearing aids is not statistically significant (Table 3).

It complies with findings of other authors that the deaf child development is influenced by multiple factors [7, 12]. Regardless of the type and quality of amplification some kids are better comunicators than others. All of the children in both experimental groups (E1 and E2) were enrolled in continuos speech and hearing rehabilitation, so that their vocabulary was fairly developed and they were capable of solving these tasks. When it comes to abstract words there is a significant difference in hearing in cochlear implanted over children with hearing aids (Table 4). Cochlear implanted children were so far superior to deaf children with hearing aids regading speech perception. They have much better auditory skills (detection, discriminaton, identification and comprehenssion) and they perform better on the Ling 6-sound test, phonemes, logatomes, short words, polysyllables, sentences [7]. Congenitally deaf children develop speech perception and consistency in the first three

years following cochlear implantation. Children implanted before the age of 2 years could reach their hearing peers by the age of 6, if they are subjected to intensive speech and hearing rehabilitation [13–15]. Comparison of the results in this study with other authors findings was not possible, because of language differences. There are no available studies in Serbian language or similar languages like Croatian or Bosnian. Complete results for 100 words showed significant difference between hearing and deaf children (Tables 1 and 3). The overall success rate was 77.93% for the hearing children (C), 26.87% for the cochlear implanted (E1) and 20.32% for the deaf children with hearing aids (E2) (Table 1). The difference for abstract words was statisticlly significant (Mann-Whitney *U*-test, $p = 0.019$) between the groups E1 and E2 which suggest that cochlear implant significantly contributes to speech development in profoundly deaf children.

Conclusion

Cohlear implantation has statistically significant impact on speech and improves auditory skills and language development in the deaf children. There is an abstract words acquisition, with no statistically significant difference in vocabulary of the cochlear implanted and the deaf children with hearing aids, although the cochlear implanted children show better overal scores of the described and defined words.

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O P Š T I P R E G L E D

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Kompjuterizovane tehnologije za dijagnostiku i terapiju impaktiranih zuba

Computer-aided technologies in diagnostics and therapy of impacted teeth

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Ključne reči:
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Key words:
oral medicine; tooth, impacted; diagnosis, computer-assisted; telemedicine.

Uvod

Nicanje zuba predstavlja čudesno delo prirode koje je savršeno projektovano u nameri da obezbedi normalnu funkciju žvakanja kod čoveka, ali koje, nažalost, u svom toku može da naiđe na mnogobrojne nezgode. Jedna od nepravilnosti nicanja zuba je impaktiranost u različitim oblicima. Kao takva učestvuje u velikom procentu u oralnohirurškoj kazuistici, kako po učestalosti, tako i po posledicama koje izaziva. Klinička slika prisustva impaktiranih zuba može biti različita: od oskudne i asimptomatske, do vrlo teške, praćene dramatičnim komplikacijama koje mogu ugroziti život pacijenta [1]. Danas, savremene kompjuterizovane i robotizovane tehnologije postaju sastavni i neizostavni deo zbrinjavanja impaktiranih zuba, i to, počev od same pretpostavke postojanja moguće impakcije, preko tačne i precizne dijagnostike, pomoći u analizi postojećeg stanja i mogućih rešenja, asistiranja kod donošenja konačne odluke o izboru terapije, pomoći kod planiranja izvođenja terapije i aktivnom učešću kod intraoperativne navigacije i postoperativnom, odnosno posttretmanskom periodu [2]. Uz praćenje i pomoć kod kliničkog dela, ovi moćni kompjuterizovani sistemi staraju se i za administrativno-dokumentacioni deo, postavljajući pri tom, osnove sistema za statističko-analitičke procese – (*Evidence Based Dentistry*) koji su baza za postavljanje novih naučno prihvatljivih zaključaka zasnovanih na dokazima o problematici impaktiranosti zuba [3]. Kako upotreba kompjuterskih sistema značajno poboljšava kvalitet zbrinjavanja pacijenata sa impaktiranim zubima, dužnosti lekara koji se svakodnevno susreću sa ovom patologijom: oralnih hirurga, ortodonata, maksilofacijalnih hirurga i protetičara, jeste da budu upoznati sa mogućnostima koje ovi napredni sistemi pružaju. Ovaj rad ima za cilj da lekarima zainteresovanih specijalnosti predstavi upotrebu kompjuterizovanih tehnologija u dijagnostici i terapiji impaktiranih zuba.

Telestomatologija impaktiranih zuba

Telestomatologija je novo područje stomatologije koje se zadnjih godina ubrzano razvija i u sebi sjedinjava telekomunikacione tehnologije, digitalno snimanje, kompjutersko analiziranje, elektronsku evidenciju i Internet, a kao rezultat daje kvalitetan daljinski pristup lečenju u ruralnim, nepristupačnim i udaljenim oblastima [4]. Primena u oralnoj i maksilofacijalnoj hirurgiji postaje sve prisutnija. Duka i sar. [5] u kliničkoj eksperimentalnoj studiji sprovedenoj 2009. godine, pokazali su da oralni hirurzi metodom telestomatologije bazirane na prenosu kompjuterizovanih podataka preko Interneta, podjednako dobro kao i u realnom vremenu, mogu sagledati problematiku izniklosti, položaja i stanja impaktiranih umnjaka, a dalje, na osnovu dobijenih informacija, može se daljinski sačiniti plan oralnohirurškog zbrinjavanja u smislu lečenja ili ekstrakcije. Telestomatologija pruža i veliku uštedu troškova, jer omogućava udaljenu analizu panoramskih snimaka, digitalnih fotografija i video zapisa, a zadovoljavajući rezultati postižu se i u proceni ortodontsko-hirurškog lečenja impaktiranih zuba, što je posebno važno za osobe sa posebnim potrebama i ograničenom pokretljivošću [6, 7].

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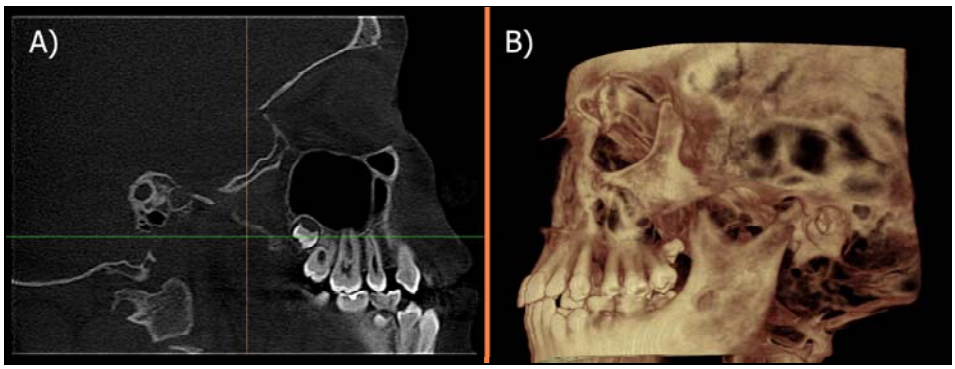
Trodimenzionalna kompjuterizovana dijagnostika

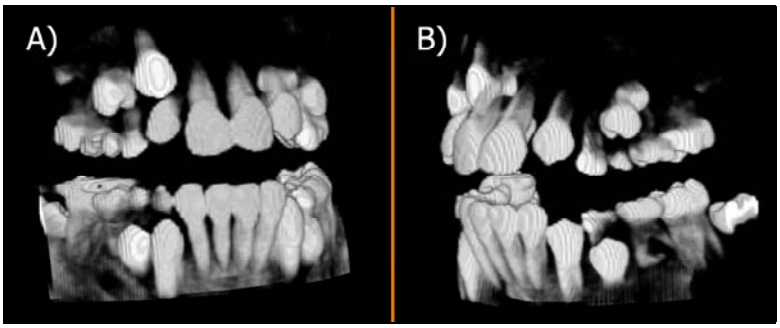
Panoramska radiografija, retroalveolarno i aksijalno snimanje najčešće su korišćene radiografske tehnike u dijagnostici impaktiranih zuba, a lekaru daju dvodimenzionalnu (2D) predstavu snimanih struktura. Vizuelne informacije dobijene na ovaj način pružaju globalni pogled na oblik i visinu ciljanih struktura, ali preciznost izostaje jer imaju faktor deformisanja oko 25% [8]. Trodimenzionalna (3D) kompjuterizovana dijagnostika u vidu kompjuterizovane tomografije (KT) i *Cone Beam Computed Tomography* (CBCT) tehnologije, omogućava preciznost i do 1 mm i ne dovodi do distorzije slike (slika 1) [9]. Na 3D modelu koji se dobija rekonstrukcijom poprečnih snimaka, matematički precizno i vizuelno potpuno prihvatljivo, prikazani su impaktirani zubi i njihovi odnosi sa susednim anatomskim strukturama (slika 2). Detaljan i tačan 3D prikaz stvara specijalisti neophodne uslove za razmatranje svih mogućnosti rešavanja impaktiranog zuba shodno njegovom položaju i obliku i eventualnom patološkom procesu koji ga prati (ciste, tumori i dr), pa je specijalista u stanju da planira put izvlačenja, tj. ekstrakcije tako da ne ošteti bliske osetljive anatomske strukture poput mandibularnog kanala i njegovog sadržaja, foramena mentale sa sadržajem, maksilarnog sinusa, poda nosa i dr [10, 11]. Rekonstruisani 3D model pruža prave informacije i za donošenje odluke o mogućnosti i prihvatljivosti hirurško-ortodontskog izvlačenja impaktiranog zuba, jer se kost na modelu može digitalno ukloniti i trasirati put zubu, te se na ovaj način spasiti od ekstrakcije i velikih koštanih defekata, pre svega gornji kanini [12, 13]. Sawamura i sar. [14] pokazali su da je tačnost 3D KT slike u odnosu na klasičnu radiografiju 91 : 61. Pregled rekonstruisanog 3D modela daje jasne i nedvosmislene odnose impaktiranih zuba i pratećih odontogenih cisti prema resorpciji korenova susednih zuba, ali i moguće resorpcije korenova samih impaktiranih zuba [15]. I, na kraju, 3D model služi kao osnova za navigacioni proces u toku same intervencije hirurške ekstrakcije impaktiranih zuba.



Sl. 1 – *Cone Beam Computed Tomography* (CBCT) analiza impaktiranih zuba u softveru *Medica Systems & Products* 3DCT
A) dvodimenzionalni poprečni snimak; B) trodimenzionalna rekonstrukcija

Trodimenzionalna ortodontska analiza

Za ortodontsku analizu stanja impaktiranih zuba i mogućnost izvlačenja može se koristiti 3D rekonstrukcija bazirana na KT/CBCT tehnologiji, ali i 3D rekonstrukcija bazirana na CAD (*Computer-aided design*) 3D skeniranju izlivenog ortodontskog studijskog modela [2]. Ova rekonstrukcija



Sl. 2 – Priprema za merenje odnosa impaktiranih zuba i susednih zuba i anatomskih struktura (Softver *Medica Systems & Products* 3DCT)

umnogome pomaže kliničkoj dijagnostici impaktiranog zuba u sve tri ravni, pri tom dajući informacije o gustini kosti i kvalitetu prolaska zuba kroz predstojeće tvrdotkivne i mekotkivne prepreke, pa se može koristiti u tretmanu i posttretmanskom periodu radi određivanja uspešnosti primenjenog metoda [16]. Kada je impaktiran zub hirurški oslobođen i spreman za ortodontsko izvlačenje, uzima se otisak hidrokoloidnim alginatom i skenira u prostoru čime se procesom digitizacije dobija digitalni 3D model [17, 18]. Sada je ortodont u mogućnosti da kompjuterizovanom analizom napravi plan najboljeg izvlačenja impaktiranog zuba, što se čini profesionalnim softverima za ortodontsko planiranje: *Ortho*CAD-om i *Emodels*™-om. Softveri daju i po pet istovremenih pogleda

na model, te se model gleda istovremeno iz više perspektiva, uz opcije rotiranja i uvećavanja radi određivanja pozicije zuba prema drugim zubima i radi izvođenja različitih merenja u bilo kom prostornom okviru.

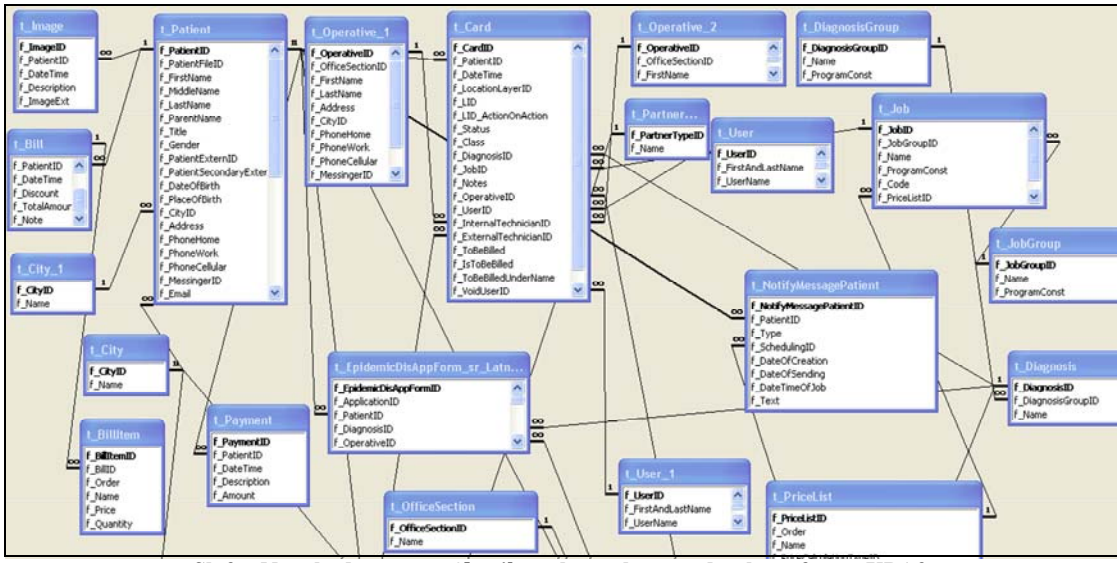
Intraoperativna navigacija

Intraoperativni navigacioni sistemi pomažu terapeutu da stekne precizan uvid u poziciju operativnih instrumenata ili sonde tokom hirurškog zahvata. Najbolje mogućnosti pružaju navigacioni sistemi bazirani na kontinuiranom radiografskom snimanju tokom intervencije, ali su jako neprijatni po terapeuta jer ga izlažu povećanom rendgen zračenju, te kao takvi nisu poželjni za stalnu upotrebu u lečenju impaktiranih zuba [19]. Od sistema koji rad baziraju na preoperativnom KT/CBCT 3D snimanju i kasnijem korišćenju dobijenog snimka za mapiranje trase i intraoperativne lokacije instrumenata, poznate su sledeće vrste: mehanički (pozicija se izračunava preko zupčanika i uglova pomeranja); elektromagnetni (pozicija se detektuje preko polja promena sa kalemovima); ultrazvučni (pozicija se određuje merenjem zvučnih signala u realnom vremenu) i video optički (preko kaluklisanja pozicije infracrvenim diodama ili prepoznavanjem paterna CCD-kamerama) [20–22].

Kod hirurškog vađenja impaktiranih zuba, kao i celoj oralnoj i maksilofacijalnoj hirurgiji najviše se koristi video optička navigacija, jer ima najbolja svojstva i laka je za rukovanje. U toku izvođenja intervencije, operator prati kretanje instrumenata kroz dublje partije tkiva na monitoru kompjutera koje, inače, nije u stanju da vidi golim okom. Kretanje se usmerava ka adekvatnom terapijskom efektu, ali uz istovremeno očuvanje osetljivih anatomskih detalja, poput neurovaskularnih spletova, pneumatizovanih šupljina, susednih zuba i dr. Nedostatak ovakve intraoperativne navigacije je što se ona bazira na preoperativnom KT snimanju i kompjuterskoj rekonstrukciji, te izmene na tkivu koje se u toku operacije dese nisu vidljive na pregledu koji prati intervenciju.

Digitalna evidencija pacijenata

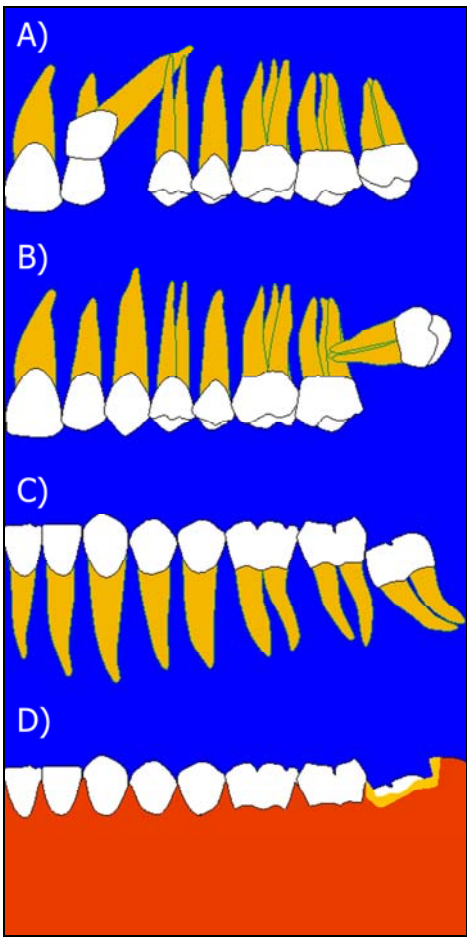
U oralnoj hirurgiji sve više se koristi digitalna evidencija pacijenata, digitalno se zapisuju oralnohirurška patologija ustanovljena kod pacijenata, i terapijske procedure sprovedene kod njih [23]. Digitalnu evidenciju vrše specijalizovani softveri za *Evidence Based Dentistry*. Ovim su u stanju da predstave tematiku impaktiranih zuba u punom tekstualnom obliku, u *master-detail* strukturi svojih baza podataka i vizuelno, grafičkim iscrtavanjem u dve ili tri



Sl. 3 – Neophodna *master/detail* struktura baze podataka softvera XPA3 (prolom za indeksiranje i pretragu sistema *Evidence Based Dentistry*)

dimenzije (slika 3) [3, 24, 25]. Grafička komponenta razlikuje se od softvera do softvera i može da prikazuje status impaktiranog zuba: impaktiran (da ili ne), nivo impakcije (poluimpaktiran ili impaktiran), položaj (prema Winterovoj klasifikaciji) [26] ili slobodan položaj u 3D prostoru, status periodontalnih džepova oko zuba, moguće komplikacije (perikoronitis akutni i hronični), druge patološke promene koje mogu pratiti impaktiran zub (ciste i tumori) (slika 4). Softveri se mogu koristi lokalno, na kompjuterima u ambulanti lekara gde se izvršava intervencija, kada govorimo o desktop softverima za upravljanje ordinacijom, ili se mogu izvršavati centralno, na Internet serveru, kada govorimo o *online* softverima [27].

Osim što imaju prednost u brzini i kvalitetu pristupa zdravstvenim kartonima i pratećoj dokumentaciji, oni mogu da učestvuju i u stvaranju neophodnih *database* informacija za jednu od najperspektivnijih grana stomatologije, *Evidence Based Dentistry*, koja na osnovu kompjuterske pomoći u statističkoj analizi postojećih podataka iz zdravstvenih kartona omogućava lekaru da donosi nove zaključke u vezi sa određenom problematikom [28]. Na ovaj način Mettes i sar. [29] izvršili su analizu kompjuterizovane digitalne evidencije oralnohirurške patologije i našli su da nije opravdano profilaktičko vađenje asimptomatskih impaktiranih umnjaka kod odraslih i da profilaktičke ekstrakcije asimptomatskih umnjaka ne sprečavaju kasniji nedostatak prostora u predelu inciziva [29].



Sl. 4 – Vizuelizacija pozicije impaktiranih zuba u softveru XPA3 Prolom
A) impaktiran gornji očnjak; B) impaktiran gornji umnjak; C) poluimpaktiran donji umnjak; D) perikoronitis u predelu poluimpakcije donjeg umnjaka

Procena jačine mandibule

Ekstrakcija duboko impaktiranih zuba može biti praćena komplikacijom frakture mandibule, jer može biti neophodno da se ukloni veliki deo kosti ili sama impakcija može biti udružena sa većim patološkim lezijama (ciste, tumori) [30, 31]. U ovakvim slučajevima kompjuterska analiza i procena jačine mandibule pre i/ili posle ekstrakcije impaktiranog zuba pomaže u donošenju kvalitetnih odluka u vezi planiranja ekstrakcije, toka postoperativnog lečenja i moguće neophodnosti za terapijskom profilaksom izbegavanja moguće frakture donje vilice. Kompjuterska procena jačine mandibule vrši se pomoću metode konačnih elemenata (*finite element method*) kojom se mogu odrediti različiti parametri pre- i postoperativnog toka i proceniti opšti uticaj sila u vidu Von Mises-ovog stresa mandibule [32, 33]. Trodimenzionalni model mandibule kompjuterski se dobijaju iz KT snimaka, pa se na njega softverski nanose konačni elementi koji uključuju puno i parcijalno opterećenje prilikom žvakanja, ali i drugih mogućih stanja mandibule [34]. Posle simulacije opterećenja na 3D modelu donje vilice vide se polja različite obojenosti koja predstavljaju opterećenja koje vilica može podneti, zajedno sa lokacijama i verovatnoćom mogućeg frakturisanja. Ukoliko se procena vrši preoperativno, ona će dati uvid u rizik od frakture i definisaće kritičnu visinu mandibule koja mora biti održana prilikom hirurške ekstrakcije impaktiranog zuba, kako bi kost bila zaštićena od mogućeg preloma.

Sticanje znanja bazirano na kompjuterizovanoj tehnologiji

Svetska kompjuterska mreža (Internet) ima dominantnu ulogu u celokupnoj informacionoj i komunikacionoj tehnologiji koju koriste studenati osnovnih i postdiplomskih studija, kao i postdiplomaci oralne hirurgije u cilju sticanja novog znanja u vezi dijagnostike i terapije u oralnoj hirugiji [35]. *Google Scholar* i drugi provajderi elektronskih knjiga, u stanju su da u delić sekunde stave na raspolaganje studentima kako standardne i tradicionalne metode dijagnostike impaktiranih zuba, tako i primenu novih metoda i tehnologija u ovoj regiji. Na ovaj način dobija se celokupna školska literatura vezana za impaktirane zube koja uključuje: članke, teze, knjige, apstrakte, hipoteze i razmišljanja akademskih izdavača, profesionalnih udruženja, univerziteta i dr. Pretragom ključnih reči: „ simptomi impaktiranih zuba“ (*impacted teeth symptoms*), za 0,26 sekundi pronalazi se 628 000 članaka koji su važni za učenje o simptomima impaktiranih zuba. Najveća svetska kompjuterizovana medicinska baza podataka MEDLINE pretražuje se preko Internet interfejsa PUBMED, i na upit „simptomi impaktiranih zuba“ na engleskom daje apstrakte 2 629 naučnopriznatih članaka indeksiranih od 1948. godine do danas. Takođe, dostupne su *web* strane manjih, nezavisnih kompanija koje su provajderi za mnoge specijalizovane elektronske knjige, udžbenike, ili interaktivne i video kurseve procedura i tretmana impaktiranih zuba. *Web* portal www.*OralCamera*.it. sadrži 126 video demonstracija hirurških ekstrakcija impakiranih zuba. Pored elektronskih resursa za učenje, svetska kompjuterska mreža pruža studentima, stomatolozima i specijalistima oralne hirurgije pristup specijalizovanim diskusionim grupama, gde se mogu *online* konsultovati o određenom slučaju impakcije zuba i dobiti relevantne savete. Predstavnik diskusionih foruma je: www.medhelp.org.

Restaurativni sistemi u stomatologiji sa kompjuterskim upravljanjem

U situacijama u kojima nema adekvatne mogućnosti za hirurško-ortodontsko lečenje nepotpuno izniklih zuba, tzv. poluimpaktiranih zuba, a isti su indikovani za fiksnu protetsku nadoknadu kao samostalne krunice ili, u praksi češće, kao neophodni nosači mostova, može se učiniti kompjuterizovana protetska rekonstrukcija sa kompjuterizovanom izradom fiksne nadoknade. U pitanju su CAD/CAM sistemi (dizajniranje vođeno kompjuterom i izrada upravljana kompjuterom (*Compjuter-Assisted Design/Compjuter Assisted Manufacturing in Dentistry*) koji omogućavaju kompjuterizovano snimanje stanja u ustima, detaljnu analizu, 3D dizajn i kompjuterom izradu fiksne protetske nadoknade upravljanu na ovim poluimpaktiranim zubima. Glavne komponente CAD/CAM sistema su: uređaj za dobijanje 3D površina prepariranih zuba (3D kamera, mehanički kontaktni skener, op-

tički nekontaktni skener/laser); softver za analizu, dizajniranje i simuliranje restauracije i mašina za precizno mikroglodanje keramike (materijala) upravljana kompjuterom prema dizajniranoj restauraciji [2, 36–39].

Glavne prednosti ovakvog načina izrade fiksne nadoknade su: mogućnost finog dizajniranja morfologije krune na poluimpaktiranom zubu, simulacija sila fizičkog opterećenja i nosivosti nadoknade na 3D digitalnom modelu, 3D simulacija moguće defiksacije nadoknade sa poluimpaktiranog zuba, brza izrada nadoknade u toku jedne posete pacijenta i ekonomska isplativost [40–43].

Zaključak

Dvadesetprvi vek je vek kompjutera i ogromnog tehničkog razvoja. Kompjuteri nezadrživo ulaze u sve oblasti medicine i doprinose velikom povećanju kvaliteta zdravstvene zaštite stanovništva. Prodor kompjutera u stomatologiju i užu specijalnost zbrinjavanja impaktiranih zuba, kako sa oralnohirurškog, tako i sa ortodontskog ili protetskog stanovišta nesumnjivo vodi u promenu načina učenja, dijagnostikovanja i novom pristupu u donošenju odluka.

Za ostvarivanje prave koristi od kompjutera, neophodno je obezbediti dobre tehničke uslove, ali još bitnije obrazovati osoblje. Investicije u kupovinu adekvatnih uređaja i softvera predstavljaju lakši deo posla koji se može jednokratno rešavati. Međutim, za ispravno obrazovanje ljudi potrebne su godine, te je neophodno obrazovanje sprovoditi kontinuirano još od redovnih studija, preko postdiplomskih studija i postspecijalističkog usavršavanja. Neophodno je obavezno obučavanje studenata stomatoloških fakulteta u okviru predmeta Oralna hirurgija i obavezan predmet Kompjuterizovana stomatologija, jer smo u vremenu u kome kompjuteri postaju podjednako bitni kao i sve druge osnove oralnohirurških intervencija, kao anestezija, operativna tehnika, hemostaza, antibiotska zaštita. Ne koristiti pomoć kompjutera, vrlo brzo može imati posledice kao i ne koristiti pomoć antibiotika. Oni daju veoma značajan doprinos učenju i specijalizaciji, i sastavni su i neodvojivi deo hirurškog zbrinjavanja impaktiranih zuba, pa je, stoga, neophodno angažovati što više ljudi i resursa u cilju usavršavanja na ovom polju.

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C A S E R E P O R T S

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Metastasis of hepatocellular carcinoma presented as a tumor of the maxillary sinus and retrobulbar tumor

Metastaza hepatocelularnog karcinoma kao tumor u maksilarnom sinusu i retrobulbarnom prostoru

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Abstract

Introduction. Hepatocellular carcinoma (HCC) is the most frequent primary malignant tumor of the liver. It is usually seen in the 6th and 7th decades of life and chronic hepatitis B is the most frequent cause. Extrahepatic metastasis of HCC is an indicator of a poor prognosis and the most common sites are lungs, bones, lymph nodes, kidneys and adrenal glands. We reported a case of isolated metastasis in the right maxilla, which had been found initially, before the tumor in the liver was diagnosed. **Case report.** A 70-year-old man underwent dental surgery of the upper right molar. Prolonged bleeding control was difficult for up to two weeks, so the biopsy was performed. Histopathological analysis revealed a metastatic hepatocellular carcinoma. Computerized tomography (CT) of the abdomen revealed a diffusely heterogeneous liver parenchyma with irregular borders and two foci of mass lesions. There were metastasis in the spleen and also two pathological retroperitoneal lymph nodes were detected, but no ascit, liver cirrhosis, cholestasis or portal vein thrombosis were seen. CT of the orbital and maxillary regions revealed a tumor mass in the right maxillary sinus, spreading to the alveolar sinus, nasal cavity and partially infratemporal space. A tumor mass was in the right orbit as well, infiltrating the surrounding bones and muscles. Clinically, there was proptosis of the right eye accompanied by amaurosis. The treatment started with chemotherapy based on 5-fluorouracil (sorafenib was not available). After three cycles, control CTs showed a stable disease in the liver, but progression in the right maxillary sinus and orbit. Enucleation of the right eye was performed and postoperative radiotherapy was planed. The patient deteriorated rapidly and died, about 6 months after the disease had been diagnosed. **Conclusion.** Extrahepatic metastasis of HCC represents a progressive phase of the disease with poor prognosis, so the main aim of the treatment should be palliation and care of symptoms.

Key words:
carcinoma, hepatocellular; neoplasm metastasis; jaw; orbit; diagnosis; treatment outcome.

Apstrakt

Uvod. Hepatocelularni karcinom (*hepatocellular carcinoma* – HCC) predstavlja najčešći primarni maligni tumor jetre koji ima agresivan tok sa preživljavanjem od svega nekoliko meseci. Kod oko 80% bolesnika glavni uzročnik HCC je ciroza jetre u kombinaciji sa hroničnim virusnim hepatitisom, pretežno tipa B i C. Prisustvo ekstrahepatičkih metastaza je indikator loše prognoze. Metastaze se najčešće javljaju u plućima, kostima, limfnim čvorovima, bubrezima i nadbubrežnim žlezdama. Predstavili smo bolesnika sa metastazom hepatocelularnog karcinoma u gornjoj vilici i očnoj duplji. **Prikaz bolesnika.** Bolesnik, star 70 godina, bio je podvrgnut ekstrakciji gornjih desnih kutnjaka. Nakon ekstrakcije nastalo je krvarenje koje nije moglo da se zaustavi tokom dve nedelje. Urađena je biopsija okolnog tkiva, a patohistološka dijagnoza potvrdila je da se radi o metastazi hepatocelularnog karcinoma. Na kompjuterizovanoj tomografiji abdomena viđene su hipodenzne fokalne promene u jetri i slezini kao i izmenjeni limfni čvorovi u retroperitonealnom prostoru paraaortalno. Kompjuterizovana tomografija srednjeg masiva lica pokazala je tumor koji u potpunosti ispunjava desni maksilarni sinus, razara alveolarni produžetak i širi se u nosnu šupljinu i infratemporalni prostor. U desnoj očnoj duplji tumor se u potpunosti infiltrisao u spoljašnji pravi mišić pokretač očne jabučice i razarao je spoljašnji zid orbite. Kliničkim pregledom registrovana je protruzija desne očne jabučice. Lečenje je započeto prvom linijom sistemske hemoterapije baziranoj 5-fluorouracila. Nakon tri ciklusa registrovana je stabilizacija bolesti u jetri, ali i dalja progresija u gornjoj vilici i očnoj duplji. Učinjena je enukleacija desnog oka. Planirana zračna terapija predela maksilarnog sinusa i orbite desno nije sprovedena, s obzirom na to da je bolesnik egzitirao. **Zaključak.** Ekstrahepatične metastaze hepatocelularnog karcinoma predstavljaju odmaklu fazu bolesti i indikator su loše prognoze. Iz tih razloga, glavni cilj terapije treba da bude usmeren ka palijativnom lečenju i zbrinjavanju simptoma bolesti.

Ključne reči:
hepatom; neoplazme, metastaze; vilice; orbita; dijagnoza; lečenje, ishod.

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Introduction

Metastatic hepatocellular carcinoma (HCC) is a very aggressive disease with poor prognosis and only a few months' survival [1]. It is the most common primary liver cancer [2]. It is usually seen in the sixth and seventh decade of life. About 80% of HCC is caused by cirrhosis combined with chronic viral hepatitis B or C [3–5]. Second cause of HCC is alcoholism, with or without viral infection presence [6]. At the moment of diagnosis, about 50% of patients have distant metastases, while the occurrence of extrahepatic metastases indicates a bad prognosis [7]. Most common metastatic sites are lungs, bones, lymph nodes, kidneys and adrenal glands [4, 5, 8–10]. Rarely, HCC can be manifested as metastatic deposits without primary tumor in liver [11, 12]. Unlike HCC metastases in the upper jaw which are described more often in the literature [13–17], the metastases to the maxilla have been seen only in one case [18].

In this paper we presented the patient with hepatocellular carcinoma metastases in the upper jaw and maxillary sinus additionally spreaded to the orbit. It manifested as a prolonged bleeding after tooth extraction.

Case report

A 70-year-old patient underwent surgical removal of upper molars. Two weeks later, biopsy of surrounding tissue due to prolonged bleeding after the applied haemostasis, was performed. Routine histopathological analysis showed malignant metastatic tumor, most probably hepatocellular or renal carcinoma metastasis (Figure 1). In order to reveal the

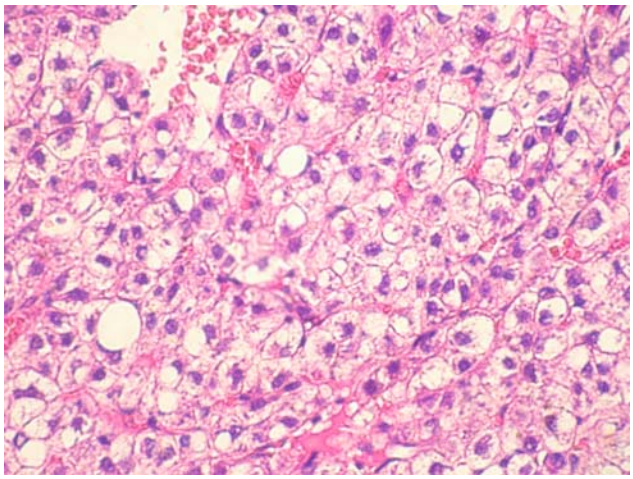

Fig. 1 – Tumor composed of solid sheats of large cells (HE ×100)

metastasis origin, we used additional immunohistochemical staining: HepPar (OCH1E5, 1:25), Vimentin (V9, 1:200), TTF-1 (8G7G3/1, 1:10), CK7 (OV-TL12/30, 1:50), CK20 (Ks20.8, 1:50), made by DAKO and RCC (66.4C2, 1:50) made by Novocastra lab. LSAB+system visualisation. Tumor cells manifested strong immunoreactive staining for HepPar antibody (Figure 2) known as one of the most specific and most sensitive markers for HCC [19]. Cells did not show any immunoreactivity for the other used antibodies: vimentin, TTF-1, CK7, CK20 and RCC antibody.

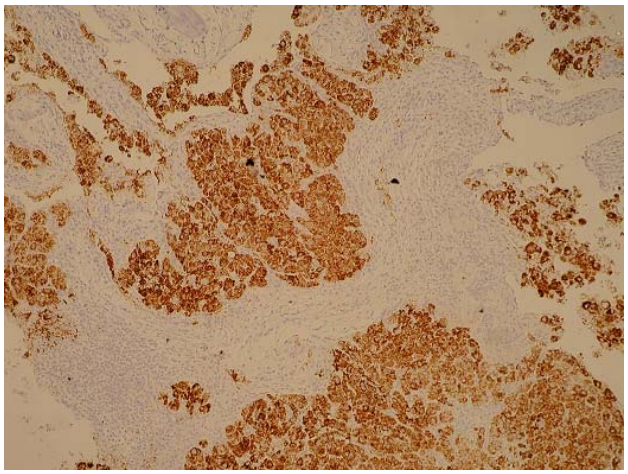

Fig. 2 – Tumor cells reveal diffuse granular staining of cytoplasm (HE ×100)

Abdomen CT showed two hypodense focal changes in the liver (segment VIII), dimensions 22 mm and 30 mm. One hypodense focal change was seen in the spleen, 22 mm diameter, as well as two soft-tissue formations around the aorta, interpreted as abnormal lymph nodes, dimension 21 mm and 44 mm. Nodular formations up to 11 mm were seen in the mesenterium.

CT of the orbital and maxillary regions revealed a tumor mass in the right maxillary sinus, spreading to the alveolar sinus, nasal cavity and partially in the infratemporal space. The tumor mass was also disseminated in the right orbit, infiltrating the surrounding bones and muscles.

Radiography of the lungs and skeletal system was normal.

Laboratory findings before the treatment were: urea 9.0 mmol/L; creatinine 122 μmol/L; total bilirubin 35.4 μmol/L; aspartat-aminotranspherase (AST) 86 UI/L; alanin-aminotranspherase (ALT) 106 UI/L; alkaline-phosphatase (ALP) 295 UI/L. Alpha-fetoprotein (AFP) was 18309 ng/mL. In blood count, a mild anemia was detected, with hemoglobin level of 102 g/L. All other parameters were within normal ranges.

Clinically, there was proptosis of the right eye, worsening over time, accompanied by amaurosis and a complete loss of vision in the right eye after two months (Figure 3). The patient had a strong pain in the right side of the head, managed by opioid painkillers.

Cirrhosis of the liver and viral hepatitis infection B were not confirmed. The patient was a non-smoker and did not consume alcohol.

Karnofsky Performance Status before the treatment was 80%, but gradually decreased to 60%.

According to the multidisciplinary team (MDT) decision, the treatment started with the first line of systemic chemotherapy based on 5-fluorouracil and leucovorin. Sorafenib was not available. Three cycles were applied, every 28 days. During the chemotherapy, hemoglobin levels

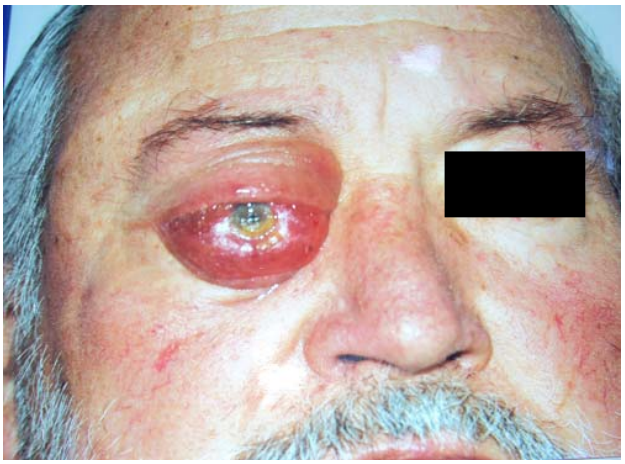

Fig. 3 – Proptosis of the right eye

were gradually decreasing to 88, 76 and 64 g/L, which required blood transfusion several times. After three cycles of chemotherapy, MDT did the evaluation of disease progress. Control CTs showed a stable disease in the liver, but apparent progression in the right maxillary sinus and orbit. Finding in the right eye was also worse with gradual loss of vision. Pain was more intensive and not manageable with morphine analgesics.

The patient was presented to MDT, who indicated enucleation of unfunctional eye with additional radiotherapy of maxillary sinus and right orbit. He was hospitalized in the Clinic for Eye Disease where the planned operation was performed, without any postoperative complications. Histopathology of the enucleated right bulbus revealed deformity caused by spontaneous rupture but no elements of tumor were found. Although all symptomatic therapy was given, the patient's condition was getting worse. Radiotherapy was not performed. The patient died six months after the diagnose had been settled.

Discussion

Hepatocellular carcinoma is a very aggressive disease with a five-year survival in 0.8% men and 4.4% women [20]. Intrahepatic metastases appear very early and more than 50% of patients have extrahepatic metastases as well [18]. A role in metastases development can be recognized in the state of liver parenchyma and the level of liver damage caused by viral hepatitis [21]. Metastases are usually spread by intrahepatic blood and lymphatic vessels or by direct infiltration. Hematogenic spreading means spreading through the hepatic portal vein or vena cava. The most common metastatic sites are: lungs (18.1%–49.2%), lymph nodes (26.5%–41.7%), bones (4.2%–16.3%) and adrenal glands (8.4%–15.4%) [4, 5, 8–10].

Treatment of patients with hepatocellular carcinoma depends mostly on the disease stage. When the disease is localized to the liver only, the main therapy option is surgical removal of the tumor. In the locally advanced and metastatic disease, the only choice is systemic chemotherapy. So far, there have been no precise protocols for patients with extrahepatic metastases, but the usage of cytostatics is very often limited by a bad performance status of a patient [22].

The patient presented in this paper had metastases in the maxillary sinus spreading to the orbit and nasal cavity. Metastases were also detected in the spleen and paraaortal lymph nodes. Liver cirrhosis and viral hepatitis infection B were not confirmed. Levels of bilirubin, transaminases and alkaline-phosphatase were doubled, while alpha-fetoprotein level was 18.000. The disease was manifested in a metastatic phase so chemotherapy was the treatment of choice. Three cycles of chemotherapy based on 5-fluorouracil were administrated with a final effect of disease progression. Due to spontaneous cornea perforation caused by lagophtalmus and tumor compression, the right bulbar enucleation was performed. Palliative radiotherapy of the maxillary sinus and right orbit was not completed because the patient died. He lived six months after the moment of diagnosis.

While metastases of hepatocellular carcinoma to the mandible have been described in many papers [13–17], metastases in the maxilla are very rare [18]. Clinical and autopsy findings of these cases revealed two ways of metastatic spreading to the maxillary-facial region. When cancer cells reach the hepatic artery and portal vein, the first metastatic site are lungs. From the lungs, dissemination goes to the maxillary area [18]. In other case, if autopsy does not show any metastases in lungs, dissemination to maxillary area is considered to be either by vertebral and azygos vein system or by lymphatic system.

The presented patient had no confirmed lung metastases, which suggested that spreading of cancer cells to the maxillary sinus and the orbit had been by lymphatic or vertebral vein system.

The presence of extrahepatic metastases is an indicator of bad prognosis. In that stage the only treatment is chemotherapy. Due to very aggressive nature of disease with rapid deterioration of performance status, it is very important to make a good assessment of treatment benefit and potential risks. The main aim of treatment should be palliation and symptom care.

Conclusion

In this paper we described an unusual hepatocellular carcinoma metastasis, as bleeding tumor in the maxillary sinus spreading to the orbit. In such case, it is quite possible to overlook the real origin of prolonged bleeding in a patient with previous intervention on molars. Due to that, it is important to make a proper diagnosis and find the cause of every unusual complication, even if they appear after small interventions.

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CASE REPORT

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Bilateral Monteggia fracture in adults

Obostrana *Monteggia* fraktura kod odraslih

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Abstract

Introduction. In 1814 Giovanni Monteggia first described two cases of fractures of the proximal third of ulna with dislocation of the radial head. These fractures are more common in children than in adults, and mutual Monteggia fracture is a rare complication. This study presents a treatment course of a patient with bilateral Monteggia fracture. **Case report.** A 55-year-old patient was injured by falling in the yard. Radiography showed bilateral Monteggia fracture type II (by the Badon classification). Operative treatment of fracture was done by a compression plate on the right side and by the *zuggurtung* technique on the left one. Closed repositioning of the radial head was done on both sides. The patient was wearing a plaster splint for the upper arm for 21 days. After removing the fixation, the function of the elbow was determined by the Broberg Morrey score (BM) which was on the right side 45.5 and on the left side 47.5. After the proper physical therapy, four months after the surgery, BM score was 100 on the right side, and 93 on the left one. **Conclusion.** Surgical treatment and early rehabilitation is the key for the return of good function of both elbows.

Key words:
ulna fractures; radius; dislocations; internal fixators; rehabilitation.

Apstrakt

Uvod. Giovanni Monteggia prvi je, 1814. god, opisao dva slučaja preloma proksimalne trećine ulne sa dislokacijom glave radijusa. Ovi prelomi češće se javljaju kod dece nego kod odraslih, a obostrani Monteggia prelom veoma se retko javlja. U ovom radu prikazali smo tok lečenja bolesnice sa obostranim Monteggia prelomom. **Prikaz bolesnika.** Bolesnica, stara 55 god, povređena je prilikom pada u dvorištu. Radiografskim pregledom dijagnostikovan je obostrani Monteggia prelom tip II (po Badovoj klasifikaciji). Urađeno je operativno zbrinjavanje preloma ulne desno polovinskom pločom, a levo *zuggurtung* tehnikom. Obostrano je urađena zatvorena repozicija glave radijusa. Nadlaktanu gips longetu bolesnica je nosila 21 dan. Nakon skidanja imobilizacije funkcija lakta određena je po Broberg Morrey skoru (BM) i iznosila je desno 45,5, a levo 47,5. Posle sprovedene fizikalne terapije, četiri meseca od operacije, BM skor desno iznosio je 100, a levo 93. **Zaključak.** Hirurški tretman i rana rehabilitacija su ključni za povratak dobre funkcije oba lakta.

Ključne reči:
ulna, prelomi; radijus; iščašenje; fiksatori, unutrašnji; rehabilitacja.

Introduction

Fracture of the proximal ulna with dislocation of the radius is known by its eponym as Monteggia fracture. It was first described by Giovanni Monteggia in 1814. It is more common in children, while in adults it occurs as a result of the effects of a direct force, after being hit by a stick and trying to protect the forearm, namely, by falling to the stretched hand with the forearm extremely proned [1, 2].

Monteggia fracture was classified by Badou (1976) into four types of fracture depending on the side of the luxated radial head. The most common are type I, where there is anterior dislocation of the radial head and type II where there is posterior or posterior-external dislocation of the radial head. Until the appearance of AO plates and stable osteosynthesis, these fractures were treated conservatively and, in general, functional results after treatment used to be very unsatisfactory [3–8].

Bilateral Monteggia fracture in adults is very rare. Kloen et al. [5] described a bilateral Monteggia fracture that had been treated surgically, as in the patient we presented.

Case report

A 55-years-old female patient was injured when falling on the flexed forearm with a burden in the hands. After the fall, the patient did not lose consciousness nor vomited, and referred immediately to the Traumatology Center. The patient was inspected clinically and radiographinally. Based on

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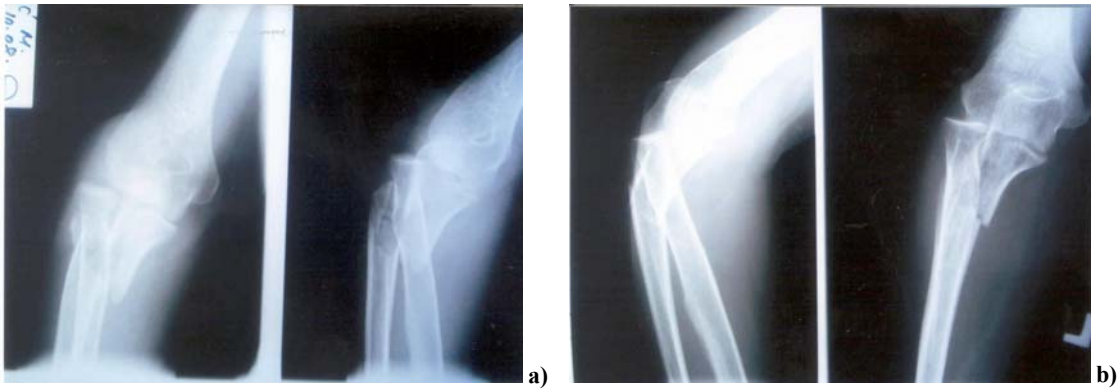
the findings, the diagnosis was bilateral Monteggia fracture (Figures 1a and 1b). There were no neurovascular outages and hospitalization was suggested. The patient refused hospitalization, so an attempt of orthopedic repositioning the upper arm was made and plaster splints were applied on both sides. Subsequently, at the following check-up, the patient agreed to be hospitalized and surgically treated.

Upon addmitance and an appropriate preoperative care 8 days after the injury the patient was surgically treated. Fracture of both proximal ulnas was approached by the posterior side. After repositioning the fracture and osteosynthesis by a compression plate with seven screws on the right, repositioning of the ulna was done on the left side and ostheosynthesis was performed with *zuggurtung* tehnique. Closed repositioning of the luxated radial head was done bilaterally. Intraoperatively, we made radiography to check the position of the radial heads (Figure 2). Upper arm plaster splints were placed bilaterally. Postoperatively, cephalosporins and aminoglycosides were applied intravenously, and on the 6th postoperative day oral antibiotics were prescribed. On the postoperative day 13 stitches were removed and the patient was discharged to home treatment. On the 21st day after operation plaster splints were removed. The function of the elbows was evaluated by the Broberg Morrey (BM) score and it was on the right side 45.5, and on the left one 47.5 (BM score values were between 15 and 100). The patient was referred to physical therapy on the outpatient basis. After four months the patient came to regular check-up. X-rays showed the full consolidation of both fractures of the ulna (Figure 3). The right BM score was 100 (130° of flexion, extension 0°, 90° pronation, supination 90°), while the left elbow BM score was 93 (130° of flexion, extension 20°, 90° pronation, supination 90°); there were no neurologic events.



a) **b)**

Fig. 1 – Monteggia fracture: a) fracture of the proximal third of the ulna; b) dislocation of the radial head

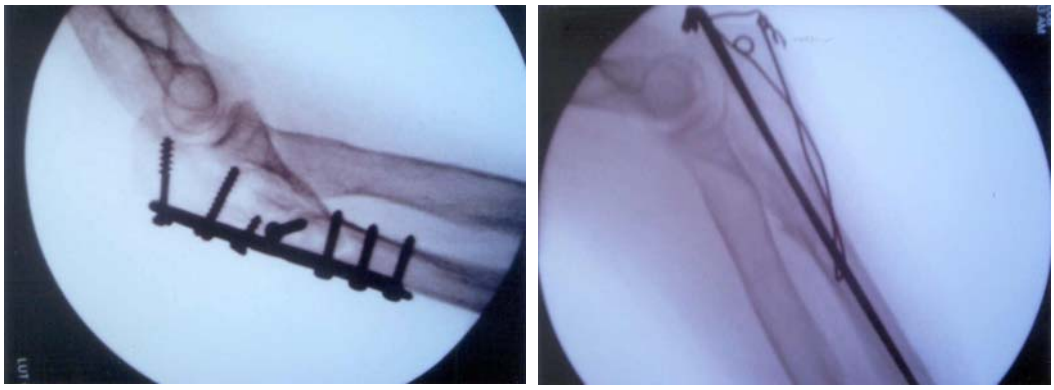


Fig. 2 – Intraoperative radiography of the radial heads

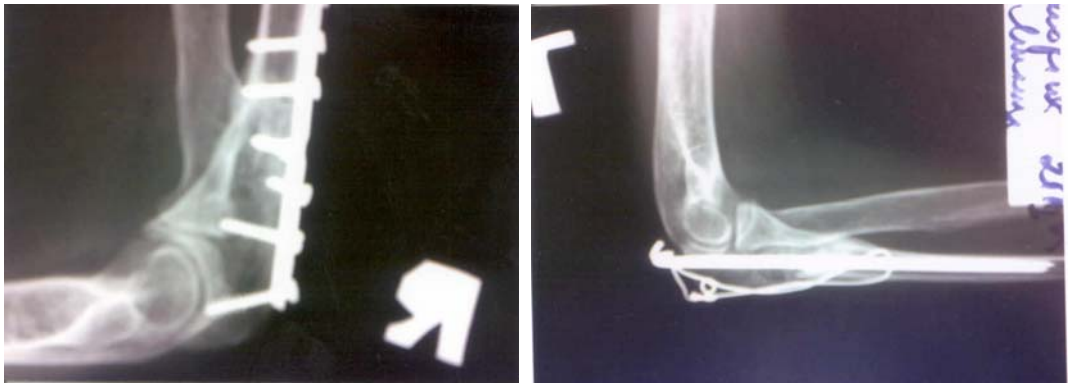


Fig. 3 – Postoperative radiography – full consolidation of both fractures of the ulna

Discussion

Fractures of the proximal end of the ulna with dislocation of the radial head are serious injuries and their treatment remains a challenge for the orthopedist.

Monteggia fractures are produced by the direct force, the most common effect of blunt force, when a patient tries to defend him (her) self. Evans [9] proved that the cause of the fracture may be forced pronation and forearm during the fall by testing it on cadavers. Fall from a scooter and traffic accidents in the modern world befall to leading cause of Monteggia fracture. The presented patient was injured by falling to the flexed arm - similar to the direct effects of blunt force as the main cause of fracture.

Until the appearance of AO plates and stable osteosynthesis, these fractures were treated nonoperatively by plaster splints. Following the rule, this kind of treatment resulted in a very poor function of the elbow, and led to a subsequent disability of the patient. The appearance of AO plate provides stable osteosynthesis of fractures, early mobilization of the elbow, so the complications are significantly rare [4, 5, 7, 8]. In addition to plate, Ring et al. [10] announce the use of *zug-gurtung* technique to achieve a satisfactory stability of fracture. In our study, for the stabilization of the ulna fracture, we used an AO plate on the right side and on the left *zug-gurtung* technique. There were no complications during the treatment. Both fractures healed for the same period regardless of the type of osteosynthesis.

Arenas et al. [11] describe nerve lesions in Monteggia fracture primarily of the anterior interoseal nerve, with spontaneous remissions. Ring et al. [4] describe lesions of *n. interosealis posterior*, *n. medianus*, *n. ulnaris* or both nerves, which are rare and occur in the open Monteggia fracture.

Konrad et al. [2] deseribed in their study the occurrence of non-healing fractures in 6 patients (out of 63) and heterotopic ossification in 7 patients. Korner et al. [8] reported a revision of the surgery in 14 patients out of 68, as well as the appearance of osteoarthritis changes at the humeroradial and radioulnar joint. In our study we noted the appearance of the described complications. Function of both elbows was completely recovered – BM score for the right elbow was 100, and for the left one 93.

Conclusion

Bilateral Monteggia fracture in adults is very rare and requires anatomical reposition of the ulna, its stable osteosynthesis, reposition of the luxated radial head and early rehabilitation. This method of treatment allows complete recovery of elbow function.

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CASE REPORT

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Preimplantation filling of tooth socket with β-tricalcium phosphate/polylactic-polyglycolic acid (β-TCP/PLGA) root analogue: clinical and histological analysis in a patient

Klinička i histološka analiza kod pacijenata sa preimplantacijskim popunjavanjem zubne alveole β-trikalcijum fosfatom/polilaktatnom-poliglikolnom kiselinom (β-TCP/PLGA)

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Abstract

Introduction. Bone resorption is a physiological process after tooth extraction. The use of bone substitutes to fill the tooth socket is suggested to prevent bone resorption and establish good bone architecture for implant placement. A pure ß-tricalcium phosphate coated with copolymer (polylactic-polyglycolic acid) as a root analogue, is suitable for filling tooth sockets. **Case report.** We presented a patient successfully treated with root analogue after extraction of the right second lower premolar. Three months later, the patient was planned for the placement of six TE® ITI dental implants into the mandible. During the sugery, the biopsy of bone-like tissue from the previously treated socket was taken. All the implants were immediately loaded due to good primary stability. Histological analysis of the specimen revealed fibrous healing in the area treated with root analogue. **Conclusion.** The use of ß-tricalcium phosphate coated with copolymers after tooth extraction enables satisfactory bone architecture for consequent implant treatment.

Key words:
tooth extraction; bone resorption; therapeutics; oral surgical procedures, preprosthetic; polymers; dental implants; histological techniques.

Apstrakt

Uvod. Resorpcija kosti je fiziološki proces koji nastaje nakon ekstrakcije zuba. Primena koštanih zamenika za popunjavanje alveola nakon ekstrakcije zuba preporučuje se u cilju sprečavanja resorpcije kosti i očuvanja njene arhitekture pre ugradnje dentalnih implantata. Analog korena zuba, sastavljen od β-trikalcijum-fosfata obloženog kopolimerom (polilaktatna poliglikolna kiselina), pogodan je za terapiju ekstrakcione alveole. **Prikaz bolesnika.** Prikazan je pacijent sa uspešnim popunjavanjem ekstrakcione alveole drugog donjoviličnog premolara sa analogom korena zuba. Tri meseca kasnije kod pacijenta je ugrađeno šest ITI TE® implantata u donju vilicu i tom prilikom izvršena je biopsija koštanog tkiva predhodno tretirane alveole. Svi implantati odmah su opterećeni zbog dobre primarne stabilnosti. Histološki nalaz bioptičkog uzorka ukazao je na fibrozno zarastanje u tretiranoj ekstrakcionoj alevoli. **Zaključak.** Upotreba β-trikalcijum-fosfata obloženog kopolimerom za popunjavanje ekstrakcione alveole zuba omogućava postizanje zadovoljavajuće arhitekture kosti za buduću terapiju dentalnim implantatima.

Ključne reči:
zub, ekstrakcija; kost, resorpcija; lečenje; hirurgija, oralna, preprotetske procedure; polimeri; zubi, implantati; histološke tehnike.

Introduction

The major sequel of human tooth loss is the loss of alveolar bone. The rate of this process is highest immediately after tooth extraction – the height of the alveolar process decreases several millimeters (mm) within the first 6 months of the healing period [1]. The unique atrophy of the alveolar process has been described as reduction of residual ridges [2] and considered to be multifactorial in origin [1]. Atrophy of the alveolar ridge may cause aesthetic and surgical problems in prosthetic dentistry, especially when implant treatment is planned.

Immediate alveolar ridge prophylaxis after tooth extraction includes preservation of the alveolar process by retention of endodontically treated roots (physiologically most accepted), immediate implant placement, guided bone regen-

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eration and the use of root analogues [3–10]. The use of root analogues as preimplant therapy can provide adequate quantity of bone and soft tissue for implant placement. Many authors showed that different bone substitute materials had been used as root analogues, some of them being: dense hydroxyapatite [5], polyglycolic acid [6], polylactic acid [7], bioabsorbable polylactic-polyglycolic acid (PLGA) [3, 4], deproteinized bovine bone mineral integrated in a 10% collagen matrix [11], β-tricalcium phosphate (β-TCP) combined with type I collagen [8] and β-TCP/PLGA [12]. RootReplica® (Degradable Solutions AG, Switzerland) consists of absorbable β-TCP granules (Ø: 500–800 μm) coated with PLGA layer (about 15 μm). It was developed in order to maintain the dimension of the alveolar process. Using the moulding technique, RootReplica® can be exactly shaped into the form of an extracted tooth root. This scaffold has an interconnected open porosity (55%) and a medium pore diameter of 280 μm. Its degradation occurs without releasing large amounts of acid products [12].

In this paper we described clinical and histological follow-up of implant stability in a patient who had received six immediately loaded titanium implants in the mandible, one of which being inserted in the extraction socket previously treated with biodegradable β-TCP/PLGA root analogue.

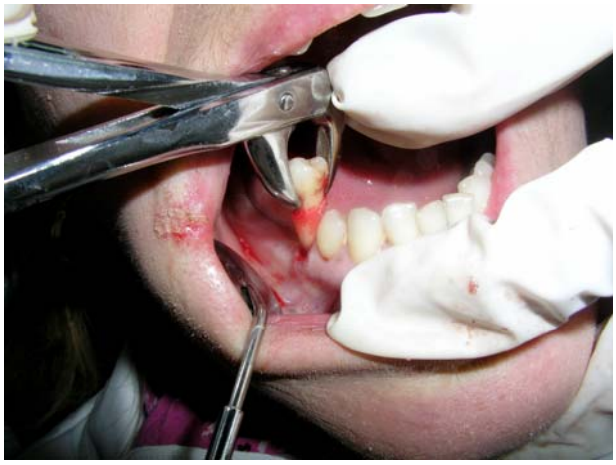
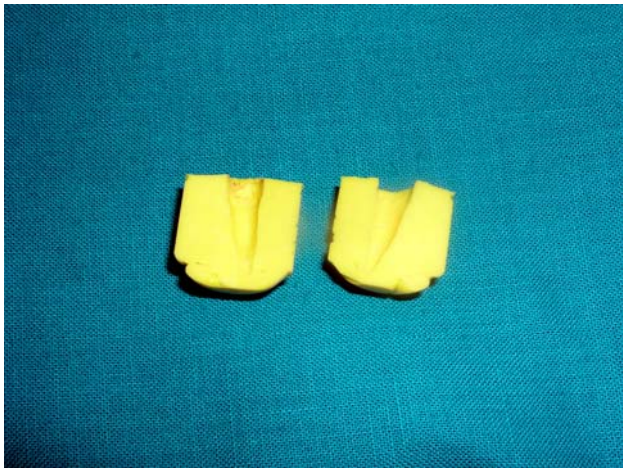


Fig. 1 – The tooth 45 removal

a preimplant treatment. The tooth 45 was atraumatically removed three months before the planned implantation and placed in the sterile impression material up to the level of cement-enamel junction as to prepare a polyvinyl siloxane model that exactly reproduces the shape of the root (Figure 2, a and b). The cavity of the mould was filled with β-

a)



b)

Fig. 2 – a) Extracted tooth placed in the sterile impression material; b) the polyvinylsiloxane model

Case report

In November 2004, a healthy 52-year-old female patient with no contraindications to surgical treatment came for prosthetic treatment with dental implants. Clinical and radiographic findings revaled bilateral partially dentate mandible (Kennedy Class I), as well as a vertical root fracture of the tooth 45. After radiographic analysis and producing diagnostic cast, we decided to remove the fractured tooth 45 (Figure 1) and to put six implants bilaterally in the regions of the second lower premolars and first and second lower molars to support a fixed prosthetic reconstruction.

To provide an adequate soft tissue contour and bone dimension for implant placement in the region of the right second premolar that had to be extracted, its socket was filled with root analogue, β-TCP/PLGA (RootReplica®), as TCP/PLGA granules. The mould with the granules was then heated at 80ºC for 2 minutes to fuse the granules together and form a mechanically stable copy of the root (Figure 3, a and b).

RootReplica® was inserted into the bleeding socket with a slight pressure (Figure 4). After filling the extraction socket, computer tomography was performed to provide vertical and horizontal dimensions of the bone in the treated area (Figure 5). The succeeding radiograph, taken three months later, immediately before the placement of the implant, showed an adequate thickness of bone walls to accommodate Ø3.3 mm diameter implants (Figure 6). The tissue in the former tooth socket was apparently firm from the clinical point of view. During implant insertion, a trephine-cylindrical biopsy specimen (using the trephine drill Ø2.0 mm) was processed for light microscopy (Figure 7).


a)

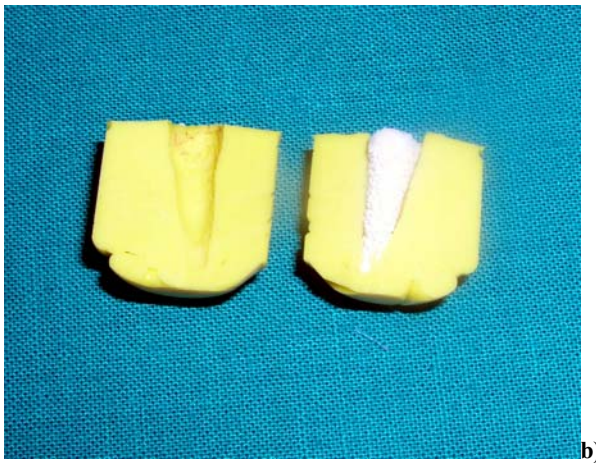

b)

Fig. 3 – a) Heating of the mould; b) β-tricalcium phosphate/polylectic-polyglycolic acid copy of the root

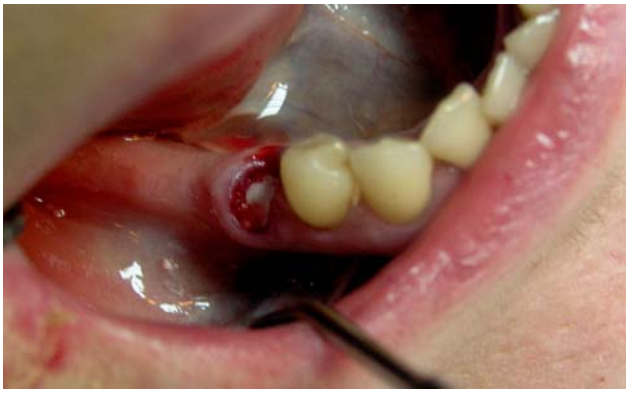


Fig. 4 – Placing of the root replica in the postextraction wound

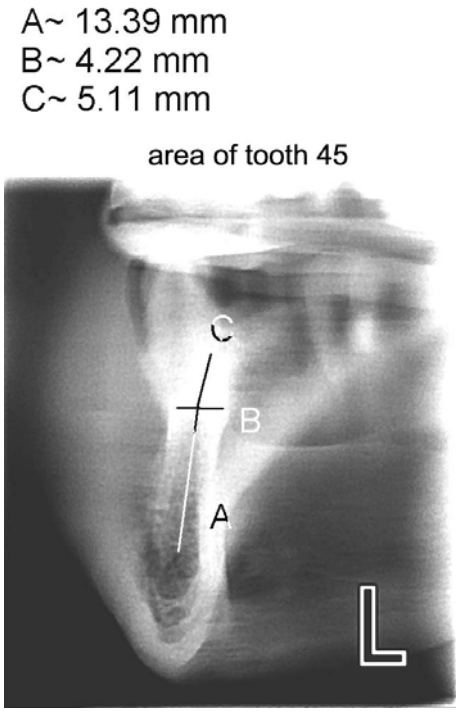


Fig. 6 – Radiograph three months later

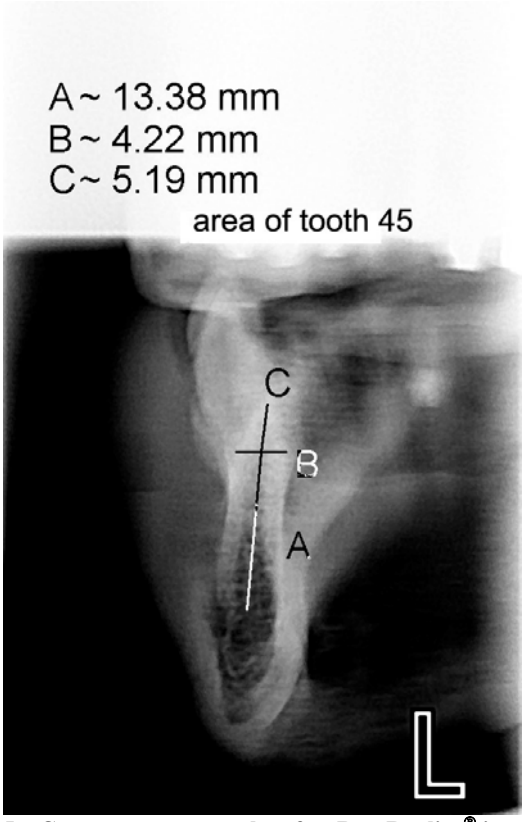


Fig. 5 – Computer tomography after RootReplica® insertion

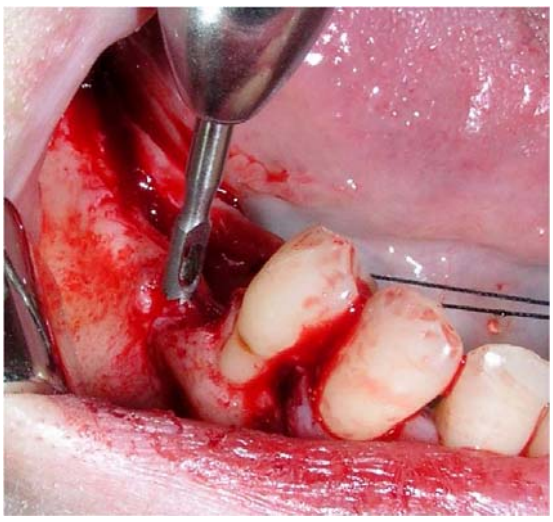


Fig. 7 – Taking of trephine-cylindrical biopsy

Placement of all implants was done using local anesthesia with 2% lidocaine with epinephrine (Xylestesin, ESPE Dental AG, Seefeld, Germany). After flap elevation, implant sites were prepared in accordance with the usual procedure for Straumann TE® implants. Straumann TE® implants (Institute Straumann AG, Waldemburg, Switzerland) of Ø3.3/4.8 mm diameter were used (Figure 8). The flap was

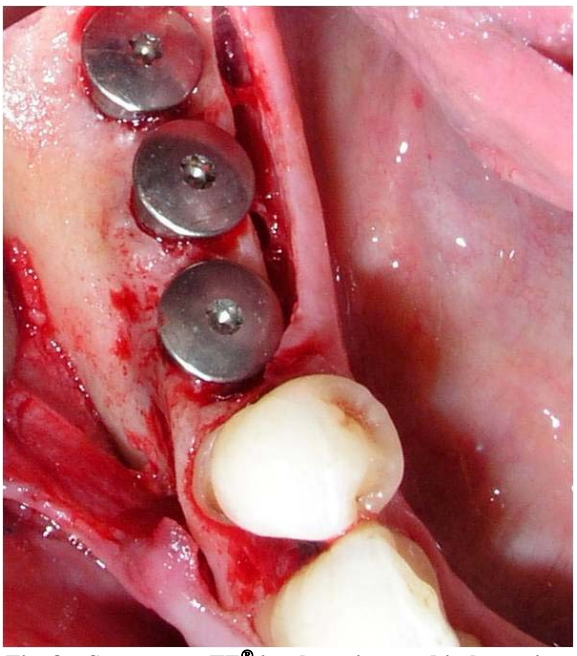


Fig. 8 – Straumann TE® implants inserted in lower jaw

closed with horizontal and single sutures. Resonance frequency analysis (RFA), done with Osstell mentor® (Integration Diagnostics AB, Sävedalen, Sweden), was used to check the primary stability of all the six implants, as well as in the follow-up period (6, 13 and 52 weeks). As all implants fulfilled the criteria for immediate loading [13], they were immediately loaded (on the same day of implant insertion). Therefore, temporary restorations were performed according to the immediate loading protocol. Three months later, the temporary restorations were changed with metal-ceramic bridges as definitive prosthetic reconstructions (Figure 9).

Fig. 9 – Definitive prosthetic reconstruction

The biopsy specimen was immediately immersed in a fixative solution of pH 7.2, containing 2.5% glutaraldehyde and 2.5% paraformaldehyde in the 0.1 mol/L phosphate-buffered saline (PBS). After washing in 0.1 mol/L PBS, the specimen was dehydrated in graded alcohol and processed for embedding in methylmethacrylate. Semithin sections were cut and stained with haematoxylin and eosin for light microscopic examination, magnification being × 40.

No signs of microbial infection, exudation or dehiscence were noted in the healing period of the post-extraction wound treated with RootReplica®. Three months later, soft tissue covered the area treated with β-TCP/PLGA root analogue, forming a nice gingival shape for implant insertion. At the same time point computer tomography showed no loss of bone dimension (Table 1).

RFA presented a successful primary stability of all the inserted implants (Table 2). After six weeks, we noted a decrease of implant stability quotient (ISQ) of the implant inserted in the area filled with β-TCP/PLGA root analogue at the level of –9. At the same time point, a slighter decrease of implant stability was observed for the most of the immediately loaded implants. However, the increase of implant stability was evident lately for all the inserted implants (Table 2).

Table 1

The mandibular bone width measured by computer tomography

Period	Bone width (mm)	Bone height (mm)
At the time of RootReplica® placement	4.22	13.38
At the time of Straumann TE® implantation	4.22	13.39

Table 2

Resonance frequency analysis of implant stability immediately after the implant placement and within a 52-week period

Area of immediately loaded implants	Implant stability quotient			
	Primary stability	6th week	12th week	52nd week
35	80	72	79	80
36	80	71	78	80
37	82	73	77	81
45 (placed in the socket filled with root analogue)	76	67	81	81
46	77	79	80	81
47	81	79	81	81

At the time of tissue specimen taking for biopsy, RootReplica® was stable in the extraction site. Histological examination of the trephine-harvested tissue of the extracted socket treated with RootReplica® showed particles of the implanted material separated by the soft connective tissue, with inflammatory cells present in the area (Figure 10).

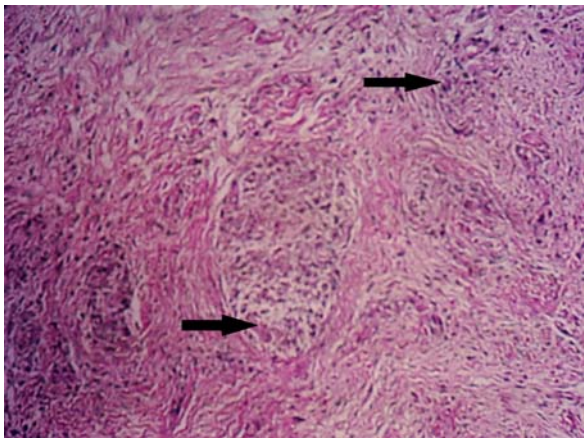


Fig. 10 – Histological view of the trephine-harvested tissue of the extracted socket treated with RootReplica® (haematoxylin and eosin ×40) – arrows point to the inflammatory cells

Discussion

Different treatment modalities have been used to prevent post-extraction atrophy of alveolar ridges and maintain their original diameter and shape. To our knowledge, there have been only a few publications referring to extraction sockets treated with root analogs [3–8]. The presented case report indicates clinical and histological findings of the process of post-extraction alveolar socket healing after the placement of biodegradable β-TCP/PLGA material as a rootcopy before definite implant placement. The final radiographic outcome in this case broadly agrees with previous reports [3, 4]. We noticed similar values of bone height and width after RootReplica® placement into the extraction socket and 3 months later. The patients provided with dense hydroxyapatite root replica implants, after a 1-year follow-up period, were found to have significantly higher and wider residual ridges than non-treated patients [3]. In the experimental study of the use of polyglycolic acid root replica for placement in extraction sockets, a significantly higher bone dimension was noticed than in the control group with empty sockets after a 5-month period [5]. A case report on the preserved ridge height after placement of polylactic acid root replica in the extraction socket, during a 21-month observation period, was also presented [6].

Histological and fine structural investigations in the experimental study confirmed the biocompatibility and absorbability of the scaffolds [12, 14], and that β-TCP/PLGA granules were subjected to complete absorption after 24 months [15]. In the report on two cases of alveolar bone regeneration with porous tricalcium phosphate, Zerbo et al. [9] histologically confirmed resorbability of that grafting material and its replacement by bone without adverse reaction.

In the presented patient, inflammatory cells were noticed in the biopsies taken three months (approximately 12 weeks) after the insertion of a root analogue. Contrary to this, histological findings in the study of Nair and Schug [3] showed no inflammatory and foreign-body giant cells, demonstrating a complete biodegradation of a root-replica material during the period of observation. In the presented patient, we found predominantly fibrous tissue in the specimen of tissue filling the post-extraction socket, which could be due to the procedure used. Namely, the manufacturer emphasizes that RootReplica® need not to be covered after insertion in the extracted socket. In an experimental study [12], the authors covered the treated areas with a flap. We did not cover the RootReplica® material after insertion, and that could be the reason for the presence of inflammatory cells in the specimen. However, this fact did not influence the possibility of titanium implant insertion and the gain of its primary stability; moreover, the shape and diameter of the alveolar ridge in the area was quite satisfactory, enabling sufficient architecture of soft tissue for further successful implant placement.

The presence of inflammatory cells in the socket previously treated with root analogue probably contributed to a slightly greater loss of implant stability estimated by RFA. However, later on, in the postoperative period all implants (including the implant inserted in the socket previously treated with β-TCP/PLGA root analogue), demonstrated a substantial gain of stability, in three implants even better than primarily after the insertion. Therefore, treatment of tooth root sockets with β-TCP/PLGA root analogue to enable better alveolar ridge morphology after tooth extraction seems not to interfere with the possibility of titanium implant insertion.

Conclusion

The use of β-TCP coated with PLGA root analogue after tooth extraction enables satisfactory bone architecture for consequent implant treatment.

Acknowledgments

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P R I K A Z S L U Č A J A

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Odloženi angioedem tokom primene inhibitora angiotenzin-konvertujućeg enzima

Delayed angioedema during therapy with angiotensin-converting enzyme inhibitors

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Apstrakt

Uvod. Primena inhibitora angiotenzin-konvertujućeg enzima predstavlja vodeći uzrok angioedema izazvanog lekovima, sa incidencijom od 0,1 do 0,2%. Angioedem nije imunske prirode, već nastaje kod predisponiranih osoba kao posledica nagomilavanja vazoaktivnih medijatora čija razgradnja je blokirana. **Prikaz bolesnika.** U ovom radu prikazan je 63-godišnji bolesnik sa hipertenzijom i hroničnom opstruktivnom bolešću pluća koji je imao dve epizode angioedema tokom petogodišnje primene enalaprila. Prva epizoda javila se posle tri godine, a druga posle pet godina od početka primene enalaprila. Oba puta bolesnik je bio hospitalizovan, pri čemu je u poslednjem trenutku izbegnuta urgentna traheotomija kao krajnja terapijska mera. **Zaključak.** Tokom terapije inhibitorima angiotenzin-konvertujućeg enzima može nastati odloženi angioedem, posebno kod osoba sa inflamacijom respiratornih puteva usled infekcije ili hronične iritacije.

Ključne reči:
angiotenzin-konvertujući enzim, inhibitori; angioneurotski edem; lekovi, neželjeno dejstvo, sistemi za izveštavanje.

Abstract

Introduction. Angiotensin-converting enzyme inhibitors are leading cause of drug-induced angioedema, with incidence of 0.1 to 0.2%. The angioedema is not of immune nature; in predisposed individuals it is caused by accumulation of vasoactive mediators due to reduced activity of angiotensin-converting enzyme. **Case report.** We presented a 63-year old male patient suffering from hypertension and chronic obstructive pulmonary disease, who had developed two episodes of angioedema during a 5-year long therapy with enalapril. The first episode happened after three, and the second after five years of the therapy. On both occasions, the patient was admitted to the hospital and tracheotomy was avoided in the last moment. **Conclusion.** Long-term therapy with angiotensin-converting enzyme inhibitors could be associated with delayed angioedema, especially in patients with inflammation of airways caused by infection or chronic irritation.

Key words:
angiotensin-converting enzyme inhibitors; angioedema; adverse drug reaction reporting systems.

Uvod

Primena inhibitora angiotenzin-konvertujućeg enzima (ACE) predstavlja vodeći uzrok angioedema izazvanog lekovima i jedan od najčešćih poznatih uzročnika angioedema uopšte [1–8]. Uzevši u obzir procenu da ove lekove koristi više desetina miliona ljudi širom sveta [9], sa stalno prisutnom tendencijom porasta upotrebe koja sledi incidenciju kardiovaskularnih oboljenja, pojava angioedema, kao potencijalno fatalne komplikacije lečenja, iako nije česta, svakako se mora smatrati značajnim zdravstveno-ekonomskim problemom savremenog doba. O značaju pomenutog neželjenog efekta svedoče podaci iz dosadašnjih epidemioloških istraživanja koji, iako nisu konzistentni, ukazuju da je prosečno 1/3 bolesnika lečenih u urgentnim centrima zbog angioedema uzimala ACE inhibitore; čak 20% takvih bolesnika bilo je neposredno životno ugroženo usled opstrukcije disanja i podvrgnuto intubaciji ili hitnoj traheotomiji [1–7, 10–16]. U ranijim izveštajima, zabeležena je stopa smrtnosti takvih bolesnika od oko 11% [14], međutim, brža i agresivnija terapija tokom poslednje 1–2 decenije dovela je do znatnog smanjenja broja smrtnih ishoda [1–3, 16].

Angioedem posle primene ACE inhibitora javlja se relativno retko: najveći broj studija procenjuje učestalost kod 0,1–0,2% bolesnika [4, 8, 10, 17], mada stvarna incidencija može biti veća imajući u vidu rastuću upotrebu ove značajne grupe

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lekova i posebno činjenicu da zbog specifičnosti kliničkog ispoljavanja ovog oblika angioedema, koja se pre svega ogleda u tome da može nastati bilo kada u toku primene leka, nezavisno od doze, znatan broj bolesnika u etiološkom smislu biva u praksi neprepoznat [2, 7, 8, 10, 18–27].

U radu je prikazan bolesnik sa odloženom pojavom angioedema posle primene enalaprila.

Prikaz bolesnika

Muškarac, star 63 godine, primljen je u večernjim satima u Urgentni centar Kliničkog centra Kragujevac zbog velikog otoka jezika i gušenja. Dva sata ranije osetio je bol i svrab u gornjoj vilici, da bi potom naglo došlo do oticanja usana, jezika i vrata.

Anamneza i uvid u medicinsku dokumentaciju bolesnika pokazali su da boluje od primarne arterijske hipertenzije i hroničnog opstruktivnog bronhitisa. Od lekova, tokom zadnjih 4–5 godina, redovno je koristio enalapril (20 mg dnevno), diltiazem retard i aminofilin retard tablete, odnosno inhalacije tiotropijum bromida, kombinaciju flutikazona i salmeterola, a prema potrebi, i kombinaciju ipratropijuma i fenoterola. Aktivan je pušač, prosečno 20 cigareta dnevno.

U fizikalnom nalazu na prijemu, pored otežanog, stridoroznog disanja, dominirao je veliki, bezbolan otok jezika i podjezičnog predela u čitavoj usnoj duplji i ždrelu, onemogućavajući uvid u dublje strukture. Bolesnik je bio bez poremećaja svesti, stabilnog arterijskog pritiska i pulsa. Rutinskim laboratorijskim analizama konstatovano je povećanje koncentracije hemoglobina (183 g/L) i hematokrita (54%) uz osmolarnost plazme od 303,4 mmol/L, izračunatu na osnovu izmerenih koncentracija natrijuma, kalijuma, ureje i glikemije (verovatno znaci hemokoncentracije) i blago povišena vrednost C-reaktivnog proteina (CRP) (10,3 mg/L).

Otorinolaringolog i alergolog postavili su radnu dijagnozu akutnog alergijskog angioedema i inicijalno ordinirali terapiju: visoke doze metilprednizolona (80 mg intravenski odmah, zatim još 60 mg posle 2 sata, a u nastavku lečenja 80 mg na 12 sati), hlorpiramin (20 mg intramuskularno, a zatim ista doza na 12 sati), ranitidin (50 mg intravenski u 500 mL 0,9% NaCl, a zatim identična doza na 12 sati) i 10% kalcijum-glubionat (1 375 mg/10 mL intravenski, u daljem lečenju ista doza jednom dnevno). Ova terapija u prva 3–4 sata nije dovela do značajnog poboljšanja, međutim, u daljem kliničkom toku postepeno došlo je do povlačenja simptoma, te nije bilo potrebe za primenom traheotomije.

Nakon 12 sati od prijema, konsultovan je klinički farmakolog koji je, prepoznajući moguću povezanost nastalog angiodema sa primenom enalaprila, savetovao prekid dalje primene svih ACE inhibitora, takođe, preporučio obustavu i drugih lekova koje je bolesnik koristio zbog osnovnih oboljenja tokom naredna 24 sata, uz intenzivno praćenje disajne funkcije i krvnog pritiska. Što se tiče lečenja hipertenzije, eliminisao je iz dalje terapije i blokatore receptora za angiotenzin 2 kao potencijalnu alternativu.

Sutradan, po stabilizaciji stanja bolesnika, ponovno uzetom anamnezom dobijen je podatak da je pre dve godine imao sličnu reakciju sa otokom lica, vrata i jezika, praćenu jakim gušenjem, za koju je mislio da je bila izazvana kardiopirinom ili „lekom za povišeni pritisak“; lečen je tada Lemod®-om i Sinopen®-om, a nakon smirivanja tegoba nastavljeno je sa primenom prethodne antihipertenzivne terapije, uključujući i enalapril.

Sedmog dana bolesnik je otpušten iz bolnice u dobrom opštem stanju, sa urednom disajnom funkcijom, a ACE inhibitor je zamenjen fiksnom kombinacijom diuretika (amilorid + metiklotiazid).

Detaljnim pregledom istorije bolesti bolesnika nismo došli do podataka da su ordinirajući lekari indikovali dopunsko imunološko ispitivanje etiologije nastalog angioedema, u smislu merenja nivoa IgE antitela u krvi, odnosno procene funkcionalne aktivnosti sistema komplementa uključujući i inhibitor C1 esteraze.

Na osnovu svih iznetih činjenica ovom bolesniku, može se reći da se radi o umereno teškom obliku recidivantnog odloženog angioedema prouzrokovanog primenom enalaprila. Prethodna, prva epizoda, koja se javila posle dve-tri godine od početka primena leka nije prepoznata na odgovarajući način, što je u nastavku terapije dovelo do ponovnog ispoljavanja istog neželjenog dejstva. Ovakvu dijagnozu potvrđuje i odgovor Nacionalnog centra za farmakovigilancu Agencije za lekove, kome je prijava neželjenog događaja poslata odmah nakon prvog pregleda farmakologa: u proceni uzročno-posledične veze između leka i zabeležene pojave, ista je prema Naranjo skoru svrstana u kategoriju „moguća“. Međutim, kako u prijavi neželjene reakcije nije naveden i naknadno dobijeni podatak od bolesnika o prethodnoj epizodi angioedema tokom primene enalaprila, smatramo da bi ocena uzročnosti morala biti veća. Naime, prema ponovnoj proceni kauzalnosti koju smo izvršili korišćenjem istog instrumenta (Naranjo algoritma), povezanost je vrednovana kao „verovatna“ (minimalni Naranjo skor – 5: neželjeni događaj dobro je poznat i opisan u literaturi, postoji vremenska povezanost primene leka sa pojavom neželjenog događaja, pozitivan je *dechallenge* i bolesnik je ranije imao sličnu reakciju posle primene istog leka).

Diskusija

Mada patofiziologija angioedema izazvanog ACE inhibitorima nije u potpunosti razjašnjena, većina autora smatra da ta pojava, u osnovi, nije imunske prirode, već nastaje isključivo kod predisponiranih osoba kao posledica lokalizovanog vazoaktivnog delovanja bradikinina, supstance P i drugih medijatora, nagomilanih u određenim tkivnim strukturama usled smanjene razgradnje [21, 28–31]. U nastanku i evoluciji ove pojave važna je i uloga CRP: pretpostavljeni mehanizam zasniva se na njegovoj proinflamatornoj aktivnosti koja uključuje pojačano stvaranje bradikinina. Međutim, dejstvo CRP verovatno je kompleksnije i zahteva dodatna ispitivanja [32]. S obzirom na to da je CRP nespecifičan marker akutne i hronične inflamacije, mišljenja smo da njegovo merenje nije neophodno u sklopu inicijalne dijagnostike angioedema u toku primene ACE inhibitora, ali da može biti od pomoći u diferencijalnoj dijagnozi jer, prema našim saznanjima povećanje koncentracije ovog parametra ne javlja se

kod angioedema druge etiologije. Ključni patofiziološki činilac ipak predstavlja predispozicija: izgleda da ACE inhibitori povećavaju verovatnoću pojave angioedema kod bolesnika sa izvesnim stepenom sklonosti ka njegovom nastanku. Pokazana je veća učestalost kod osoba crne rase, ženskog pola, starijih bolesnika i aktivnih pušača [2, 3, 8, 16, 20, 33–36]. Ostale značajne faktore rizika čine alergija/angioedem u anamnezi (uključujući i latentne kliničke oblike deficijencije inhibitora esteraze C1 komponente komplementa) [34, 35, 37–40], smanjena aktivnost drugih enzima koji inaktiviraju bradikinin [41, 42] i, naročito, lokalna trauma tkiva (npr. izazvana spoljnom povredom, intubacijom, operacijom, bronhoskopijom i sl) [8, 21, 22, 43–45]. Učestalija pojava zabeležena je i kod bolesnika sa imunodeficijencijom nakon transplantacije srca ili bubrega [46]. Pored prethodno pomenutih faktora rizika, izvesni autori kao moguće okidače razvoja angioedema navode i infekcije, odnosno aerozagađenje [40] o čemu bi, takođe, trebalo voditi računa kada su u pitanju bolesnici koji koriste ACE inhibitore. Posebna opreznost neophodna je kod starijih i gojaznih osoba, odnosno obolelih od opstruktivnih plućnih bolesti, zbog rizika od razvoja težeg kliničkog oblika angioedema. U slučaju našeg bolesnika, predisponirajućim činiocem može se smatrati zapaljenje disajnih puteva usled hronične iritacije ili infekcije.

Angioneurotski edem posle primene ACE inhibitora uglavnom zahvata predeo glave i vrata, sa predilekcijom za jezik i usne [1, 2, 4, 16], dok je znatno ređe lokalizovan u gastrointestinalnom sistemu [47–49], što može stvarati ozbiljne dijagnostičke zabune. Obično se javlja u toku prve nedelje, ali neretko i posle nekoliko meseci do nekoliko godina od početka terapije [8, 10, 16, 17, 24, 25, 40, 50–53]. Upravo ova odložena pojava, prema izvesnim autorima moguća kod 1/4–1/2 bolesnika koji su prethodno dobro podnosili lek [17, 25], predstavlja razlog da se ne prepozna uzročno-posledična veza između leka i angioedema [2, 7, 8, 10, 19–25, 28, 53, 54], i da bolesnik nastavi sa primenom leka rizikujući nastanak težih kliničkih oblika [24]. Ponovljeni angioedem može se javljati po sličnom scenariju kao i prvobitna epizoda. Dešava se u praksi i da bolesnici imaju višestruke ponavljane napade [24, 25], ponekad praćene dužim asimptomatskim periodima [53], što dodatno može zbunjivati lekare i odložiti pravilnu dijagnozu, odnosno prekid primene leka-uzročnika [26, 28]. Blaži oblici angioedema (npr. samo sa otokom lica i usana lakog stepena), kojima često bolesnici ne pridaju značaj, te ih i ne saopštavaju prilikom poseta lekaru mogu biti posebno zahtevni kada je postavljanje dijagnoze u pitanju.

Iznenadna, nepredvidiva pojava, akutni razvoj simptoma i postepena spontana rezolucija tokom narednih nekoliko dana karakterišu klinički tok ove vrste angioedema [2, 3, 5, 10, 24, 28, 36, 55]. Uz obaveznu obustavu upotrebe leka koji je uzrokovao angioedem, što je kod blažih ispoljavanja sasvim dovoljna mera [55], u lečenju težih kliničkih oblika treba uspostaviti prohodnost disajnih puteva i hemodinamsku stabilnost [2, 3, 5, 13, 16, 56].

S obzirom na nealergijsku patogenezu, adrenalin, kortikosteroidi i antihistaminici smatraju se terapijom „druge linije“ [6, 36, 55], ali se kod ovih bolesnika gotovo uvek odmah primenjuju, imajući u vidu da u početku nije moguće jasno razlučiti etiopatogenetski mehanizam nastalog angioedema. Najteža komplikacija u vidu kompletne opstrukcije disanja zbog edema grkljana zahteva hitnu traheotomiju. Postoje i određeni izvešataji o efikasnosti sveže zamrznute plazme (SZP) u terapiji teških, kliničkih oblika rezistentnih na konvencionalnu terapiju, kada je bolesnik neposredno životno ugrožen [57, 58], za koju autori pretpostavljaju da je posledica razgradnje akumuliranog bradikinina pod dejstvom ACE iz plazme. Međutim, sa primenom SZP kod bolesnika sa angioedemom treba biti veoma oprezan i dati je jedino ako smo apsolutno sigurni da je ACE inhibitor uzrok, jer zbog sadržaja komponenti komplementa njena primena može dovesti do pogoršanja angioedema nastalog iz drugih razloga.

Kada je reč o daljem lečenju osnovne kardiovaskularne bolesti kod bolesnika kod kojih je zbog pojave angioedema prekinuta primena ACE inhibitora, svakako je značajno ukazati da se blokatori receptora za angiotenzin 2 ne mogu smatrati apsolutno bezbednom alternativom: iako u principu ređe nego kod ACE inhibitora, angioedem može pratiti upotrebu i ove grupe lekova [2, 55, 59–62], te ih treba izbegavati ili, ukoliko nemamo na raspolaganju efikasniju i bezbedniju zamenu (npr. u slučaju bolesnika sa kongestivnom srčanom insuficijencijom, nefropatijom sa proteinurijom, visokog rizika od kardiovaskularnih komplikacija, loše kontrolisane hipertenzije koja reaguje samo na supresiju renin-angiotenzin-aldosteron sistema ili npr. kod postojanja preosetljivosti na različite grupe antihipertenziva), primenjivati ih uz strogi nadzor bolesnika.

Zaključak

Prikazani bolesnik jasno odslikava sve specifičnosti angioedema izazvanog primenom ACE inhibitora, zbog kojih u praksi nije lako postaviti dijagnozu ovog retkog, ali zbog mogućih posledica po zdravlje i život bolesnika veoma značajnog neželjenog efekta. U jednom takvom složenom procesu, veoma je važna i uloga kliničkog farmakologa kao stručnjaka sposobnog da prepozna i reši probleme vezane za upotrebu lekova. S obzirom na veliki terapijski značaj ACE inhibitora, koji se ogleda u širokoj primeni ovih lekova u praksi, moguća odložena pojava angioedema tokom dugotrajne terapije svakako obavezuje kliničare na maksimalan oprez sa ovom grupom lekova.

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Delovi rada su: **naslovna strana, apstrakt sa ključnim rečima, tekst** i **literatura.**

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Tekst sadrži sledeća poglavlja: **uvod**, **metode**, **rezultate** i **diskusiju**. **Zaključak** može da bude posebno poglavlje ili se iznosi u poslednjem pasusu diskusije. U **uvodu** ponovo napisati naslov rada, bez navođenja autora. Navesti hipotezu (ukoliko je ima) i ciljeve rada. Ukratko izneti razloge za studiju ili posmatranje. Navesti samo strogo relevantne podatke iz literature i ne iznositi opširna razmatranja o predmetu rada, kao ni podatke ili zaključke iz rada o kome se izveštava.

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Primeri oblika referenci:

Đurović BM. Endothelial trauma in the surgery of cataract. Vojnosanit Pregl 2004; 61(5): 491–7. (Serbian)

Balint B. From the haemotherapy to the haemomodulation. Beograd: Zavod za udžbenike i nastavna sredstva; 2001. (Serbian)

Mladenović T, Kandolf L, Mijušković ŽP. Lasers in dermatology. In: *Karadaglić Đ*, editor. Dermatology. Beograd: Vojnoizdavački zavod & Verzal Press; 2000. p. 1437–49. (Serbian)

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: *Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG,* editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12]; 102(6): [about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

Tabele

Sve tabele štampaju se sa proredom 1,5 na posebnom listu hartije. Obeležavaju se arapskim brojevima, redosledom pojavljivanja, u desnom uglu (**Tabela 1**), a svakoj se daje kratak naslov. Objašnjenja se daju u fus-noti, ne u zaglavlju. Za fus-notu koristiti sledeće simbole ovim redosledom: ***, †, ‡, §,** ||**, ¶, **, ††, ...** Svaka tabela mora da se pomene u tekstu. Ako se koriste tuđi podaci, obavezno ih navesti kao i svaki drugi podatak iz literature.

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Examples of references:

Jurhar-Pavlova M, Petlichkovski A, TrajkovD, Efinska-Mladenovska O, Arsov T, Strezova A, et al. Influence of the elevated ambient temperature on immunoglobulin G and immunoglobulin G subclasses in sera of Wistar rats. Vojnosanit Pregl 2003; 60(6): 657–612.

DiMaio VJ. Forensic Pathology. 2nd ed. Boca Raton: CRC Press; 2001.

Blinder MA. Anemia and Transfusion Therapy. In: Ahya NS, Flood K, Paranjothi S, editors. The Washington Manual of Medical Therapeutics, 30th edition. Boston: Lippincot, Williams and Wilkins; 2001. p. 413-28.

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: *Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG,* editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12]; 102(6): [about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

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