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PREVALENCE AND DETERMINANTS OF HEMOGLOBIN VARIABILITY AND ITS IMPACT ON MORTALITY IN PATIENTS ON MAINTENANCE HEMODIALYSIS

Zorica Dimitrijević[1,2], Branka Mitić[1,2], Karolina Paunović[1], Sonja Šalinger-Martinović[2]

Treatment with erythropoiesis-stimulating agents (ESA) is the optimal therapy for renal anemia. However, maintaining hemoglobin (Hb) within narrow targets remains a significant clinical problem because during ESA treatment, the Hb levels usually fluctuate widely; this phenomenon is termed "hemoglobin variability" and is associated with higher mortality. Our study aimed to determine the prevalence and cause of hemoglobin variability in patients on chronic hemodialysis (HD) treatment and to estimate the association of Hb variability with all-cause mortality.

A prospective study was conducted on 193 chronic HD patients treated with ESA. Hemoglobin cycling was defined as Hb variability throughout at least eight weeks and amplitude of more than 1.5 g/dl from the Serbian target range of 10-11 g/dl.

During the one-year follow-up, there was 5.6 ESA dose modification per patient. 23.4% of patients had never experienced Hb cycling during the study period. The total number of 460 hemoglobin excursions were recorded in 76.6% of patients, with 2.42 ± 2.7 Hb excursions per year, mean amplitude of 2.13 ± 0.76 g/dL, and the average length of Hb excursion of 8.2 ± 2.7 weeks. The Hb cycling was not affected by the gender, age, weekly ESA dose, or the presence of diabetes or hypertension. However, Hb variability was associated with ESA dose change, CRP, and HD vascular access type. The odds ratio for 1-year all-cause mortality was 1.424 (95% CI: 1.231–1.682, P < 0.001).

Hemoglobin cycling frequently occurs in ESA treated HD patients as a result of current practice in ESA dosing, the presence of infection, and the type of vascular access for HD and these fluctuations predicted overall mortality.

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Key words: *hemoglobin variability, erythropoiesis-stimulating agents, hemodialysis*

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Introduction

Anemia is a common complication that is associated with adverse cardiovascular complications and poor outcomes in patients with chronic kidney disease (CKD) (1). The introduction of erythropoiesis-stimulating agents (ESA) has revolutionized the management of anemia in CKD, leading to substantial reductions in the blood transfusion requirements, improvement in energy, and physical function (2) and improvements in health-related quality of life (3). Even though the optimal target hemoglobin (Hb) concentration in hemodialysis (HD) patients continues to be a substantial dilemma, the European Best Practice Guidelines (EBPG) recommended that the target hemoglobin level should be determined on an individual basis, having in mind gender, age, ethnicity, activity, and comorbid conditions (4). Nevertheless, with the publication of CREATE and CHOIR studies, both the upper and lower limits for target Hb concentration was set to 10-12 g/dL (5, 6) as it was shown that targeting normal Hb levels did not result in better survival, but rather in increased cardiovascular events and mortality in HD patients. Keeping patients' Hb levels in such a narrow range is difficult considering the loss of physiological regulation of red cell generation and many other factors, such as iron deficiency, chronic inflammation, secondary hyperparathyroidism, malnutrition, and inadequate dialysis dose. The data confirm that only 30% of patients will belong to this hemoglobin range at any point in time because fluctuations in the Hb level result in frequent under-and overreaching the target level (7). This phenomenon is known as Hb

variability, and it is defined as repeated, cyclical, up and down movements of absolute Hb levels during ESA treatment. It is speculated that Hb variability may influence patients' survival. A few authors hypothesized that Hb variability increase mortality risk since fluctuations in Hb might affect oxygen delivery to tissues, thereby resulting in end-organ damage. Over the last decade, significant consideration has been given to the variability in Hb levels for dialysis patients. Several population-based studies investigating Hb fluctuation have been performed to date, but the results are controversial (7-10).

The aims of the study were to assess the prevalence and causes of Hb variability in hemodialysis patients and to estimate all-cause mortality depending on hemoglobin cycling in light of the Serbian regulatory restrictions in renal anemia management with lower target hemoglobin range of 10-11 g/d.

Patients and methods

This prospective study was carried out at the Nephrology Clinic of the Clinical Center Niš, Serbia from January 2015 to February 2016. The study was conducted in accordance with the Declaration of Helsinki for medical research. We included 193 stable patients over 18 years with end-stage renal disease who have been undergoing treatment with repeated hemodialysis for longer than three months. Patients with acute illness, malignancy, or active inflammatory diseases were excluded.

Baseline data including demographic characteristics, dialysis vintage, dialysis parameter (Kt/V), anthropometric parameters (body height, body weight), dose, type and regimen of ESA, hematological, and biochemical analyses as well as data on selected comorbidities. Hematological parameters were analyzed on Nihon Kohden Hematology Analyzer; biochemical data were measured on Siemens Dimension RXL Max Chemistry Analyser while serum measurement of PTH was done on Roche Cobas e411.

During the study period, anemia syndrome was corrected by subcutaneously applied ESA, which was used according to the current recommendations of the European Best Practice Guidelines and Health Insurance Fund of Serbia's policy for renal anemia treatment in HD patients. The nursing staff administered ESA into the left or right upper arm during the regular hemodialysis sessions. As intravenous ESA application requires higher doses, all our patients were on a subcutaneous dosing regimen.

Erythropoiesis-stimulating agents were started when the Hb level was below 10 g/dL. The starting and maintenance doses of erythropoietin alpha/beta were 50–150 and 25–75 μ/kg/week and of darbepoetin 0.25–0.75 and 0.13–0.35 μ/kg/ week. The erythropoiesis-stimulating agent was stopped after achieving an Hb level of 11 g/dL. Two hundred units of r-HuEPO is equivalent to 1 μg of darbepoetin, so we converted darbepoetin unit accordingly.

Initially, rHuEPO was administered three times a week to achieve target concentrations of Hb 10-11 g/dL while maintaining the obtained target Hb levels by an individual approach (hold, keep unchanged, increase or decrease the dose on a monthly basis). ESA therapy was stopped when hemoglobin level reached 12 g/dl. Intravenous iron sucrose was prescribed if ferritin was < 100 μg/L, or the transferrin saturation (TSAT) was < 20%, and Hb was below the target range. Patients received 100 mg intravenously over each of the next 10 HD treatments, and then every two weeks thereafter. Iron was withheld if ferritin was > 800 μg/L, or the TSAT was > 50%.

As per Fishbane and Berns (11), hemoglobin variability characterizes the fluctuation of hemoglobin above or below the target range over time. In our study group, Hb cycling was defined as an oscillation in Hb of ≥ 1.5 g/dl over > 8 weeks during which Hb levels increased or decreased and then reversed the initial trajectory in relation to target Hb of 10-11 g/dL. Patients were divided into two groups: without Hb fluctuation and with Hb fluctuation. The outcome measure was all-cause mortality during the twelve months follow-up.

The following laboratory parameters were monitored over the period of 12 months after inclusion: hemoglobin (Hb-g/L) monthly, hemodialysis dose (KT/V), TSAT (%), C-reactive protein (CRP-mg/L), ferritin (ng/ml) and serum albumin (g/L) at 3 months and parathyroid hormone (PTH-pg/ml) at 6 months prior to the mid-week hemodialysis session in the first week of the month. TSAT was calculated as the ratio of serum iron to total iron-binding capacity (TIBC). All laboratory values were measured by automated and standardized methods.

Statistical analysis

Continuous variables were presented as mean ± standard deviation (SD), and categorical variables were presented as number (N) or percentage (%). The Student's t-test was used to compare two groups of data (if there is a normal distribution of frequencies within the group), or the non-parametric Mann-Whitney Rank Sum test is used if the frequency distribution is uneven. Logistic regression analysis was performed to identify independent risk factors for Hb-variability. A Kaplan–Meier analysis was used to examine the effects of hemoglobin variability on all-cause mortality. P-value < 0.05 was considered as statistically significant. Statistics were generated using SPSS version 21.

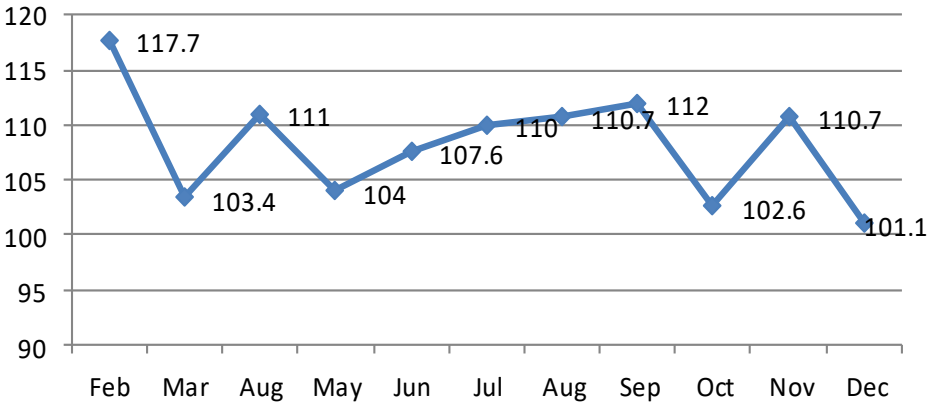
Results

The study included 193 stable ESRD treated with hemodialysis. The mean age of the patients was 63.88 ± 12.51 years, 61.1% of patients were male, dry weight was 64.3 ± 12.8 kg, and dialysis vintage was 66.67 ± 55.18 (range 14.9–284.1) months. Most of the patients (71.1%) suffered from hypertension, and 25.90% from diabetes. The characteristics of the participants are summarized in Table 1.

Table 1. Basic demographic, hemodynamic, anthropometric and biochemical characteristics of the HD subjects treated with ESA

Age (years)	63.88 ± 12.51
Men (%)	118 (61.1%)
Dialysis vintage (months)	66.67 ± 55.18
Arteriovenous fistula (%)	81.2
Kt/V	1.35 ± 0.64
Dry weight (kg)	64.3 ± 12.8
Interdialtic weight gain (kg)	2.9 ± 2.3
Body Mass Index (kg/m^2)	25.1 ± 3.2
Systolic blood pressure (mmHg)	135.2 ± 30.9
Diastolic blood pressure (mmHg)	91.7 ± 13.1
Hypertension (%)	135 (71.1%)
Diabetes mellitus (%)	50 (25.90%)
Hb (g/l)	103.17 ± 5.04
Iron (mmol/l)	13.7 ± 12.9
TIBC (µmol/L)	38.7 ± 20.4
Transferrin saturation (%)	32.6 ± 10.6
Ferritin (ng/mL)	246.1 ± 135.8
Cholesterol (mmol/l)	4.9 ± 1.5
Triglycerides (mmol/l)	2.6 ± 1.9
LDL-C (mmol/l)	3.1 ± 0.4
HDL-C (mmol/l)	1.6 ± 0.9
s. Albumins (g/l)	32.07 ± 4.37
Total protein (g/l)	66.78 ± 7.54
s. Creatinine (µmol/l)	809.64 ± 356.22
CRP (mg/l)	9.96 ± 7.55
PTH (pg/ml)	446.30 ± 294.81

Abbreviation: Hb, hemoglobin; TIBC-total iron binding capacity; LDL-C, low density cholesterol; HDL-C,high density cholesterol; CRP, C-reactive protein; PTH, parathormone.



Graph 1. Average monthly Hb level within HD study population

Graph 1 displays the mean hemoglobin values during one-year follow-up. Considering the whole study population, mean Hb was maintained within the target range most of the time.

However, apparently stable mean Hb levels in an overall study population can hide the occurrence of intra-individual variability in many patients which is shown in Figure 1.

Narrow hemoglobin target values resulted in frequent dose adjustments including the ESA withdrawal and consequent substantial hemoglobin fluctuations. During the one-year follow-up, there were an average 5.6 ESA dose changes, and 61% of patients had ≥ three dose changes (Graph 2). Regarding the number of hemoglobin cycling episodes, it was noticed that only 23.4% of patients had never experienced hemoglobin cycling during the study period.

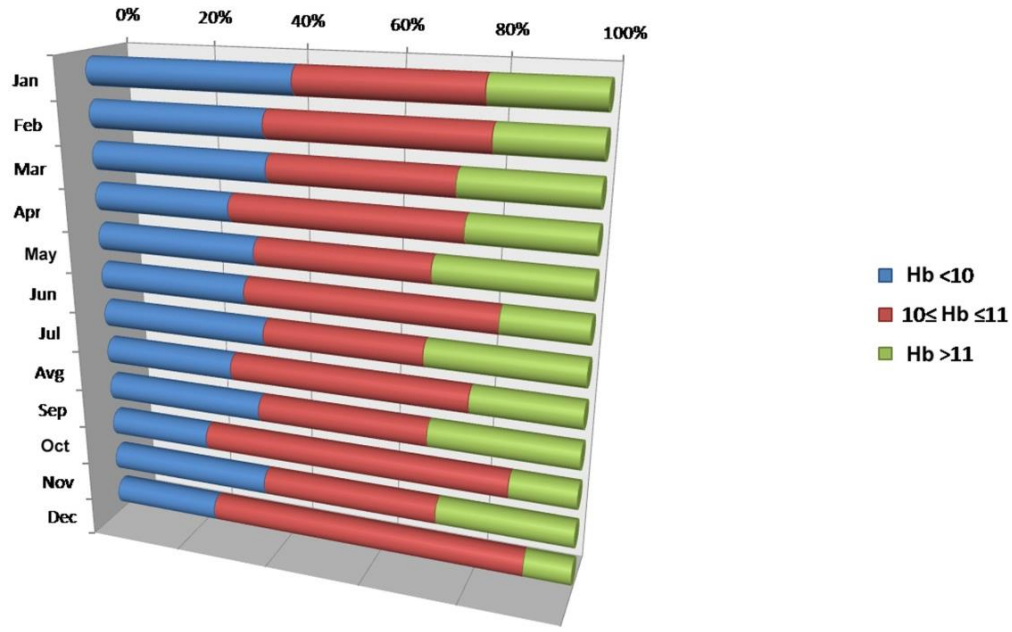
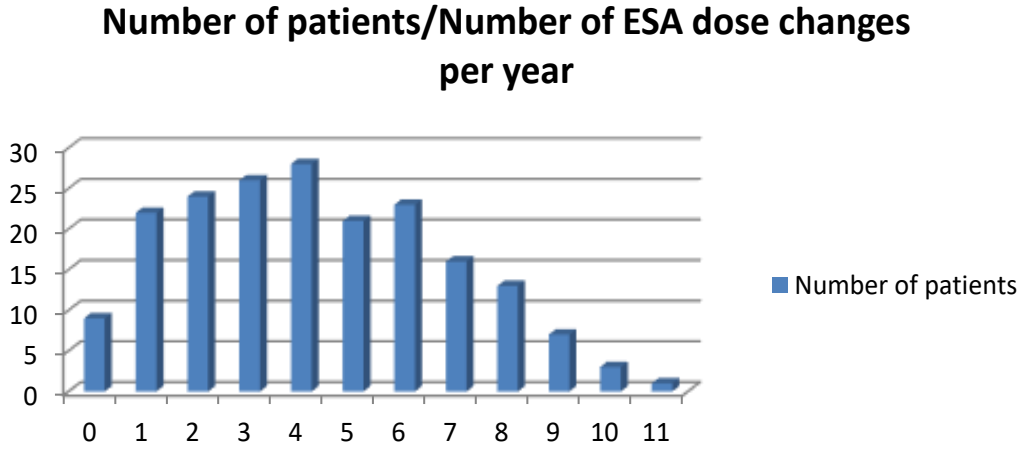


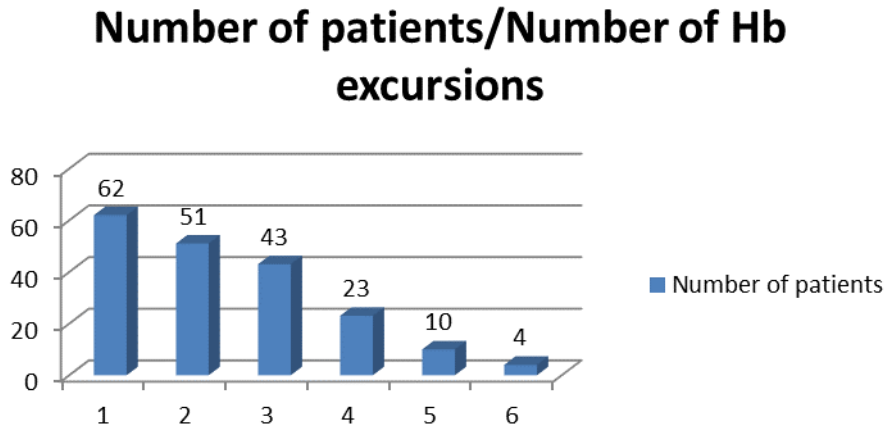
Figure 1. Distribution of monthly Hb levels in HD patients: below/within/above target percentage bar plot



Graph 2. Number of ESA dose changes per year

Simultaneously, out of 193 patients, hemoglobin excursions were recorded in 145 (76.6%). These patients experienced a mean of 2.42 ± 2.7 Hb excursions (defined as half of one full Hb cycle) per year, and the mean amplitude of excursions was 2.13 ± 0.76g/dL, while the average length of hemoglobin excursion was 8.2 ± 2.7 weeks. A total of 460 hemoglobin excursions were documented. Regarding the fluctuating pattern in hemoglobin levels over time, we recorded 188 excursions with Hb above the expected value (Hb values > 125g/dl) and 272 downward Hb excursions with Hb values < 85g/dl (Graph 3).

Finally, the study population was divided into two groups according to the presence or absence of Hb cycling during the follow-up time (Table 2).



Graph 3. Number of Hb excursions in HD patient treated with ESA

Table 2. Characteristics of patients with or without hemoglobin cycling

	Patients with Hb variability	Patients without Hb variability	p
Number of patiets	148	45	
Gender (males %)	60.7	61.7	0.578
Age (years)	61.32 ± 11.42	63.71 ± 12.02	0.612
AVF (%)	78.74	91.57	0.002
HD vintage (months)	59.76 ± 62.27	58.04 ± 55.13	0.05
DM (%)	19.2	21.8	0.246
HTA (%)	65.12	69.33	0.065
Frequency of ESA dose change	4.22 ± 2.44	2.95 ± 2.58	< 0.001
ESA dose (IU/kg/week)	70.08 ± 47.37	63.37 ± 55.52	0.822
Hb (g/dl)	101.53 ± 10.39	104.87 ± 4.57	0.004
Feritin (ng/mL)	639.37 ± 488.51	531.62 ± 319.78	0.003
TSAT (%)	30.82 ± 13.87	35.46 ± 32.87	0.758
CRP (mg/l)	14.32 ± 2.05	7.14 ± 2.4	< 0.001
Kt/V	1.31 ± 0.4	1.35 ± 0.2	0.17
PTH (pg/ml)	573.8 ± 496.2	241.6 ± 319	0.002

Abbreviation: Hb, hemoglobin; HD-hemodialysis; DM-Diabetes mellitus; HTA-hypertension; ESA erythropoiesis stimulating agents; TSAT-transferrin saturation; CRP, C-reactive protein; PTH, parathormone.

The change in Hb level was not affected by the gender, age, weekly ESA dose or the presence of diabetes or hypertension. However, the frequency of ESA dose change ($p < 0{,}001$), inflammation ($p < 0.001$), type of vascular access ($p = 0.002$) and secondary hyperparathyroidism ($p = 0.002$) significantly influenced hemoglobin variability.

Six variables with the highest correlation coefficient in the univariate analysis were included in the multiple linear regression analysis to determine the significant predictors of Hb variability. The results show that Hb variability was associated with ESA dose change (OR 1.56;95% CI 1.29–2.04, $p < 0.001$), CRP (OR 1.73; 95% CI 1.22–1.99, $p < 0.001$) and vascular access type (OR 2.13; 95% CI 1.56–3.18, $p = 0.033$) (Table 3).

Of 193 patients 31 of them (16.6%) died in 1A 2 month study period. The effects of hemoglobin fluctuation on mortality were evaluated by logistic regression analysis. Full adjustment was made with variables such as age, vascular access type, CRP, Hb, dialysis vintage, diabetes, hypertension and ESA dose.

The hazard ratios in HD patients were 1.458 (95% CI: 1.304–1.771, $p < 0.001$) for 6-month all-cause mortality, 1.424 (95% CI: 1.231–1.682, $Pp < 0.001$) for 1-year all-cause mortality after full adjustment (Table 4).

The cumulative 12-months survival rates of the two groups were statistically significant according to the Kaplan–Meier curve ($p < 0.001$ by log-rank test) (Graph 4).

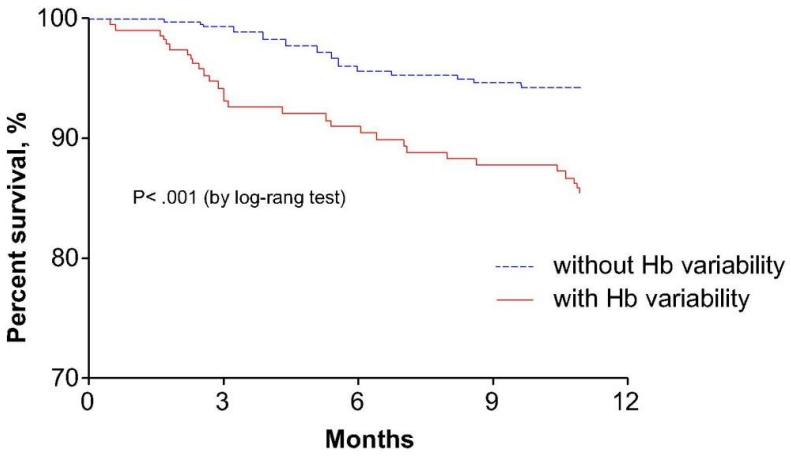
Table 3. Independent predictors of hemoglobin variability determined with logistic regression model

Variable	OR	95% Confidence Interval	p
ESA dose changes per patient (n)	1.56	(1.29 – 2.04)	< 0.001
CRP	1.73	(1.22 – 1.99)	< 0.001
Vascular access type	2.13	(1.56 – 3.18)	0.033

Table 4. Prediction of mortality according to Hb variability in HD patients***

	Age adjusted OR (95% CI)	Fully Adjusted OR (95% CI)***
6-months mortality	1.570 (1.341 – 1.897)‡	1.458 (1.304 – 1.771)‡
12-months mortality	1.420 (1.227 – 1.551)‡	1.424(1.231 – 1.682)‡

Adjusted by age, vascular access type, CRP, Hb, dialysis vintage, diabetes, hypertension and ESA dose, CI, indicates confidence interval; HR, hazard ratio; OR, odds ratio. ‡$p < 0.001$



Graph 4. The Kaplan Meier curve for the cumulative 12-month survival rates between patients with and without Hb variability. Log-rank test shows significant difference between two groups statistically.

Discussion

Keeping the constant Hb levels is obligatory to ensure continuous and sufficient oxygen delivery to tissues. In healthy subjects, individual variation in the Hb level occurs within the range of normal values, usually does not exceed 1 g/dL and have no clinical significance. However, for hemodialysis patients, substantial variability in the Hb level over time is not uncommon. Fluctuations in the Hb levels provoke repeated episodes of relative ischemia and tissue hypoxia, which may result in organ dysfunction or injury (10).

The key finding of this study is confirming that Hb cycling frequently occurs in hemodialysis patients. Over a one-year period, only 25% of patients using ESA had stable Hb levels within a target range of 10-11 g/dL. This reflects the difficulty of maintaining Hb levels within a narrow range as recommended by the most recent guidelines and hemoglobin management still remained a substantial challenge in the care of hemodialysis patients, with almost all patients moving between categories over fairly short time periods. The finding that patients receiving ESA had high variability agrees with previous studies and points to the current practice of prescribing ESA as one of the causes of Hb variability (12-14). The other possible factors that might affect patients' Hb variability were summarized in a review by Kalantar-Zadeh and Aronoff (15). These authors concluded that drug-related factors, patient demographics, iron deficiency, infections, inflammation, malignancies, and reimbursement-related factors all had an impact on Hb variability. Of these multiple factors, the ESA dose was the most actionable factor in the management of anemia for patients on dialysis therapy.

In the present study, we observed three major determinants of Hb fluctuation. The first was a frequent change in ESA dose. A positive correlation was seen between ESA dose change and amplitude of Hb excursion, implying that dose changes were causal, rather than reactive. That finding has also been published by others (16) and strongly implicates current dosing strategies and anemia management protocols in the pathogenesis of Hb cycling. Interestingly, compared with dose increases, dose reductions seemed to be a stronger predictor of cycling. We noted 272 downward Hb excursions. The Hb decline was mostly the consequence of ESA withdrawal (in 78.3% of cases) and dose reduction in 15%.

Evidence suggests that inflammation is an important factor associated with Hb variability. In a retrospective study of 225 hemodialysis patients, high CRP values were associated with less stable Hb levels (17). Likewise, Barany et al. reported a significant correlation between Hb variability and CRP levels (18). Similarly to these findings, we observed that higher CRP values significantly influence Hb variability. These results provide supporting evidence that inflammation can trigger hemoglobin variability. Thus, ESA dosage should be regularly reviewed, and patients should be monitor closely in the presence of inflammatory conditions.

Whereas the weekly dose of ESA was comparable regardless of the vascular access used, the weekly dose of ESA used in the patients with central vein catheter (CVC) was significantly higher than that used in those with AVF. This observation is consistent with other studies that indicate that CVC use as vascular accesses is associated with the need for higher doses of ESA secondly to blood loss during dialysis and possible catheter-related infections (19). Besides, the type of vascular access had an impact on Hb variability, possibly via intercurrent inflammation.

Studies about the clinical significance of Hb variability have been increased but results were conflicting. Regidor et al. (20) noticed that patients with Hb fall greater than 2 g/dL had the greatest mortality risk when compared with patients who showed Hb fall lower than 0.8 g/dL. In a cohort of 34,963 prevalent HD patients, Yang et al. (21) demonstrated that per every 1 g/dL increase of Hb variability, there is a 33% increase in mortality risk. On the contrary, Zeynep et al. found that hemoglobin variability has a modest association with morbidity and all-cause mortality in ESA treated dialysis patients (22). Persistently or transiently low Hb levels have also been associated with hospitalization and death (9, 23, 24, 25), as have downward Hb excursions (25). In our study, we likewise observed that Hb fluctuation was an independent determinant of mortality, which is in accordance with the recent study of Lin et al. (26). They also demonstrated that high Hb variability is an independent risk factor for cardiovascular mortality in HD patients and might influence the cardiac function.

Although the direct effects of Hb variation on patient outcome are still not fully understood, it is evident that large or frequent fluctuations are undesirable. Low Hb levels have a negative impact on symptoms and quality of life for patients; they also increase the requirement for blood transfusions. The myocardium may be particularly vulnerable to hemoglobin fluctuation because it has to compensate for periods of reduced oxygen delivery with increased output and myocardial cell growth. Hemoglobin levels higher than current target ranges may be associated with worse cardiovascular outcomes (7, 8), and higher Hb levels maintained with higher ESA doses have a significant cost implication. More frequent Hb fluctuations outside of target ranges require more clinician time to determine response in terms of ESA dose adjustment or of intravenous iron dosing.

Conclusion

Hemoglobin management remained a substantial challenge in the care of hemodialysis patients, with almost all patients moving between different hemoglobin categories over fairly short time periods. Our study demonstrates that both inflammation and the frequent changes of ESA dose were the major predictors of hemoglobin variability. The current ESA reimbursement practice demands constant adjustments of the ESA doses. The question is whether modification of treatment policies can contribute to reducing cycling and whether this influences the outcome. To answer this question, further studies are needed.

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PREVALENCIJA I DETERMINANTE VARIJABILNOSTI HEMOGLOBINA I NJEN UTICAJ NA MORTALITET KOD BOLESNIKA NA HRONIČNOM PROGRAMU HEMODIJALIZE

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Terapija agensima stimulacije eritropoeze (ASE) predstavlja optimalno lečenje renalne anemije. Međutim, održavanje hemoglobina (Hb) u okviru uskih ciljnih vrednosti ostaje značajan klinički problem, s obzirom na to da tokom primene ASE nivoi Hb obično značajno osciliraju; ovaj fenomen poznat je kao "varijabilnost hemoglobina", a udružen je sa povećanom smrtnošću bolesnika. Naše istraživanje imalo je za cilj da analizira učestalost i uzroke nastanka varijabilnosti hemoglobina kod bolesnika lečenih hemodijalizom (HD) i da proceni njen uticaj na mortalitet bolesnika.

Prospektivnom studijom obuhvaćeno je 193 bolesnika na hroničnoj HD, koji su lečeni ASE. Varijabilnost hemoglobina definisana je kao oscilacija koncentracije Hb u periodu od najmanje osam nedelja sa amplitudom većom od 1,5 g/dl od zadatih ciljnih vrednosti hemoglobina, koji u Srbiji za bolesnike na HD trenutno iznosi 10 g/dl — 11 g/dl.

Tokom jednogodišnjeg praćenja, bilo je 5,6 modifikacija doze ASE po bolesniku. 23,4% bolesnika nije imalo značajne oscilacije Hb tokom studijskog perioda. Ukupno 460 oscilacija (ekskurzija) hemoglobina zabeleženo je kod 76,6% bolesnika, sa 2,42 ekskurzije ± 2,7 ekskurzija godišnje, prosečne amplitude 2,13 g/dL ± 0,76 g/dL i prosečne dužine trajanja 8,2 nedelje ± 2,7 nedelja. Na osilaciju Hb nije uticala starost, pol, nedeljna doza ASE, kao ni prisustvo dijabetesa ili hipertenzije. Međutim, varijabilnost Hb zavisila je od promena doze ASE, CRP-a i tipa vaskularnog pristupa za HD.

Varijabilnost hemoglobina često se javlja kod bolesnika na HD lečenih ASE, kao posledica prakse učestalih promena doze ASE, prisustva infekcija i vrste vaskularnog pristupa za HD. Ove fluktuacije hemoglobina uticale su na povećanje mortaliteta kod naših bolesnika. Procena rizika za jednogodišnji mortalitet bila je 1,424 (95% CI: 1,231 – 1,682; P < 0,001).

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Ključne reči: *varijabilnost hemoglobina, agensi stimulacije eritropoeze, hemodijaliza*

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THE JUSTIFICATION OF CLINICAL PHARMACY SKILLS AND KNOWLEDGE FOR MODERN COMMUNITY PHARMACIST

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The course of pharmacy education has undergone a radical change as it aims to become more patient oriented. The primary objective of this study was to assess the association between clinical pharmacy education and daily activities of the community pharmacist (CP) including recognition and solution of drug-related problems (DRP). Furthermore, we wanted to investigate CPs' attitudes regarding knowledge and skills that can be required for routine community pharmacy practice. The simple questionnaire was formed to evaluate the significance of the implementation of clinical pharmacy course in the education of CPs. The questionnaires were sent by post or email or they were provided directly by one of the researcher. Data acquired from 234 CPs were divided into two groups: clinical pharmacy education group (CPEG) and non-clinical pharmacy education group (NCEG). The most frequent DRP recognized by respondents (CPEG and NCEG) were drug interactions, followed by suboptimal efficacy of the treatment and inappropriate dosage selection. Additionally, CPEG statistically more frequently than NCEG recognized low adherence ($p < 0.05$), while NCEG more frequently recognized inappropriate dosing interval ($p < 0.05$) and omitted drugs, that should have been prescribed ($p < 0.05$). The respondents agreed that knowledge of drug therapy, therapeutic planning skills and critical evaluation of drug information skills were the most important clinical pharmacy skills and knowledge required for modern community pharmacy practice. Still, CPEG gave advantage to the knowledge of laboratory and diagnostic skills compared to NCEG ($p < 0.05$). This study indicated that clinical pharmacy education can move the focus of the CPs towards a patient, but still positions drug in the center of their activity. The pharmacists with the clinical pharmacy education considered the knowledge of laboratory and diagnostic skills to be of significant importance, which confirms the ongoing change in pharmacist orientation.

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Key words: *clinical pharmacy, pharmacy practice, community pharmacy, pharmacy education*

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Introduction

A community pharmacist (CP) has numerous responsibilities regarding patients and their medications in everyday pharmacy practice. A medication review is assumed to be the first activity, which is done by the pharmacists during their routine work in the pharmacy (1, 2). This activity implies recognition of potential or existing drug-related problems (DRP), such as no indication for a drug, the choice of drug, formulation of a drug, dosage regimen, drug interactions, adverse effects and drug's contraindication. Pharmacists' roles are evolving and therefore they are meant to deal not only with DRPs, connected to drug per se, but also with patients' adherence, quality of life, pharmacoeconomy issues and giving an appropriate and acceptable solution and a piece of advice as well (2, 3). Furthermore, the increasing number of new medications and dietary supplements requires critical evaluation, processing and sharing the information regarding that novelty from the pharmacists towards other healthcare professionals and patients (3, 4). To sum up, this all represents a real challenge for the modern pharmacist, which requires multidisciplinary skills and knowledge in order to handle it. Skills and knowledge that can be acquired in undergraduate or postgraduate course of clinical pharmacy, seem to be important and helpful for accurate, effective and prompt response to

defined problems (5, 6). The course of pharmacy education has undergone a radical change as it aims at become a more patient oriented profession (7). Therefore, required knowledge and skills of a modern CP are communication skills, knowledge of drug therapy, nondrug therapy and complementary medicine, knowledge of the disease, laboratory and diagnostic skills, physical assessment skills, therapeutic planning skills and critical evaluation of drug information skills (8, 9). The successful communication seems to be the crucial regarding patient counseling, a leading tool in everyday pharmacy practice (10, 11). Additionally, academic pharmacy program can develop stronger collaborative relationships with practice sites, resulting in access to diverse patient care environments (12). Lots of information available nowadays enables patients to be more involved in their treatments, which gives an emphasis on the self-care/self-medication and consequently CP involvement. Self-medication risks may be severe unless they are controlled by a healthcare professional, who can notice potential harmful effects in appropriate time frame (13, 14). Also, there is always an urge of the patients to solve their problem as soon as possible in an acceptable manner, which makes community pharmacy first place to step in. The healthcare system needs an emphatic CP, who possesses an active knowledge and could provide pharmaceutical care for achieving this goal (15-17).

The aim of this study was to assess the association between clinical pharmacy education and daily activities of the CP including recognition and solution of drug-related problems. Furthermore, we wanted to investigate CPs' attitudes regarding knowledge and skills that can be required for routine community pharmacy practice.

Novelty of the Work

- This article indicated that clinical pharmacy skills and knowledge should have more important role in the curriculum of undergraduate studies of pharmacy and continuous education for community pharmacist as well.
- The course of clinical pharmacy may shape patient-focused pharmacist, who should be able more effectively to recognize and solve drug-related problems in everyday community pharmacy practice.
- The skills acquired through clinical pharmacy course will improve inter-professional co-operation, but will not transform pharmacist into physician.

Respondents and methods

The investigation was performed among CPs in the region of southeastern Serbia. The simple questionnaire was formed to investigate the significance of the implementation of clinical pharmacy course in education of the CPs. The questionnaire contained questions regarding demographic characteristics (gender, age) of the CPs and their education (clinical pharmacy course or not), the frequency of DRP recognized by CP and pharmaceutical interventions provided to handle DRP and pharmacists' attitudes about required knowledge and skills for community pharmacy practice. The research was conducted between January and March 2015. Data were acquired from 234 Serbian CPs affiliated to Pharmaceutical Chamber of Serbia. The sample represents 22.7% of all CP in the region of southeastern Serbia (1030 members, data were reviewed on 24.03.2016). The pharmacists were supposed to voluntarily fill and return the questionnaire in two weeks. The questionnaires were sent to 282 CPs by post or email or they were provided directly by one of the researcher as well. Of all sent questionnaires, 238 were returned, but only 234 were valid for statistical analysis and included into study. Respondents were divided into two groups based on their education of clinical pharmacy: clinical pharmacy education group (CPEG), who had undergraduate or postgraduate education in clinical pharmacy and nonclinical pharmacy education group (NCEG), who did not have any previous education in clinical pharmacy. The chi-square test was used to compare data between CPEG and NCEG. All analyses were performed with SPSS statistical analysis software, version 16.0 (SPSS, Chicago, IL, United States). The significance level was set at $p < 0.05$.

Ethics approval: This type of study does not have ethics approval due to it was conducted on voluntary basis.

Results

Demographic data regarding respondents and number questionnaires are given in Table 1.

The results showed that high percentage of CPs (84.4%) were willing to participate in this research. Female pharmacists were significantly more involved in the survey, with 90.6%, while the CPEG contributes with 45.3% in the research.

The results of this study demonstrated that almost 40% of the CPs reported DRP and provided interventions more than three times a week, while 53% of the respondents recognized DRP once a week (Graph 1).

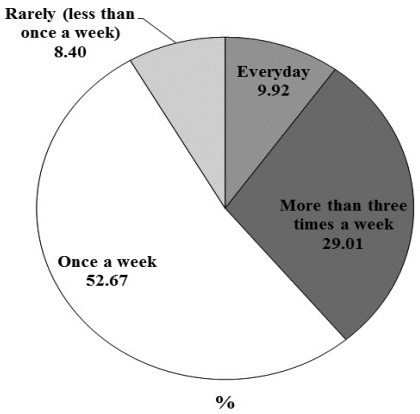
Graph 2 shows that the most frequent DRP were drug interactions, followed by suboptimal efficacy of the treatment and inappropriate dosage selection. Additionally, inappropriate drug selection and inappropriate dosing interval selection had high percent share in pharmacists' answers. Conversely, low adherence was present with a very small percentage.

Deffined groups of pharmacist (CPEG and NCEG) showed significant difference in frequency of the recognized DRPs (Graph 3). Both groups pointed out drug interactions (17% vs. 14%) suboptimal efficacy of the treatment (14% vs. 12%) and inappropriate dosage selection (12% vs. 13%) as the most significant, but CPEG statistically more frequently than NCEG recognized low adherence (6% vs. 3%, $p < 0.05$), while NCEG more frequently recognized inappropriate dosing interval (8% vs. 12%, $p < 0.05$) and omitted drugs, that should have been prescribed (2% vs. 5%, $p < 0.05$).

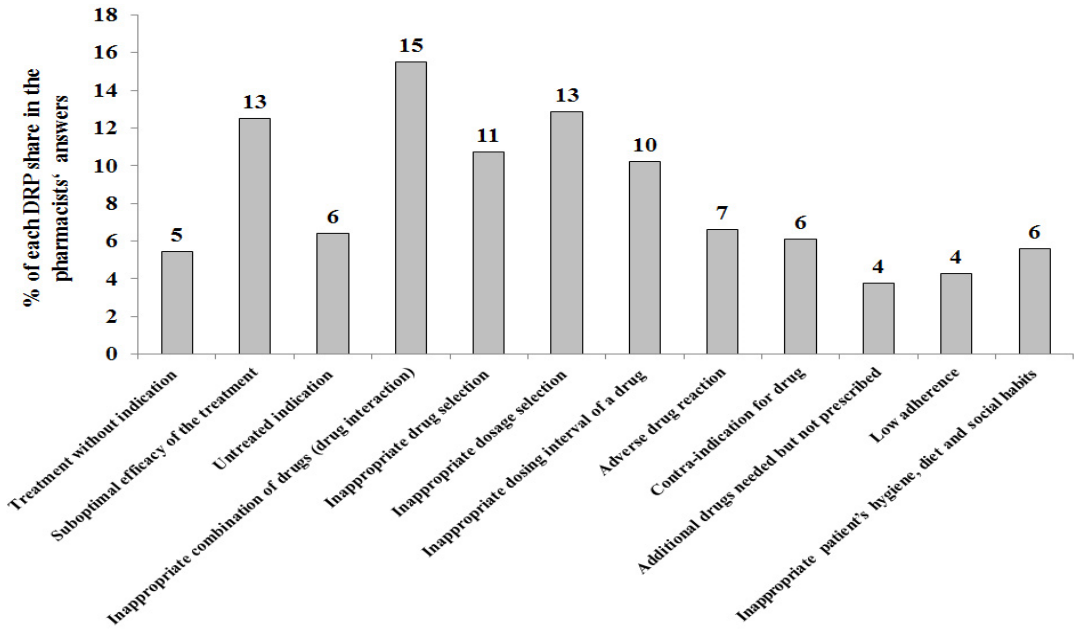
Table 1. Statistical data regarding respondents and questionnaires

	Number	Percentage
Questionnaires (Returned/Sent)	238/282	84.4/100
Questionnaires (Valid/Total)	234/238	98.3/100
Respondents (CPEG / NCEG)	106/128	45.3/54.7
Gender (M/F)	22/212	9.4/90.6
Age	Years (median [range])	
CPEG	27 (25-45)	
NCEG	36 (29-60)	

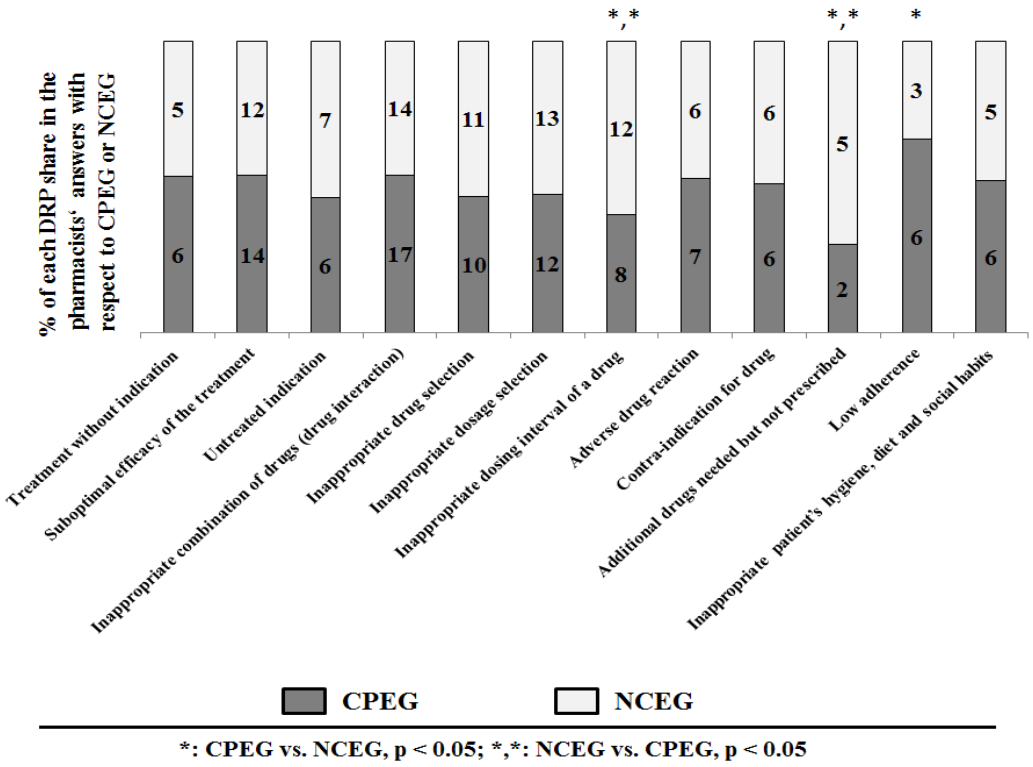
CPEG –group of community pharmacists with undergraduate or postgraduate education in clinical pharmacy; NCEG – group of community pharmacists with no prior education in clinical pharmacy



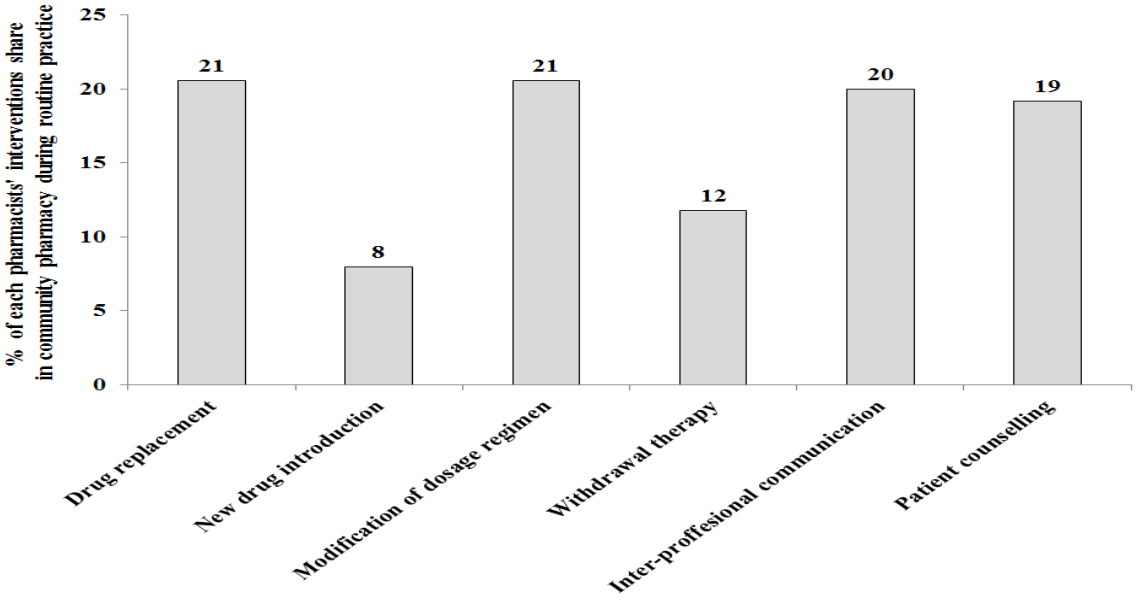
Graph 1. Frequency of recognized DRP and pharmaceutical interventions



Graph 2. The DRP recognized by CP (CPEG+NCEG)



Graph 3. The DRP recognized by CP in relation to previous clinical pharmacy education (CPEG vs. NCEG)



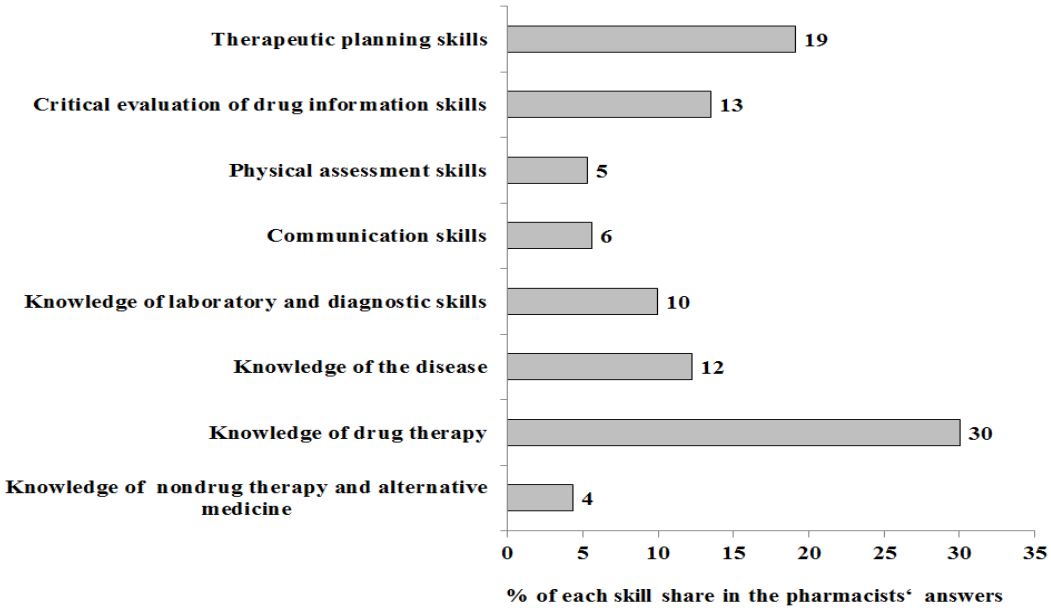
Graph 4. The frequency of pharmaceutical interventions provided by CPs

Graph 4 describes the pharmaceutical interventions provided by CPs. Drug replacement, modification of dosage regimen, interprofessional communication and patient counselling were almost equally provided.

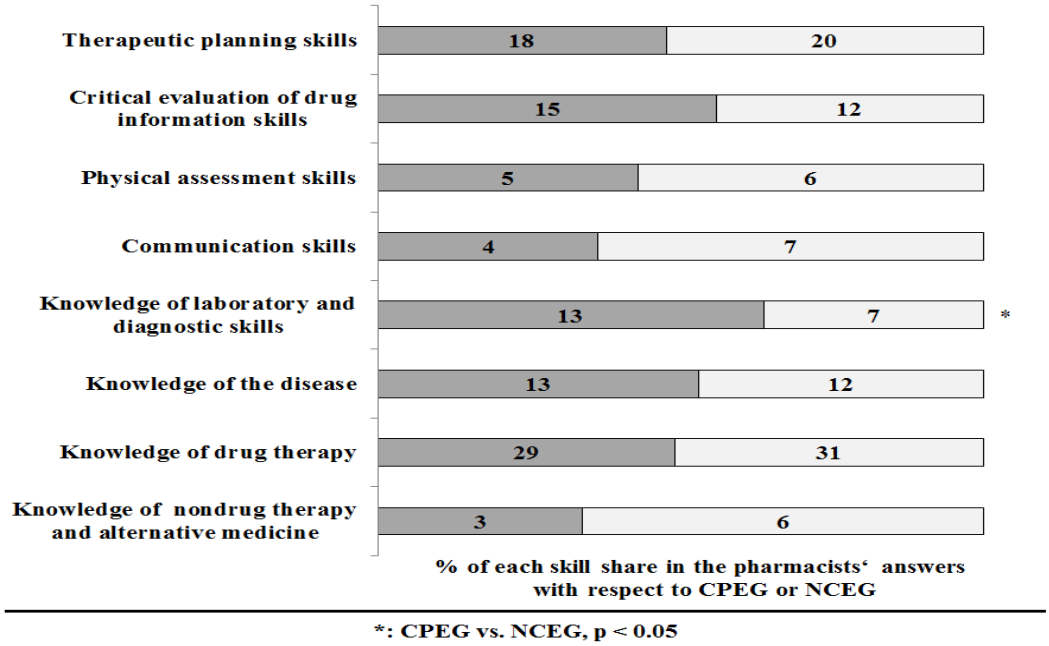
In order to recognize and solve DRPs pharmacists need certain skills and knowledge. Graph 5 shows CPs attitudes regarding required skills and knowledge for routine pharmaceutical practice.

The results of this study showed that knowledge of drug therapy (30%), therapeutic planning skills (19%) and critical evaluation of drug information skills (13%) were the most important with respect to CPs for everyday practice.

Still, CPEG gave advantage to the knowledge of laboratory and diagnostic skills compared to NCEG ($p < 0.05$) (Graph 6).



Graph 5. Pharmacists' attitudes regarding required skills and knowledge (CPEG + NCEG)



Graph 6. Pharmacists' attitudes regarding required skill and knowledge with respect to CPEG and NCEG

Discussion

The modern pharmacists realize that community pharmacy is a place which offers exchange of the information and experience, and therefore contributes to the better relationship and communication between patient and his pharmacist, which lead to patients' adherence improvement (2, 12, 16). The ongoing globalization impacts the pharmaceutical practice creating pharmacy competence framework (18). "How should pharmacists be educated to increase the quality of their practice - by improving their knowledge of drugs' pharmacology, or by improving their managing and communication skills?", is important question nowadays (11). Community pharmacy in Serbia is the place for drug dispensing, prescribing over the counter drugs, and counselling about rational pharmacotherapy and chronic noncommunicable disease as well. There was ultimate need to increase the number of pharmacists in the world (19, 20). In Serbia, CPs with a clinical knowledge and skills is even more needed healthcare profession, considering the fact that undergraduate courses of clinical pharmacy have existed less than a decade. According to the studies of Lakic at al., Serbia had less pharmacists per 100,000 inhabitants than Bulgaria, Croatia and Slovenia, countries from the same European region (19).

Besides the need for more pharmacists, the CPs require continuous improvement of knowledge and skill important for patient-focused approach in pharmaceutical practice. Recent researches confirmed the need for constant promotion of pharmaceutical knowledge and skills for problem solving interventions in everyday practice (21, 22). Accordingly, the results of this study showed insufficient frequency of the recognition of DRP and consequently pharmaceutical interventions among study enrolled CPs (Graph 1). These findings can be partly explained by the fact that clinical pharmacy course has existed less than a decade in undergraduate pharmacy studies in Serbia. Also, this indicates that ongoing reforms of pharmacy educational system should be more pronounced regarding knowledge and skills and most importantly these reforms should be more patient-oriented (23). The introduction of electronic evidence of pharmaceutical interventions will be a new tool for gathering information regarding pharmaceutical service in Serbia and may contribute to overall improvement of community pharmacy service.

The results of the conducted study showed that the most frequent DRPs among respondents were drug interactions, followed by suboptimal efficacy of the treatment and inappropriate dosage selection. The research, conducted in Ireland, showed that medication review and check of the physician's decision can also be useful screening tools that determined prescribing errors and it may reduce unnecessary medication in prescriptions, and adverse event (24). In accordance to these findings, an association was found between increasing number of drugs and supplements and the appearance of the drug interactions and adverse effects among the older population (25).

The obtained results indicated that whether pharmacist had clinical pharmacy education or not, DRPs, such as drug interactions, treatment efficiency and inappropriate dosage selection were noticed most frequently. Nevertheless, clinical pharmacy may transform CP to the more patient-focused health professional, who perceives a patient in its entirety. In our investigation CPEG recognized low adherence statistically more frequent than NCEG, while NCEG recognized more often inappropriate dosing interval and omitted medication as well. This (leads to the conclusion) explains that CPEG is more oriented towards patients' problems and their behaviour during prescribed treatment. Conversely, NCEG is more focused on medication review and medication itself, with less interpersonal communication.

According to Westerlund et al., CPs noticed that patients' incomprehension of the aim of the therapy might cause low adherence and suboptimal treatment efficacy (26). Considering this, community pharmacy should be the place, which can provide additional explanation about patients health problems and treatment-disease association in order to achieve better compliance with the patients and therefore desirable therapeutical outcomes (27, 28).

The most frequent pharmaceutical interventions were done on medication, including its replacement and modification of dosage regimen. This finding is in accordance with the results of other authors, who investigated the knowledge and skills of clinical pharmacy required in everyday practice. Tasaka et al., noticed that implementation of clinical pharmacy skills in pharmaceutical practice led to positive therapeutic and financial outcomes, which had benefit both for community and the patient (6). Additionally, interprofessional communication and patient counselling were also highly rated in routine practice of CP.

The results of this study reported that both groups had the same attitudes regarding required knowledge and skills, emphasizing that knowledge of drug therapy (30%) and therapeutic planning skills (19%) were the most important. This medication-focused approach was followed by critical evaluation of drug information skills (13%). Elliott et al. demonstrated that managing the therapeutic plan is one of the methods for better adherence (29). Still, pharmacists with previously acquired clinical pharmacy education gave a slight advantage to the knowledge of laboratory and diagnostic skills. It indicates that education of clinical pharmacy may cause the change from a medication- to a patient-focused pharmacist. Previous researches showed that CPs had less knowledge about signs and symptoms of the diseases, which was needed for appropriate pharmaceutical intervention and care of the patients as well (21, 30). This supports our finding that clinical pharmacy education may contribute to rational and timely decision regarding pharmacy-led interventions. The ultimate benefit of the introduction of these skills and knowledge can be demonstrated through improved patients' quality of life and reduced healthcare costs (18, 31). In accordance with this, the efforts on the national level have been made to improve Serbian pharmaceutical practice.

The most important improvement will be electronic documentation of pharmaceutical intervention towards patients.

Considering these results, it was shown that clinical pharmacy education and courses had a significant influence on forming of a modern CP with a patient in its focus. Still, pharmacist, both CPEG and NCEG highlighted the importance of drug therapy skills, which indicated that they would not cross their initial obligations and try to be physicians. This can represent a real background for interprofessional cooperation between pharmacist and the other healthcare professionals. The newly obtained knowledge and skills may help pharmacist to deal with his or her tasks in responsible manner and therefore to optimize pharmacotherapy and cooperate with physician in order to provide a maximum safety and benefit for the patient.

Conclusion

In conclusion, CPs deal with different DRPs and provide pharmaceutical interventions within their daily activity. The results of this study indicated that education of clinical pharmacy skills and knowledge can move the focus of the CPs, from medicine towards patient, but still position the drug in the center of their activity. Modern pharmacist with changed skills in his or her portfolio gives a huge contribution to interprofessional approach with a view to achieve desired health outcomes. Pharmacist with clinical pharmacy education were more familiar with the knowledge of laboratory and diagnostic skills, necessary for the risk therapy management, which confirms the ongoing change in pharmacist orientation. Therefore, it is desirable that clinical pharmacy course should be part of the undergraduate curriculum of pharmacy students or continuous medical education for experienced pharmacist as well.

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OPRAVDANOST STICANJA VEŠTINA I ZNANJA IZ KLINIČKE FARMACIJE TOKOM OBRAZOVANJA SAVREMENOG FARMACEUTA

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Savremeni farmaceut u apoteci suočava se sa brojnim zahtevima vezanim za pregled terapije i uočavanje problema vezanih za terapiju (drug-related problems – DRP) bolesnika. Pored poznavanja leka, farmaceut danas aktivno učestvuje u ukupnoj terapiji bolesnika, savetuje i pomaže rešavanju manjih zdravstvenih problema izborom leka u režimu izdavanja bez lekarskog recepta. Sledeći nove obaveze farmaceuta u apoteci, akademsko obrazovanje farmaceuta transformisano je u nameri da se fokus pomeri sa leka na bolesnika. Primarni cilj ovog istraživanja bila je procena povezanosti obrazovanja iz kliničke farmacije i dnevnih aktivnosti farmaceuta u apoteci (community pharmacist – CP), uključujući prepoznavanje i rešavanje DRP. Osim toga, cilj je bio i izvršiti analizu stavova CP-a u pogledu znanja i veština koje mogu biti korisne u svakodnevnom radu. Istraživanje je sprovedeno pomoću jednostavnog upitnika formulisanog za procenu značaja uvođenja veština kliničke farmacije u akademske studije. U istraživanju je čestvovalo 234 CP-a, koji su podeljeni u dve grupe: grupa sa edukacijom iz kliničke farmacije (CPEG) i grupa bez edukacije iz kliničke farmacije (NCEG). Najčešći DRP, koji su prepoznali ispitanici (CPEG i NCEG), su interakcije lekova, praćene suboptimalnom efikasnošću lečenja i neodgovarajućim doziranjem. Dodatno, CPEG su značajno češće uočavali nisku adherencu ($p < 0,05$), a NCEG neprikladan interval doziranja ($p < 0,05$) i lekove koji nedostaju, a mogli bi biti korisni u terapiji ($p < 0,05$). Ispitanici su se složili da su poznavanje lekova, veštine planiranja i rukovođenja terapijom i kritička procena informacija o lekovima najvažnije veštine i znanja iz kliničke farmacije potrebne za svako-dnevni rad u apoteci. Ipak, CPEG su dali prednost poznavanju laboratorijskih i dijagnostičkih veština ($p < 0,05$). Uočena razlika u važnosti poznavanja laboratorijskih i dijagnostičkih parametara potvrđuje pomeranje fokusa CPEG. Sprovedeno istraživanje pokazalo je da obrazovanje iz kliničke farmacije može pomeriti fokus CP-a ka bolesniku, ali i dalje sa lekom u centru farmaceutske aktivnosti.

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Ključne reči: *klinička farmacija, farmaceutska praksa, obrazovanje farmaceuta*

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DETERMINING THE CONTENT OF Cd, Cu, Pb AND Zn IN THE LEAVES OF DANDELION (*TARAXACUM OFFICINALE* WEBB.) AND IN THE SOIL BY ICP-OES

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Dandelion (Taraxacum officinale Webb.) is a plant capable of accumulating a certain quantity of metals. The aim of this study was to determine the content of Cd, Cu, Pb and Zn in dandelion leaves and soil that have been sampled from different locations. One group of samples has been influenced by pollution sources of these metals and the other one has not been exposed to the pollutants. The amount of metals in the tested samples has been determined by inductively coupled plasma optical emission spectrometry (ICP-OES).The content of detected metals was higher in leaves and soil samples that were exposed to the negative effect of environmental pollutants, compared to those samples that were not under the influence of contamination sources. The increased content of detected metals in the samples of dandelion leaves that were under the influence of the pollutant may be the result of a synergistic effect, soil, on which this plant species thrives, and the air, that is contaminated by the effects of motor traffic and other forms of pollutants. The results of this study have shown that dandelion can provide a data of environmental pollution by the content of detected metals in its tissue. As dandelion is used in human nutrition, and since heavy metals (Pb, Cd) with cumulative and toxic effects have been detected in it, it is necessary, in order to protect human health, to check the presence and content of these metals in the dandelion plant that is used in human nutrition.

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Key words: *dandelion, metals, soil, pollutants, ICP-OES*

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Introduction

The development and advance of technology, in all spheres of life, are processes that have been rapidly evolving since the industrial revolution in the 18th century. Today we are witnesses of a huge and constant development of technological and industrial processes that leads to a significant improvement in the life of an individual in all social aspects. Unfortunately, such an unstoppable advance, with all the benefits, also brings different negative effects, that can also cause very serious and unsolvable problems for all of the living beings on the planet. Global and local pollution of the environment that harms all the living organisms is definitely one of the biggest problems, and a direct result of constant industrial development of the human species.

Environmental pollutants are of organic and non-organic origin. They can have a bigger or smaller negative effect on the living world. In the big group of non-organic pollutants cadmium and lead must be pointed out. They are especially dangerous, as they are toxic, even in small quantities (1). An increased amount of metals in the environment, taking into consideration the fact that they are ecotoxicological danger, creates a need to find and develop economical chemical methods whose aim would be to detect the presence of metal in tested samples and discover the dangers that living organisms are exposed to because of these pollutants. One of the ways to detect these pollutants is to identify the organisms that, thanks to their ability to accumulate different metals, can give adequate information about the assessment of environmental contamination with these metals. These organisms that give quantitative data that is connected to the environment, for example, how much pollutants are present at the tested location. Every kind of organism that is distributed over a wider geographical area, that is toxitolerant to a large number of metals

and that can accumulate them, can be used for metal content determination. These accumulated metal contents reflect the contamination quantity when compared to monitored variables (2).

A large number of different plants shows the capability to accumulate various metals from the environment, and this characteristic qualifies them as adequate indicators of environmental pollution with some toxic metals. Herbaceous plants are capable of accumulating a significant quantity of different metals, and one of those is also dandelion (*Taraxacum officinale* Webb.). Dandelion is a plant species that produces new leaves every year. It is widespread in nature and farmland and one of its characteristics is that it is capable of accumulating traces of elements (2, 3). By reviewing the literature, it is possible to see that dandelion has been used in a number of studies as preferable plant species for determining the level of environmental pollution, based on the metal content in its tissue (4-9). Numerous studies have shown that there is a connection between the quantity of metals present in the tested part of the environment (soil, air, water...) and the detected amount of these elements in dandelion tissue (4, 5, 10). Detected quantities of metals in dandelion samples that have been collected at different distances from the metals pollution source (landfills, roads, industrial plants...) have shown that the amount of metals in the tissue of this plant depends on the distance from the pollution source. Those dandelion samples that have been taken near the pollutant had increased levels of metals compared to the samples taken from areas that haven't been exposed to them (2, 4, 8, 11).

Aim of the study

Taking into consideration these facts, the aim of this study was to determine the content of individual metals (Cd, Cu, Pb and Zn) in dandelion leaves that have been sampled at 6 locations on the territory of the city of Niš and that have been close to the pollution source with these metals (main city roads, a gas station). In order to compare the level of detected metals in dandelion leaves that have been sampled near the pollution source, the level of these metals has also been determined in dandelion leaves samples from 3 locations (local excursion spots) that haven't been impacted by motor traffic. Furthermore, since the amount of detected elements also depends on the chemical composition of soil where this plant grows, the amount of Cd, Cu, Pb and Zn has been determined in the soil from these locations. The amount of cadmium, copper, lead and zinc in the tested samples of soil and dandelion has been determined by inductively coupled plasma optical emission spectrometry (ICP-OES).

Materials and methods

Reagents and chemicals

- ICP multi-element standard solution (*Ultra scientific* (*USA*)), γ = 20.00 ± 0.10 mg/L;
- 65% nitric acid, p.a. (*Merck*, *Darmstad*);
- 36% hydrochloric acid, p.a. (*Merck, Darmstad*);
- 30% hydrogen peroxide (*Fluka*) and
- High purity water, conductivity 0.05 μScm-1(*MicroMed high purity water system, TKA Wasseraufbereitungssysteme GmbH*).

Vials of PVC material were used to store the test samples. In order to remove traces of possibly present metals, the vials were previously washed with 5% nitric acid, then tap water and finally with deionized water.

Instrumentation

The overall analysis was conducted by an iCAP 6000 inductively coupled plasma optical emission spectrometer (*Thermo Scientific, Cambridge, United Kingdom*) which combines an Echelle optical design and a charge injection device (*CID*) solid state detector. iTEVA operating software for iCAP 6000 series was used to control all functions of the instrument. The optimal operating parameters of instrument are given in Table 1.

Table 1. The optimal operating parameters for ICP-OES measurement

Parameter	
Flush Pump Rate	100 rpm
Analysis Pump Rate	50 rpm
RF power	1150 W
Nebulizer gas	0.7 L/min
Coolant Gas Flow	12 L/min
Plasma View	Axial mode

Analytical balance (*Mettler Toledo*) was used to measure the mass.

Samples mineralization was carried out in a VIMS electric (*Serbia*) furnace equipped with a microprocessor programmatic temperature control IVIGOS3123 (± 1 ^{O}C).

A pH meter (*Hanna Instruments, USA*) was used to determine the soil pH value.

Samples

The content of Cd, Cu, Pb and Zn was determined in leaves of dandelion (*Taraxacum officinale* Webb.), which were sampled from different localities in the area and surrounding of the city of Niš. Nine sites (marked 1-9) considered for this study are shown in Table 2. The leaves of dandelion plants of the same developmental stage, were cut with a plastic knife and than were placed in plastic bags. Samples of dandelion, grown near the streets, with high traffic intensity (samples 1 to 6) were taken at two different distances from the street, 1 m (a samples) and 5 m (b samples). In Table 2, the localities of dandelion samples that were not under the impacted to traffic, were marked from 7 to 9. The coordinates of all points from which the samples were collected were determined by GPS.

The soil samples were collected from the same locations as dandelion leaves samples. Soil samples were collected just at the bottom of each sampled plant by plastic spatula. Only the first 5 cm of soil was collected in the root area. Each soil sample was placed in a separate plastic bag and stored at a room temperature.

Table 2. Site identification and its coordinates

No.	Site	Coordinates
1	Street	N43°19'11.7300'' E21°54'0.0324''
2	Street	N43°18'57.9348'' E21°54'55.2816''
3	Street	N43°19'14.3940'' E21°54'1.0080''
4	Roundabout	N43°18'53.9172'' E21°53'52.6848''
5	Street near the railway	N43°18'56.5272'' E21°52'45.2064''
6	Street near the gass station	N43°19'0.9624'' E21°53'35.7720''
7	Park „Čair"	N43°18'0.4612'' E21°54'21.6720''
8	Park „Bubanj"	N43°18'15.4224'' E21°52'21.2160''
9	Natural Park „Kamenički vis"	N43°24'45.3168'' E21°56'5.1468''

Samples preparation

Mineralization of plant material

The plants leaves were cut with a plastic knife and stored in plastic bags. Then, they were washed with deionized water and left to dry in the air. After air drying, they were dried in a kiln at a temperature of 70 ^{O}C. The samples were then homogenized; a mass of 3.0 g of the sample was analyzed. For the detection of metals, the samples were prepared by a dry digestion method (12). Portion of each sample was then weighed and heated at a temperature of 600 ^{O}C for a period of 12 h in porcelain crucible. For complete mineralization of the samples, 1 ml of 65% HNO_3 was added; the samples were heated to dryness and then returned into the furnace. The obtained ash was digested in 3 ml of 65% HNO_3 and filtered through a Whatman No 541 tape in a 50 ml flask.

Pseudo-total cation content determination in soil samples

Soil samples were collected with a plastic ladle, in the immediate vicinity of the dandelion plant from a depth of 0-10 cm. All of the soil samples were spread on plastic trays in fume cupboards and allowed to dry at ambient temperature for 8 days. The pseudo-total amounts of Cd, Cu, Pb and Zn were determined by the digestion of the samples using HNO_3–HCl (aqua regia) by means of the conventional wet acid digestion method (13).

Soil pH value determination

The dry soil sample was homogenized in the spindle and diluted through the sieve 0.5 mm, then weighed 20 g and transferred to a 50 ml flask, then poured with 20 ml of deionized water. The sample than was covered with watch glass and mixed for 5 minutes, then left to stand for one hour. Subsequently, the suspension was filtered. Measurement of the pH value was carried out pH-metric (Hanna Instruments, USA), calibration of pH-meter was carried out with buffers of known concentrations (pH = 4 and pH = 7) (14).

Results

For the determination of the metal content in the samples of dandelion, the calibration method was used. The basic calibration parameters for each test element were determined using deionized water for the concentration of zero and standard solutions of the corresponding concentrations obtained by diluting the base reference multi-elemental standard. Parameters of the analytical calibration curves such as wavelength λ, limits of detection and quantification (LOD and LOQ), correlation coefficient R^2, intercept (b), slope (m), average RSD for repeatability of calibration solutions measurements are shown in Table 3.

Table 3. Parameters of the analytical calibration curves: wavelength λ (nm), limits of detection and quantification (LOD and LOQ, ppm), correlation coefficient (r), intercept (b), slope (m), average RSD (%) for repeatability of calibration solutions measurements

Element	λ (nm)	LOD (ppm)	LOQ (ppm)	r	b	m	RSD (%)
Cd	228.802	0.0828	0.2759	0.999946	0.76	1906	4.07
Cu	324.754	0.1181	0.3936	0.999889	46.67	6072	8.22
Pb	220.353	0.2191	0.7304	0.999619	0.09	153	0.87
Zn	202.548	0.0779	0.2598	0.999952	5.33	9825	1.98

The applied ICP-OES technique allows the element to be viewed at different wavelengths. All analytes were determined on four wavelengths. In this way, it is possible for each element to select the wavelength at which the spectral and matrix interferences are minimized (15). The optimal wavelength for each of the detected elements is determined by comparing the inclination of the corresponding calibration curves.

Table 4. The metal content (mg/kg) in dandelion leaves samples collected from selected locations

	The metal content ± SD			
Site	Cd	Cu	Pb	Zn
1*a*	0.25 ± 0.01	20.02 ± 1.23	0.48 ± 0.02	62.71 ± 1.56
1*b*	0.27 ± 0.02	24.11 ± 1.09	0.33 ± 0.03	58.16 ± 1.27
2*a*	0.22 ± 0.02	17.83 ± 1.12	0.39 ± 0.05	48.36 ± 1.38
2*b*	0.18 ± 0.01	19.22 ± 1.37	0.41 ± 0.03	52.88 ± 1.52
3*a*	0.22 ± 0.01	18.23 ± 1.03	0.52 ± 0.11	51.18 ± 1.09
3*b*	0.21 ± 0.01	21.12 ± 1.29	0.49 ± 0.08	49.35 ± 1.17
4*a*	0.29 ± 0.01	16.25 ± 1.11	0.54 ± 0.09	57.26 ± 1.27
4*b*	0.32 ± 0.02	14.32 ± 1.03	0.47 ± 0.10	56.27 ± 1.09
5*a*	0.36 ± 0.02	24.07 ± 1.01	1.84 ± 0.02	56.28 ± 1.86
5*b*	0.32 ± 0.01	19.72 ± 1.26	1.51 ± 0.09	48.39 ± 1.62
6*a*	0.49 ± 0.02	22.12 ± 0.98	2.03 ± 0.04	59.01 ± 2.41
6*b*	0.44 ± 0.03	24.52 ± 1.27	1.88 ± 0.12	56.58 ± 1.88
7	0.15 ± 0.02	9.72 ± 0.68	0.26 ± 0.04	44.68 ± 1.87
8	0.13 ± 0.01	11.14 ± 0.92	0.15 ± 0.03	34.08 ± 1.07
9	0.09 ± 0.02	9.25 ± 0.74	0.18 ± 0.03	36.12 ± 1.41

Table 5. The metal content (mg/kg) in soil samples collected from selected locations

	The metal content ± SD				pH
Site	Cd	Cu	Pb	Zn	
1*a*	0.41 ± 0.12	32.22 ± 2.08	22.34 ± 1.12	98.12 ± 2.80	7.61
1*b*	0.42 ± 0.13	33.16 ± 1.86	21.56 ± 1.23	96.20 ± 3.38	7.52
2*a*	0.50 ± 0.11	28.70 ± 1.12	32.65 ± 1.17	101.37 ± 3.22	7.38
2*b*	0.50 ± 0.12	29.10 ± 1.18	31.30 ± 1.45	100.04 ± 4.15	7.35
3*a*	0.38 ± 0.10	25.19 ± 2.12	27.75 ± 1.16	108.63 ± 3.60	7.56
3*b*	0.38 ± 0.15	24.66 ± 1.75	28.02 ± 1.04	107.22 ± 2.44	7.48
4*a*	0.46 ± 0.08	22.46 ± 1.11	25.85 ± 1.24	98.67 ± 5.17	7.60
4*b*	0.48 ± 0.13	24.20 ± 1.06	25.10 ± 1.05	99.04 ± 4.35	7.63
5*a*	0.60 ± 0.12	34.69 ± 1.09	28.55 ± 1.30	126.52 ± 3.20	7.52
5*b*	0.61 ± 0.15	35.26 ± 1.35	29.06 ± 1.12	125.20 ± 2.18	7.48
6*a*	0.67 ± 0.21	30.97 ± 1.71	38.28 ± 1.52	120.88 ± 5.17	7.28
6*b*	0.66 ± 0.17	29.56 ± 2.05	37.76 ± 1.13	121.02± 4.35	7.30
7	0.33 ± 0.11	8.58 ± 1.36	8.49 ± 0.09	92.13 ± 4.12	7.59
8	0.28 ± 0.09	12.71 ± 2.02	7.10 ± 0.14	75.76 ± 3.92	7.48
9	0.17 ± 0.02	9.05 ± 1.16	3.27 ± 0.16	53.52 ± 4.37	6.69

The obtained values of the content of the detected metals in the dandelion samples, sampled from different localities, as well as the corresponding standard deviations, are shown in Table 4.The contents of the detected metals are shown as the mean value (milligram per kilogram of the test sample, mg/kg) obtained for three successive measurements.

The soil is one of the major pathways for metal absorption by plants, for this reason, in this study, the soil underlaying the tested dandelion leaves samples was analyzed.

Availability of metals from soil for a plant, that is, its root, depends on the ability of metals to absorb, desorb and complex with one of the components in the soil matrix. These processes are conditioned by soil characteristics such as pH, composition and soil structure. Heavy metal mobility is higher in soil with a lower pH value.

The metal concentration in the soil samples and pH values are presented in Table 5. The contents of the detected metals are shown as the mean value (milligram per kilogram of the test sample, mg/kg) obtained for three successive measurements.

Discussion

Cadmium is an element that is not a part of any compound that has a metabolic significance, and also belongs to the most dangerous environmental pollutants. Plants adopt cadmium mainly through the root, because this heavy metal has great mobility through the soil on which the plant grows (3). It is considered that the normal Cd concentration in plant tissue is between 0.2 and 0.8 mg/kg, while the contents of this metal of 5 to 30 mg/kg are considered to be toxic (3, 16).

Small amounts of *copper* are essential for plant growth, and this element also makes the structure of many enzymes. The phytotoxic level of this metal is 30 mg/kg (18). The normal content of Cu in plants is about 4-15 mg/kg, while the contents of this element over 25 mg/kg are toxic to the plant (16).

Lead is an element that can be delivered to the plants either by soil or by air. The normal contents of this metal in plants range from 0.1 to 10 mg/kg. The toxic contents of Pb are from 30 to 300 mg/kg (16).

Zinc is an essential component for a large number of (> 300) enzymes involved in the synthesis and decomposition of carbohydrates, lipids, proteins and nucleic acids, as well as in the metabolism of other micronutrients. Zn is an element that is not considered to be highly toxic and toxin levels of this metal (300-400 mg/kg), depending on both the plant species and its level of maturity. High zinc content in plants can cause leaf losses, while on the other hand Zn deficiency leads to their deformation. It is believed that the environment is contaminated with zinc when the content of this element, detected in plants, is about 100 mg/kg (16).

The content of cadmium and lead, as the two most toxic metals in the group of determined elements, which are detected in this study, ranged from 0.09 to 0.15 mg/kg (Cd) and 0.15 to 0.26 mg/kg (Pb) for samples collected in the no traffic impacted areas, i.e., from 0.18 to 0.49 mg/kg (Cd) and from 0.33 to 2.03 mg/kg in samples that were impacted by motor traffic (Table 4). Regarding to the content of detected metals (Cd and Pb), in dandelion samples from different locations, it can clearly be seen, that exposure to one of the sources of pollution (traffic, petrol pump, trapping) contributes to the increase of these pollutants in the dandelion plant tissue in relation to samples that were not exposed to the effects of the pollutant of the environment.

The results of this study are in line with the conclusions presented by other authors, that the content of cadmium and lead in the dandelion leaves is lower in samples taken from sites that were protected from traffic (Parks and Natural Parks), in relative to the contents detected in the samples that were impacted by the source of these heavy metals (4, 5, 10, 11).

According to Kloke et al. (17) and Kabata and Pendias (4), the normal contentration of Cd in plants are 0.2-0.8 mg/kg and 0.1-10 mg/kg for Pb. Results for Cd and Pb in this study corresponded to those values. That indicates that detected contents in this paper are below to the toxic limit for those pollutants in analyzed dandelion species.

However, the given fact is that dandelion can also be used mainly as a salad in human nutrition, the contents of Cd and Pb detected in this study are compared to the maximum allowed content of these metals in the fresh dandelion leaves (0.2 mg/kg for Cd and 0.3 mg/kg for Pb) prescribed by the World Health Organization (WHO) (1). In all samples of dandelion taken from the sites impacted to the pollutants, the contents of cadmium and lead were above the maximum allowed (Table 4), while the contents of these metals in the dandelion leaves taken from locations that were not exposed to the sources of contamination were below the prescribed limits for this plant species. Detected contents of Cd and Pb in this study, in samples from uncontaminated sites, are low, but due to the cumulative effect these metals have, as well as due to their ability to deposit in the vital organs of humans, it is necessary to monitor the content of these metals in the dandelion plant, which are used in human nutrition. As this plant species has the ability to accumulate Cd and Pb from the environment, particular attention should be paid to the location from which dandelion is taken, and if used for the human nutrition, the plant has to be taken from sites that are not impacted by motor traffic and other environment pollutants.

The content of copper and zinc in the tested dandelion samples ranged from 9.25 to 11.14 mg/kg (Cu) and from 34.08 to 44.68 mg/kg (Zn) from uncontaminated sites relative to the content of these elements from the sites that were exposed to the source of pollutants, which ranged from 14.32 to 24.52 mg/kg (Cu) or from 48.36 to 62.71 mg/kg (Zn). As in the instance of Cd and Pb, the content of Cu and Zn was higher in dandelion samples that were sampled near the source of pollution compared

to samples that were not exposed to the effects of these sources.

These results confirmed that elevated contents of some metals can be found in the dandelion leaves, if this plant species is exposed to the source of the pollutant. The concentration of Cu and Zn in the samples tested was lower than that which would be toxic to this plant species (16).

By comparing Cd, Cu, Pb, and Zn content in analyzed dandelion samples taken at two different distances (1 m and 5 m) from the sites that were exposed to sources of pollution, samples 1-6 (Table 4), it can be seen that there is no regular trend of concentration of detected metals in this study, with the distance from the source of pollution. This is not in agreement with the authors who perceived such a trend in their studies (2, 5, 6, 8, 18) but it is in agreement with the Gicomina et al. study where there was also no correlation between metal content and distance from source of pollution (1).This may be due to the fact that such studies regarded point sources of pollution and/or that the total distance from the street considered in our research (max of 5m) was too short.

The soil is one of the major pathway for metal accumulation in plants, because of that, in this study was analyzed the soil underlaying the dandelion samples.

The content of metals detected in the soil is given in Table 5, while Table 6 gives the maximum limit values, remediation values and maximum permissible values of Cd, Pb, Cu and Zn content in the soil, which are prescribed by the respective Regulations and Regulation of the Republic of Serbia (19, 20).

Table 6. Maximum limit, remediation and maximum permissible values of Cd, Cu, Pb and Zn in the soil expressed as mg/kg

Metals	Maximum limit values	Remediation values	Maximum permissible values
Cd	0.8	12	up to 3
Cu	36	190	up to 100
Pb	85	530	up to 100
Zn	140	720	up to 300

Regarding the detected content of Cd, Cu, Pb and Zn in the soil samples, it can be seen that the concentrations of these metals are higher in soil sampled from the sites that are exposed to the pollutants, compared to those soil samples from locations that are not near polluted sources (Table 5). Also, the content of detected metals in all tested soil samples is below the maximum limit values prescribed by the legislation of the Republic of Serbia (Table 6).

The soil's metal availability for the plant, i.e., its root, depends on the ability of the metal to adsorb, desorb, and compile with one of the components in the soil matrix. These processes are conditioned by soil characteristics such as pH, composition and soil structure.

The results of the pH measurement of the soil samples are part of Table 5. It can be seen that the samples are mildly basic to neutral. The lowest pH value of all samples shows the soil from location 9 and this value was 6.69. The soil pH value is very important for the mobility of the metal ions in it, and therefore for the resorption of plants. Namely, if the value of the pH of the soil is lower, the soil is more acidic, and the mobility of the metal ion is higher.

Based on the obtained results (Table 5), it can be seen that soil samples from contaminated sites had higher content of detected metals regarding the soil sampled from locations that were not impacted by the source of contamination. According to this, it can be concluded that proximity to the source of pollution increases the content of detected metals in the soil on which the analyzed plant species grows.

Conclusion

The results of this study have shown that dandelion leaves can provide a good data of environmental pollution, because the content of detected metals (Cd, Cu, Pb and Zn) was higher in samples that were exposed to the negative effect of environmental pollutants compared to those samples that were not under the influence of pollution's sources. The metal concentration in the samples of dandelion leaves is proportional to urbanization, industrial activity, and density of traffic.

The content of detected metals in all dandelion samples was below the level that would be considered toxic to this plant species.

The amount of Cd, Cu, Pb and Zn in soil samples was higher in soil exposed to atmospheric and exhaust gas pollution, compared to samples that were not under this negative impact. The content of detected metals in soil samples was below the maximum limit values prescribed by the legislation of the Republic of Serbia.

The increased content of detected metals in the samples of dandelion that were under the influence of the pollutant, may be the result of a synergistic effect, soil on which this plant species thrives and the air that is contaminated by the effects of motor traffic and other forms of pollutants.

As dandelion is used in human nutrition, and since heavy metals with cumulative and toxic effects have been detected in it, Pb and Cd, it is necessary, in order to protect human health, to check the content of these metals in the dandelion used in human nutrition. Also, due to the ability of this plant species to adsorb Pb and Cd, if it is additionally exposed to them, it is necessary to take into account from which site this plant species is taken for nutrition.

The ICP-OES method proved to be a good method for determining the content of cadmium, copper, lead and zinc in this type of samples.

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ODREĐIVANJE SADRŽAJA KADMIJUMA, BAKRA, OLOVA I CINKA U LISTOVIMA MASLAČKA (*TARAXACUM OFFICINALE* WEBB.) I ZEMLJIŠTU ICP-OES METODOM

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Maslačak (*Taraxacum officinale* Webb.) je biljna vrsta koja može da akumulira određenu količinu metala. Cilj ove studije bio je da se odredi sadržaj Cd, Cu, Pb i Zn u listovima maslačka i zemljištu, koji su uzorkovani sa različitih lokaliteta. Jedna grupa uzoraka bila je izložena uticaju izvora zagađenja ovih metala, dok druga grupa nije bila pod uticajem zagađivača. Količina metala u ispitivanim uzorcima određena je primenom tehnike indukovano kuplovane plazme optički emisione spektrometrije (ICP-OES). Sadržaj detektovanih metala bio je veći u uzorcima lista maslačka i zemljišta koji su bili izloženi negativnom uticaju zagađivača životne sredine, u odnosu na one uzorke koji nisu bili pod uticajem ovih izvora zagađenja. Povećani sadržaj detektovanih metala u uzorcima listova maslačka, koji su bili pod uticajem štetnog dejstva zagađivača, može biti rezultat sinergističkog efekta zemljišta, na kome ova biljna vrsta uspeva i vazduha, koji je zagađen motornim saobraćajem i drugim vidovima zagađenja. Rezultati ove studije pokazali su da maslačak, na osnovu količine detektovanih metala u biljnom tkivu, može pružiti podatke o zagađenju životne sredine. Kako se maslačak koristi u ljudskoj ishrani, a kako su u listu ove biljne vrste detektovani teški metali (Pb i Cd), koji imaju kumulativno i toksično dejstvo, neophodno je, u cilju zaštite ljudskog zdravlja, proveravati prisustvo i sadržaj ovih metala u maslačku koji se koristi u ljudskoj ishrani.

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Ključne reči: *maslačak, metali, zemljište, zagađivači, ICP-OES*

Original article

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ANALYSIS OF GENE AMPLIFICATION IN PAPILLARY THYROID CARCINOMAS

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Thyroid cancer is the most common endocrine malignancy and its rate has been steadily increasing all over the world. Papillary thyroid carcinoma (PTC) represents the most common histological subtype of thyroid cancer, accounting for about 75-85% of the cases. Until now, the mechanisms underlying the tumorigenesis of PTC still remain unclear.

To estimate BRAF and MYC gene amplifications in papillary thyroid carcinomas by FISH analysis and their link with the development of T1 tumor stage and their possible role in etiopathogenesis of this disease through chronic activation of MAPK pathway.

Tumor tissue specimens from 10 female patients with PTC were analysed by immunohistochemistry (CK19 and Ki67) and FISH analysis. FISH probes were applied on 5 μm thick histological sections and covered with glass and sealed with rubber cement. After denaturation at 75 ^{O}C for 5 min, hybridisation process at 37 ^{O}C during 3 h was done. After washing, DAPI containing mounting medium was applied.

In all tumor nuclei two signals of BRAF and two signals of MYC were found, indicating that gene amplifications were not found in the study group.

Although observed at a low number of patients, amplification of BRAF and MYC genes was not involved in PTC tumorigenesis in the development of T1 tumor stage.

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Key words: *papillary thyroid carcinoma, gene amplification, BRAF, MYC, immunohistochemistry, T1 tumor stage*

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Introduction

Thyroid cancer is the most common endocrine malignancy and its rate has been steadily increasing all over the world. Papillary thyroid carcinoma (PTC) represents the most common histological subtype of thyroid cancer, accounting for about 75-85% of the cases. Until now, surgical strategy is the major treatment for patients with thyroid cancer, due to its high resistance to standard chemotherapy. However, the extensive resection, postoperative recurrence and metastasis may lead to limited therapeutic effects and poor outcomes (1).

Thyroid cancer mutations develop most often in genes encoding components of the MAPK/ERK and PI3K/AKT pathways (2). In PTCs, the most common mutations are BRAF mutations, which were reported in 40-90% of cases from various geographical areas. RET/PTC rearrangements are the second most common genetic alteration in PTC (3). On the other hand, RAS mutations have increased sharply, and RET/PTC rearrangements have steadily decreased over the years (4). cMYC regulates approximately 15% of human genes and is involved in up to 20% of all human cancers. Reports discussing cMYC protein expression in thyroid carcinomas are limited, with controversies pertaining to cMYC expression patterns noted in the literature.

For well differentiated types of thyroid carcinomas (PTC, FC, and OvFC), there was a non-significant trend toward lower cMYC expression from stages pT1 through pT3 (5).

From the clinical point of view, 10% to 15% of patients with this carcinoma exhibit poor prognosis, related to still insufficiently identified features of tumor biology which may be uncovered by further expression profiling (6).

Until now the mechanisms underlying the tumorigenesis of PTC still remain unclear, and prediction of disease progression is a great challenge for PTC cases.

Aim

The aim of this study was to investigate BRAF and MYC gene amplifications in papillary thyroid carcinomas by FISH analysis in T1 tumor stage and their possible role in etiopathogenesis of this disease through chronic activation of MAPK pathway.

Materials and methods

Patients

In the current study, PTC tissues and adjacent normal tissues specimens were collected from 10 female patients who underwent thyroid surgery in Clinical Center of Niš, Serbia. All of the patients were pathologically diagnosed with PTC according to World Health Organization classification, and the tissues specimens were reviewed by two pathologists. All of the patients were diagnosed with T1 stage, without lymph node metastases.

Immnohistochemical analysis

Formalin-fixed and paraffin-embedded tumor sections (4-5 μm) were made for immunohistochemical analysis. Slides set aside for immunohistochemical evaluation after deparaffinization and endogenous peroxidase blocking (3% solution of H_2O_2 for 15 min) were submitted to microwave treatment (20 min at 620 Win 0.01 M citrate buffer, pH 6.0). MIB-1 monoclonal antibody for Ki-67, dilution 1:100, and RCK108 monoclonal antibody for cytokeratin 19 (DAKO, Glostrup, Denmark), was applied for 60 min at room temperature. Immunohistochemical staining was performed by the streptavidin-biotin method using an LSAB kit (DAKO, Glostrup, Denmark) according to the manufacturer's instructions (LSAB Kit, DAKO, Glostrup, Denmark). The chromogen was 3.3'-diaminobenzidine (DAB). Tissue sections were lightly counterstained with Mayer's hematoxylin (Merck, Germany). During the tissue staining, positive and negative control samples were simultaneously stained.

FISH analysis

Formalin fixed paraffin embedded tissue from 10 patients with papillary thyroid carcinomas were screened for gene amplification. For BRAF gene (7q34) locus specific SureFish BRAF-CN orange-red probe and chromosome 7 centromere locus specific SureFISH green probe were used (Agilent Technologies, West Cedar Creek, USA). For MYC gene (8q24.21) locus specific SureFish MYC red probe, and chromosome 8 centromere locus specific SureFISH green probe were used (Agilent Technologies, West Cedar Creek, USA).

After deparaffinization and denaturation at 80 ^{O}C during 10 min, hybridisation process at 45 ^{O}C during 2 h was done. FISH probes were applied on 5 μm thick histological sections and covered with glass and sealed with rubber cement. After washing, DAPI containing mounting medium was applied. Slides were then analysed with LEICA DM1500 fluorescent microscope with green and blue filters.

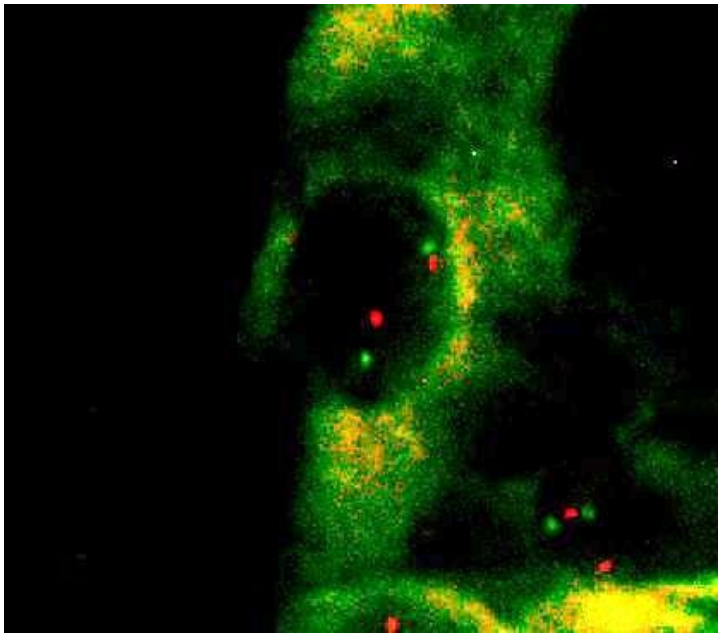

Figure 1. BRAF FISH analysis. In all tumor nuclei two signals of BRAF (smaller signal) and two signals of centromere (larger signal) were found

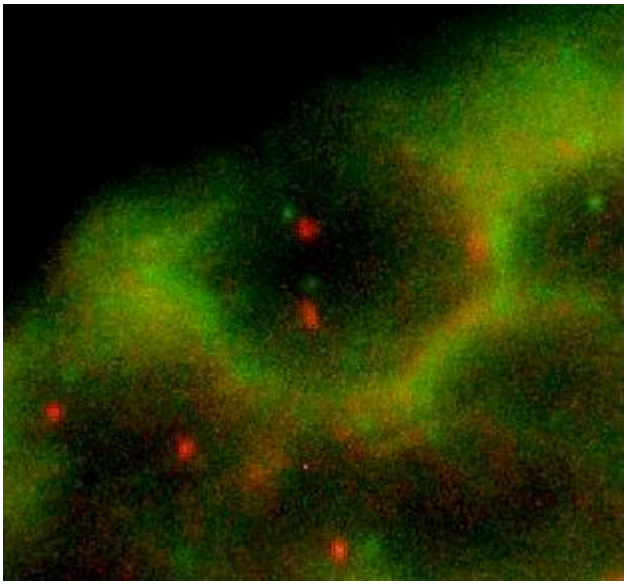

Figure 2. MYC FISH analysis. In all tumor nuclei two signals of BRAF (smaller signal) and two signals of centromere (larger signal) were found

Results

Pathological features

In all cases of papillary thyroid carcinoma, tumor cell nuclei were large, pale and crowded. Tumor cells were cytokeratin 19 positive.

FISH analysis

In all tumor nuclei two signals of BRAF and two signals of centromere were found (Figure 1). Similarly, in all tumor nuclei two signals of MYC (smaller signal) and two signals of centromere (larger signal) were found (Figure 2), indicating that BRAF and MYC gene amplifications were not found in the study group.

Discussion

The demographic and clinicopathological characteristics and molecular profile of PTCs have been changing over the past few decades. These modifications suggest changes in etiologies and risk factors of thyroid cancer that influence the tumorigenesis of PTCs (3).

The reliable tools are urgently needed to predict tumor progression and prognosis for patients with PTC. PTC is a complex disease, and both genetic and environmental factors such as iodine intake and radiation exposure are implicated in etiology of the cancer. Growing evidence has demonstrated that genetic/epigenetic factors play an important role in PTC development and treatments.

Whole exome sequencing revealed that mutational genes were mainly implicated in MAPK, PPAR, and p53 signal pathways, suggesting their functional roles in tumorigenesis of PTC (1). The MAPK/ERK pathway is activated in response to a diverse array of stimuli, such as mitogens, growth factors, and pro-inflammatory cytokines, and it regulates cell proliferation, differentiation, apoptosis, and survival. Genetic alterations in the MAPK/ERK pathway result in constitutive activation of signaling and can therefore be pro-tumorigenic. The PI3K/AKT pathway promotes cell cycle progression and is a key regulator of survival during cellular stress. Activation of growth factor receptor protein tyrosine kinases results in autophosphorylation of tyrosine residues, PI3K recruitment to the cell membrane, and allosteric activation of the catalytic subunit encoded by a gene PIK3C (2).

The majority of papillary thyroid carcinomas (PTC) are characterized by mutations in genes for components of the MAPK/ERK pathway: RET/PTC and BRAF. The BRAF gene encodes an intracellular serine-threonine kinase that phosphorylates and activates downstream targets of MAPK/ ERK signaling such as MEK. BRAF gene mutations are the most prevalent genetic alterations observed in thyroid cancer. A point mutation at nucleotide 1,799 produces a change from a valine to a glutamine at amino acid residue 600 (BRAF V600E) of the resulting protein that leads to constitutive BRAF dimerization and chronic activation of the MAPK pathway. Other BRAF gene mutations such as K601E, small in-frame deletions and insertions near codon 600 and even AKAP9/BRAF gene fusions, have been described, but constitute less than 2% of all mutations of this gene in a sporadic thyroid cancer (2).

The genetic signature in pediatric patients with PTC was remarkably different than that observed in adults (7, 8). The prevalence of the BRAF V600E mutation, the most common genetic event found in adult PTC, is significantly lower in sporadic and radiation-exposed pediatric PTC, and the aggressiveness of PTC is likely associated with other

genetic events. RET/PTC rearrangement, the second most common event in adult PTC, is the major genetic alteration found in sporadic and radiation-exposed pediatric PTC (9). Moreover, RAS mutations are a rare genetic event in pediatric PTC (8). These findings suggest that there are differences in tumor biology according to age.

The MYC protooncogene encodes a DNA-binding factor that can activate and repress transcription. Via this mechanism, MYC regulates expression of numerous target genes that control key cellular functions, including cell growth and cell cycle progression. MYC also has a critical role in DNA replication. Deregulated MYC expression resulting from various types of genetic alterations leads to constitutive MYC activity in a variety of cancers and promotes oncogenesis (10).

The results of our study indicate that BRAF and MYC genes are not amplified in PTC.

There are some limitations to our study. First, our study is limited by the inherent biases of a retrospective analysis and, therefore, the lack of a proper follow-up with all of the patients. Second, it is limited by a small sample size.

Conclusion

Although observed at a low number of patients, according to our results, amplification of BRAF and MYC genes was not involved in PTC tumorigenesis in T1 tumor stage.

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ANALIZA GENSKIH AMPLIFIKACIJA U PAPILARNOM KARCINOMU ŠTITNE ŽLEZDE

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Rak štitne žlezde najčešći je endokrini malignitet i njegova stopa je u stalnom porastu u celom svetu. Papilarni karcinom štitne žlezde (PTC) najčešći je histološki podtip karcinoma štitne žlezde, koji čini oko 75% — 85% slučajeva. Do sada su mehanizmi na kojima se temelji tumorigeneza PTC još uvek nejasni.

Procena BRAF i MYC amplifikacije gena u papilarnim karcinomima štitne žlezde izvršena je FISH analizom.

Uzorci tumorskih tkiva 10 ženskih bolesnika sa PTC analizirani su imunohistohemijski (CK19 i Ki67) i FISH analizom. FISH probe nanesene su na histološke preseke debljine 5 μm, prekrivene staklom i zapečaćene gumenim cementom. Nakon denaturacije na 75^{O}C u trajanju od 5 minuta, sproveden je postupak hibridizacije na 37^{O}C tokom 3 sata. Nakon ispiranja, primenjen je DAPI, koji sadrži medijum za montiranje.

U svim tumorskim jedrima pronađena su dva signala BRAF i dva signala MYC, što ukazuje na to da amplifikacije gena nisu nađene u ispitivanoj grupi.

Iako je primećena kod malog broja bolesnika, amplifikacija BRAF i MYC gena nije bila uključena u PTC tumorigenezu T1 stadijuma tumora.

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Ključne reči: *papilarni karcinom štitne žlezde, amplifikacija gena, BRAF, MYC, imunohistohemija, T1 stadijum tumora*

Original article

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EARLY POSTOPERATIVE OUTCOMES OF SURGICALLY TREATED AORTIC DISSECTION IN MARFAN SYNDROME PATIENTS

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Acute aortic dissection remains the leading cause of morbidity and mortality of Marfan syndrome patients. The study aimed to investigate the early postoperative outcomes of surgically treated aortic dissection in patients with Marfan syndrome. Study included all patients operated due to aortic dissection at the Cardiac Surgery Department, Dedinje Cardiovascular Institute in Belgrade during a six year period (2012–2017). Patients were divided regarding the diagnosis of Marfan syndrome. Preoperatively general data and detailed medical history were taken from every patient. Upon admission, patients underwent a thorough clinical and cardiologic examination. Patients were surgically treated according to current protocols. Postoperatively, patients were followed up for one month. During this follow-up period, we registered all complications and fatality. All pre and postoperatively collected data were compared and statistically analyzed. Study included 246 patients out of which 7.7% had Marfan syndrome. Marfan syndrome patients were significantly younger than control group patients ($p = 0.001$). There were no significant differences between patient groups regarding sex and smoking status. All patients with Marfan syndrome had dissection of type I. Marfan syndrome patients had fewer preoperative chronic illnesses and complications ($p = 0.001$). There were no significant differences in mortality ($p = 0.702$) and frequency of postoperative complications ($p = 0.231$) between patients with and without Marfan syndrome. In conclusion, it can be seen that prompt and adequate diagnosis and surgical treatment of aortic dissection can enable mostly good early postoperative outcomes in patients with Marfan syndrome.

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Key words: *Marfan syndrome, aortic dissection, surgical treatment, outcome*

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Introduction

Marfan syndrome is an autosomal dominant inherited disorder of the connective tissue in which cardiovascular, skeletal, ocular and other anomalies may be present to a variable degree (1). Its prevalence is around 1 in 5000 individuals. The combination of structural microfibril matrix abnormalities, deregulation of matrix homeostasis and inadequate cell-matrix interactions present the basis for clinical characteristics of the Marfan syndrome (2, 3).

The most common cardiovascular manifestations in patients with Marfan syndrome are progressive aortic dilatation and mitral valve prolapse. These anatomical abnormalities may cause mitral insufficiency as well as aortic insufficiency, aneurysm, dissection and even rupture (4). Surgical replacement of the dilated aortic root and ascending aorta has significantly increased the life expectancy of patients with Marfan syndrome. Nevertheless, acute aortic dissection remains the most important complication and the leading cause of morbidity and sudden death in these patients (5, 6).

Therefore, the study aim was to investigate the early postoperative outcomes of surgically treated aortic dissection in patients with Marfan syndrome.

Materials and methods

Study included all consecutive patients who were operated due to aortic dissection at the Cardiac

Surgery Department, Dedinje Cardiovascular Institute in Belgrade during a six year period (January 1st 2012 to December 31st 2017). All patients were divided into study and control group based on the diagnosis of Marfan syndrome. Moreover, during preoperative assessment, fenotypical characteristics of Marfan syndrome followed in accordance with Ghent criteria were used for the preoperative diagnosis of Marfan syndrome.

For the study purpose, we took into consideration all parameters that could potentially impact the outcome of patients with aortic dissection, while the main tested outcomes were complications and fatality in early postoperative period.

Preoperatively, general data (age, sex and smoking status) as well as detailed medical history were taken from every patient. We registered if patients had chronic illnesses and/or symptoms/ complications associated with Marfan syndrome that could be the risk factors or signs of coronary illness such as hypertension, hyperlipoproteinemia, cerebrovascular insults, periphery vascular disease, chronic kidney insufficiency before operation and other minor symptoms/complications (fatigue, shortness of breath, heart palpitations, chest pain, cold extremities). Upon admission for operation, patients underwent a thorough clinical and cardiologic examination with echocardiography for visualization of the dissection localization and measuring the diameters of ascending, descending and abdominal aorta. Only dissections of the A type (Stanford classification) were included in the study. Dissections were further divided into type I and II according to DeBakey classification system. The heart ejection fraction and EuroSCORE (www.euroscore.org) were determined for each patient. Moreover, we registered if the patient had cerebrovasular insult or heart tamponade on admission to our institution as the gravest (major) preoperative complications.

Patients were all surgically treated according to current protocols for their condition (Bentall procedure; Interposition tube graft and resuspension of the aortic valve; Tirone David procedure; hemiarch replacement and arch replacement) (7, 8). We registered deep hypothermic cardiac arrest time, cross clamp time, cardiopulmonary bypass duration.

Postoperatively, patients were followed up for one month. During that period all complications were registered such as myocardial infarction, cerebrovascular insult, paralysis, kidney insufficiency, other minor complications (prolonged intensive care; need for intubation; revision of hemostasis; uncomplicated urinary infection; sternal wound infection) and/or lethal outcome.

All pre and postoperatively collected data were compared and statistically analyzed in the whole sample as well as in regards to the diagnosis of Marfan syndrome by applying methods of descriptive (mean, standard deviation, frequency and percent) and analytical statistics (ANOVA and Kuskal Wallis χ^2 test). Spearman correlation was used to test the associations of investigated parameters and postoperative outcomes in the study group of patients with Marfan syndrome. We used SPSS 20 statistical software for Windows and p value of 0.05 was set as the level of significance.

Results

Study included 246 patients out of which 19 (7.7%) had Marfan syndrome while 227 were in the control group. Investigated patients were more often males (p = 0.001), 16 to 86 years of age. At least one preoperative chronic illness and/or symptom/ complication (major or minor) were registered in 84.6% of our patients. Postoperative complications were also rather frequent (44.7%), but in the overall sample, outcome was good for majority of patients (80.9%; p = 0.001).

Preoperatively collected data as well as postoperative outcome of investigated patients in the study and control group are presented in Tables 1 and 2.

Table 1. Descriptive data of investigated patients in the study and control group

Parameters	Marfan syndrome		Control group		F	p
	Mean	Standard Deviation	Mean	Standard Deviation		
Patients age	55.00	34.84	86.00	59.44	86.211	**0.001**
Ejection fraction	65.00	55.27	65.00	54.46	0.164	0.686
Ascending aorta diameter mm	90.00	56.66	83.00	53.38	1.902	0.169
Descending aorta diameter mm	35.00	26.16	55.00	32.85	5.711	**0.018**
Abdominal aorta diameter mm	22.00	22.01	61.00	25.26	0.070	0.793
EuroSCORE	18.45	6.52	18.45	7.69	0.025	0.983
EuroSCORE 2	65.47	9.81	32.62	8.39	0.306	0.581
EuroSCORE + Log	38.43	11.73	38.41	13.65	0.033	0.862
DHCA time min	28.00	7.23	34.00	9.71	0.199	0.656
Cross clamp time min	64.00	8.11	65.00	6.21	0.454	0.501
Cardiopulmonary bypass min	141.00	49.68	132.00	61.31	0.398	0.529

Legend: mm – millimeters; min – minutes; DHCA – deep hypothermic cardiac arrest time

Table 2. Frequency of investigated parameters in study and control group

Parameters		Marfan syndrome		Control group		KW χ^2	p
		Number	%	Number	%		
Patients sex	male	14	73.7	168	74.0	0.001	0.975
	female	5	26.3	59	26.0		
Smoking	no	8	42.1	119	52.4	0.744	0.388
	yes	11	57.9	108	47.6		
Dissection type	one	19	100	194	85.5	**7.177**	**0.035**
	two	0	0	33	14.5		
Hypertension	no	16	84.2	59	26.0	**27.927**	**0.001**
	yes	3	15.8	168	74.0		
Hyperlipo - protainaemia	no	19	100	186	81.9	**4.101**	**0.043**
	yes	0	0	41	18.1		
CVI before	no	18	94.7	211	93.0	0.087	0.769
	yes	1	5.3	16	7.0		
Periphery vascular disease	no	18	94.7	207	91.2	1.815	0.178
	yes	1	5.3	20	8.8		
Chronic kidney insufficiency	no	19	100	218	96.0	**3.779**	**0.049**
	yes	0	0	9	4.0		
Coronary illness before operation	no	19	100	194	85.5	**7.177**	**0.035**
	yes	0	0	33	14.5		
Tamponade on admission	no	16	84.2	167	73.6	1.038	0.308
	yes	3	15.8	60	26.4		
Cerebrovascular insult on admission	no	18	94.7	207	91.2	0.227	0.529
	yes	1	5.3	20	8.8		
Other symptoms and complications	no	17	89.5	196	86.3	0.147	0.701
	yes	2	10.5	31	13.7		
Had some preop. complications	no	10	52.6	28	12.3	**21.709**	**0.001**
	yes	9	47.4	199	87.7		
Myocardial infarct postoperatively	no	19	100	222	97.8	**3.425**	**0.049**
	yes	0	0	5	2.2		
Cerebrovascular insult postop.	no	18	94.7	196	86.3	1.087	0.297
	yes	1	5.3	31	13.7		
Postoperative paralysis	no	18	94.7	225	99.1	2.783	0.095
	yes	1	5.3	2	0.9		
Postop. kidney insufficiency	no	17	89.5	200	88.1	0.031	0.859
	yes	2	10.5	27	11.9		
Other postop. complications	no	8	42.1	154	67.8	5.143	**0.023**
	yes	11	57.9	73	32.2		
Had some postop. complications	no	8	42.1	128	56.4	1.441	0.231
	yes	11	57.9	99	43.6		
Postoperative lethal outcome	no	16	84.2	183	80.6	0.146	0.702
	yes	3	15.8	44	19.4		

Legend: preop – preoperative; postop – postoperative

In our sample patients with Marfan syndrome were significantly younger than patients from the control group. There were no significant differences between patient groups regarding sex and smoking status. All patients with Marfan syndrome had dissection of type I.

Investigated patients with Marfan syndrome in general were healthier preoperatively than patients from the control group. Compared to patients from the control group significantly fewer patients with Marfan syndrome had hyperlipoproteinemia and hypertension, while there were no patients with Marfan syndrome who had chronic kidney insufficiency and coronary illness. Periphery vascular disease and cerebrovascular insults were found with similar frequency in both patient groups.

On examination upon admission for operation, patients from both groups had comparable ejection fraction as well as diameters of ascending and abdominal aorta preoperatively. On the other hand, descending aorta had larger diameter in patients of the control group than with Marfan syndrome. Still, EuroSCORE for both patient groups - with and without Marfan syndrome was 18.45.

Table 3. Correlations of investigated parameters and postoperative outcomes

Parameters		Early postoperative lethal outcome	Early postoperative complications
All early postoperative complications	rho	**0.963**	/
	p	**0.012**	/
Patients age	rho	-0.171	0.156
	p	0.483	0.524
Patients sex	rho	-0.259	-0.217
	p	0.285	0.373
Hypertension	rho	-0.188	0.369
	p	0.442	0.120
Smoking status	rho	**0.508**	-0.295
	p	**0.026**	0.219
Cerebrovascular insult before	rho	-0.102	-0.276
	p	0.678	0.252
Ejection fraction	rho	0.106	0.045
	p	0.676	0.858
Ascending aorta diameter (millimeters)	rho	0.446	-0.630
	p	0.063	0.094
Descending aorta diameter (millimeters)	rho	-0.630	-0.169
	p	0.094	0.689
Abdominal aorta diameter (millimeters)	rho	0.369	0.369
	p	0.120	0.120
Tamponade on admission	rho	0.208	0.077
	p	0.392	0.754
Cerebrovascular insult on admission	rho	-0.102	0.201
	p	0.678	0.409
Other symptoms and complications preoperatively	rho	-0.149	0.293
	p	0.544	0.224
Had some preoperative complications / symptoms	rho	-0.122	0.382
	p	0.620	0.106
EuroSCORE	rho	-0.630	0.369
	p	0.094	0.120
EuroSCORE 2	rho	0.045	0.318
	p	0.858	0.313
EuroSCORE Log	rho	0.369	0.201
	p	0.120	0.409
Deep hypothermic cardiac arrest time (minutes)	rho	**0.522**	0.159
	p	**0.032**	0.543
Cross clamp time (minutes)	rho	0.072	0.216
	p	0.777	0.390
Cardiopulmonary bypass duration (minutes)	rho	0.145	**0.477**
	p	0.554	**0.039**

Legend: Bold – significant

There were no significant differences in duration of hypothermic cardiac arrest as well as the time on the cross clamp between groups.

In the early postoperative period patients from the control group more often developed myocardial infarction, while patients with Marfan

syndrome postoperatively more often had other minor complications. Still, when all postoperative complications were assessed together there were no significant differences in their frequency between groups.

Lethal outcome after the operation for aortic dissection was generally not very frequent in our sample and occurred in similar percent of patients with and without Marfan syndrome.

Finally, in the study group of patients with Marfan syndrome postoperative lethal outcome significantly correlated with having early postoperative complications, being a smoker and longer duration of hypothermic cardiac arrest, while early postoperative complications were associated with longer duration of cardiopulmonary bypass (Table 3).

Discussion

Marfan syndrome is caused by more than a thousand currently described mutations in the fibrillin-1 (FBN1) gene (on chromosome 15) encoding a glycoprotein which is a principal component of the extracellular matrix microfibril (2, 3). Therefore, major clinical features of this syndrome result from weaker connective tissues caused by defects in fibrillin-1, but the extent of signs and symptoms are highly variable because of varying genotype expression (1). Moreover, some studies have shown that in Marfan syndrome, function of transforming growth factor beta (TGF-β), stimulator of inflammation, fibrosis and activator of matrix metalloproteinase 2 and 9, is abnormal (9). Increased TGF-β activation in tissues can cause failure of lung septation, development of a myxomatous mitral valve and aortic root dilation. In addition, as the destruction of the elastic and collagen lamellae progresses over time, the loss of elasticity in the media causes increased aortic stiffness and decreased distensibility. Therefore, due to the loss of appropriate medial layer support, aortic dissections are facilitated (2, 3, 9). Therefore, it is considered that nearly all patients with Marfan syndrome will develop cardiovascular disease over their lifetimes, most frequently aortic root enlargement with associated aortic regurgitation, thoracic aortic aneurysm, aortic dissection or mitral valve prolapse (10).

Although nowadays there are well established clinical criteria and available genetic tests, Marfan syndrome patients are still diagnosed late. Consequently numerous patients do not get specialist care before they develop complications (6, 7). According to the data of a large study covering 20 years period conducted by The International Registry on Acute Aortic Dissections around 4% of patients with acute aortic dissection had Marfan syndrome (1). Studies on Marfan syndrome patient cohorts reported that around 36% of patients have an aortic event. The incidence of aortic complications at the age of 20 is less than 5% and it increases up to 50% by the age of 50 years (5, 10, 11). In our sample Marfan syndrome was somewhat more frequent reaching 7.7%.

Different studies indicate that patients with Marfan syndrome have aortic dissection more often at a significantly younger age compared to patients without Marfan syndrome. On the other hand, because of their younger age, they were found to generally have less other comorbidities (12, 13). Patients with Marfan syndrome mostly have lower incidence of hypertension, atherosclerosis and diabetes mellitus, acute renal failure and stroke, while they more frequently have aortic aneurysms, aortic insufficiency grade 3 or 4 and prior cardiac surgery (1, 6). The same was confirmed in our study as well.

Males were found to have a significantly increased risk of aortic events compared with women. In addition, aortic complications happened earlier in males than in females (8, 11). Our study also showed that male Marfan patients were significantly more often affected by acute dissection than women (73.7% vs. 26.3%). It is not clear why aortic dissection in Marfan syndrome occurs more frequently in men, but there are several hypotheses, including a still unknown protective effect of the X chromosome in women. This is suggested by the fact that women with a deficiency of the X chromosome (Turner syndrome) have a significantly increased risk of aortic disease, including dissection (2, 14).

Available data show that distribution of type A and B dissection seems to occur with a similar frequency in both patients with and without Marfan syndrome (11). In our sample, type II dissection was only registered in the control group patients. Moreover, the diameter of descending aorta was significantly larger in the control group patients than in patients with Marfan syndrome. In some investigations, significantly larger diameters of the aortic annulus and root were registered in Marfan syndrome patients with aortic dissections, but not larger diameters more distally (1, 15). In our sample, patients with and without Marfan syndrome had similar diameters of ascending and abdominal aorta. However, neither of the preoperatively measured diameters of the aorta was associated with early postoperative complications or outcomes in Marfan syndrome patients.

Another interesting observation from the literature is that the incidence of aortic rupture appears to be lower in Marfan syndrome, as suggested by the lower incidence of pericardial effusion and periaortic hematoma on preoperative imaging (1, 10). Contrary, in our study no significant differences in preoperative tamponade occurrence were noted between patients with and without Marfan syndrome.

Open aortic repair is the gold standard for treating patients with aortic dissection. The aortic root is commonly replaced while some patients may also need distal aortic repair (7, 8, 15, 16). Despite improvement in prevention and surgical techniques, aortic aneurysm repair is still a high-risk operation for patients with Marfan syndrome (17). Failure to extend the primary surgery to aortic root or arch repair was found to correlate with more postoperative complications (13, 18). However, elective repair of aortic aneurysms in Marfan syndrome patients usually has excellent survival and postoperative quality of life. Mortality in urgent or emergent repair is somewhat higher, but still quite acceptable (18-20).

The mortality rates in the first month after surgery for aortic dissection vary in literature data. Some centers managed to achieve very low early postoperative adverse events (2 to 6% of Marfan syndrome patients) which mostly occurred due to intraoperative ruptures (10, 11). In the USA authors reported 4.4% 30-day mortality in patients with Marfan syndrome who underwent urgent ascending aortic repair. Postoperative survival is usually above 60% even in case of reintervention (18). Other findings showed that the overall in-hospital mortality rate after surgery is 10.9% in patients with Marfan syndrome compared to 16.9% in patients without Marfan syndrome (1, 19). The early mortality in type A dissection was not significantly different in patients with or without Marfan syndrome, but in case of type B dissection mortality was lower in patients without Marfan syndrome (4, 13). In our sample, in-hospital mortality was not high and comparable with the mortality currently reported for the overall aortic dissection population, which ranges between 8% and 20% in high-volume centers (1, 20). In our study there were no significant differences in early postoperative mortality of patients with and without Marfan syndrome.

According to the literature data, risk factors for adverse postoperative outcome in Marfan syndrome patients were younger age at surgery, post-operative use of ECMO, post-operative hemorrhage, neurologic complication and having additional coronary artery procedure (10, 16, 17). Sex and race/ethnicity were not associated with mortality after surgery for aortic dissection regardless of having or not the Marfan syndrome (5, 10). We found that in Marfan syndrome patients, postoperative lethal outcome was associated with having early postoperative complications, being a smoker and longer duration of hypothermic cardiac arrest, while early post-operative complications were associated with longer duration of cardiopulmonary bypass.

Postoperative complications after surgery for aortic dissection are mostly common in available studies reaching 45% (5, 20). The most frequently reported complications were arrhythmia, post-operative hemorrhage, pericardial complications and respiratory complications (10). The most common complications in Marfan syndrome patients are pulmonary complications. Postoperative neurological complications occurred only in patients with Marfan syndrome (1). On the other hand, permanent stroke and paraplegia, gastrointestinal ischemia as well as renal failure were found to occur rarely in Marfan syndrome patients (16, 19). In our study postoperative complications were registered in around 50% of patients, but there were no significant differences in their frequency between patients with and without Marfan syndrome. Moreover, Marfan syndrome patients postoperatively more often had only minor complications. These good results are probably related to the relatively young age at the time of surgery and low prevalence of comorbidities in patients with Marfan syndrome.

Conclusion

Results of our study show that with prompt and adequate diagnosis and surgical treatment of aortic dissection early postoperative outcomes in patients with Marfan syndrome are mostly good. Mortality rates of Marfan syndrome patients are low and postoperative complications are minor. Moreover, the outcomes of surgically treated aortic dissections are generally comparable in patients with and without Marfan syndrome. The parameters that impact Marfan syndrome patients' postoperative outcome are longer duration of cardiopulmonary bypass and hypothermic cardiac arrest, having early postoperative complications and being a smoker.

Conflict of interest

Authors declare that they have no conflict of interest.

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RANI POSTOPERATIVNI ISHODI HIRURŠKI TRETIRANE AORTNE DISEKCIJE KOD BOLESNIKA SA MARFANOVIM SINDROMOM

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Akutna disekcija aorte ostaje vodeći uzrok morbiditeta i mortaliteta bolesnika sa Marfanovim sindromom. Studija je imala za cilj da ispita rane postoperativne ishode hirurški tretirane disekcije aorte kod bolesnika sa Marfanovim sindromom. Studija je obuhvatila sve bolesnike operisane zbog disekcije aorte na Klinici za kardiohirurgiju Instituta za kardiovaskularne bolesti "Dedinje" u Beogradu, tokom perioda od šest godina (2012. – 2017.). Bolesnici su bili podeljeni u odnosu na postojanje dijagnoze Marfanovog sindroma. Preoperativno od svakog bolesnika uzeti su opšti podaci i detaljna medicinska istorija. Nakon prijema, bolesnici su bili podvrgnuti temeljnom kliničkom i kardiološkom pregledu. Bolesnici su hirurški tretirani prema aktuelnim protokolima. Postoperativno, bolesnici su praćeni mesec dana. Tokom ovog perioda praćenja registrovane su sve komplikacije i registrovana je smrtnost. Svi preoperativno i postoperativno prikupljeni podaci upoređeni su i statistički analizirani. Studija je obuhvatila 246 bolesnika od kojih je 7,7% imalo Marfanov sindrom. Bolesnici sa Marfanovim sindromom bili su značajno mlađi od bolesnika kontrolne grupe ($p = 0,001$). Nije bilo značajnih razlika između grupa bolesnika u pogledu pola i pušačkog statusa. Svi bolesnici sa Marfanovim sindromom imali su disekciju tipa I. Bolesnici sa Marfanovim sindromom imali su manje preoperativnih hroničnih bolesti i komplikacija ($p = 0,001$). Nije bilo značajnih razlika u mortalitetu ($p = 0,702$) i učestalosti postoperativnih komplikacija ($p = 0,231$) između bole-snika sa Marfanovim sindromom i bez Marfanovog sindroma. U zaključku se može videti da brza i adekvatna dijagnoza i hirurško lečenje disekcije aorte mogu omogućiti uglavnom dobre rane postoperativne ishode kod bolesnika sa Marfanovim sindromom.

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Ključne reči: *Marfanov sindrom, disekcija aorte, hirurški tretman, ishod*

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RELATIONSHIPS BETWEEN QUALITY OF SLEEP AND INSOMNIA WITH DEPRESSION AND ANXIETY SYMPTOMS IN MEDICAL UNIVERSITY STUDENTS IN SERBIA

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Since sleep problems are very common among university students, this study explored the interplay between symptoms of depression, anxiety, quality of sleep, and insomnia.

The cross-sectional study was carried out at the University of Niš, Faculty of Medicine (Serbia) in 2016 and included 600 students of both genders. Students completed the questionnaire, which was compiled and developed from the Depression Anxiety Stress Scale, the Pittsburgh Sleep Quality Index, and the Insomnia Severity Index.

Sleep problems are very frequent among university students: 432 (72%) and 258 (43%) students reported poor sleep quality or sub-threshold insomnia problems, respectively. Even 66 students (11%) reported moderate or severe insomnia. Above-threshold depression symptoms were reported by 168 students (28%) and anxiety symptoms by 180 of them (30%). Depression was strongly associated with poor sleep quality (χ^2 = 20.35; df = 1; p < 0.001), and insomnia severity (χ^2 = 13.05.; df = 1; p < 0.001). Above-threshold anxiety was associated only with insomnia severity (χ^2 = 16.42; df = 1; p < 0.001).

It has been found that an anxiety pathway was strongly associated with insomnia severity, while a depression was more relevant for worsening the quality of sleep.

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Key words: *quality of sleep, insomnia, depression, anxiety*

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Introduction

Sleep problems are very common among university students. They are particularly pronounced in students of medical sciences, and among them, primarily in students at the state universities. In the private faculties, which have become more and more frequent in recent times in Serbia as well as in other ex Yu territories, the students generally do not have problems that the students of the Faculties of Medicine of the Universities of Niš, Belgrade, Novi Sad are facing. The regime of study in these Universities is quite rigorous, and students often resort to various strategies that can influence their further psychosocial development in order to achieve and demonstrate their maximum in exams.

Unfortunately, this reflects on their psychosocial status, in a negative sense (1, 2).

In addition to exhaustion and comorbid physical or psychological problems, various psychoactive substances are often used by medical students for these reasons.

Students who suffer from insomnia often have severe depressive symptoms and/or some anxiety disorders (3, 4).

Persistent insomnia can be a risk factor or an early symptom of bipolar, depressive, and anxiety disorders (1, 3). Accordingly, depression and anxiety can be risk factors for insomnia (2, 5, 6).

Insomnia often occurs at about the same time or immediately after the onset of anxiety distress (3-5). However, the nature of the link between depression and developing insomnia is controversial. Loneliness is a disease of modern society! Since sleep problems are very common among university students, this study explored the interplay between the symptoms of depression and anxiety, quality of sleep, and insomnia.

Materials and methods

Participants and Ethics

The participants were 600 undergraduate students of the Faculty of Medicine of the University of Niš. Their mean age was 22.18 years (SD = 2.52). The survey was performed in the classrooms by trained assistants (interviewers) and was intended to last a maximum of 20 min, including the time needed for instructions. We have got verbal consent from all participants before data collection. The study procedures were carried out in accordance with the Declaration of Helsinki, and the approval of the Ethical Committee of the Faculty of Medicine of the University of Niš (14-5785-3).

Measures

The DASS device is a set of three self-reporting scales designed to measure the negative emotional states of depression, anxiety, and stress. Each of the three scales contains 14 items, divided into the subscales of 2–5 items with similar content. DASS is designed to be used for further defining, understanding, and measuring all present and clinically significant emotional states in the examinees (7, 8).

The students were asked to mark from 0 (none) to 3 (mostly or almost always) the extent to which they have experienced each of the listed conditions during the previous week. The score results of depression, anxiety, and stress were calculated by adding the points for each relevant scale. The result was then calculated for every student and for each of the subscales, according to the score matrix, and then evaluated as per the severity-rating index.

The reliability scores of the scales in terms of Cronbach's alpha scores rate the depression scale at 0.91, the anxiety scale at 0.84, and the stress scale at 0.90 in the normative sample. The means for each scale are 6.34 (SD 6.97) for depression, 4.7 (SD 4.91) for anxiety, and 10.11 (SD 7.91) for stress.

Pittsburgh Sleep Quality Index measures retrospective sleep quality and disturbances over one month (9). It comprises nineteen questions that yield seven clinically derived component scores: sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunctions. All component scores range from 0 to 3, and the Global Score (α = .78 in the present study) ranges from 0 to 21. A conventional cutoff score of > 5 is used to separate poor sleepers from good sleepers.

The Pittsburgh Sleep Quality Index (PSQI) is an effective instrument used to measure the quality and patterns of sleep in adults. It differentiates "poor" from "good" sleep quality by measuring seven areas (components): subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medications, and daytime dysfunction over the last month.

Insomnia Severity Index consists of seven items reflecting DSM-V criteria for an insomnia disorder (10). These are sleep onset, sleep maintenance, early morning awakening problems, sleep dissatisfaction, the extent to which sleep problems interfere with daytime functioning, whether one's sleep problems are noticeable to others, and the distress caused by sleep difficulties. Respondents rate symptom severity during the past two weeks using a five-point scale (0 = affected me very little to 4 = affected me a lot). The ISI yields a total score ranging from 0 to 28 (α = .82 in this study). The guidelines for interpretation are as follows: 0–7 = no clinically significant insomnia; 8–14 = subthreshold insomnia; 15–21 = clinical insomnia of moderate severity; 21–28 = severe clinical insomnia.

Data analysis

All of the data were entered into Excel spreadsheets (Microsoft Office 2003, Microsoft, Redmond, DC, USA) by several teams each consisting of two people, whereby cross-checking was done for every given survey. The statistical analysis was performed using the SPSS 17.0 program (SPSS Inc., Chicago, IL, USA) in Windows 7 Ultimate. The research results were presented in tables.

The statistical analysis of the data included the application of descriptive tests and analytical parametric tests, as well as binary logistic regression tests and correlation tests. The descriptive statistics were performed to report the analysis of the data that were presented as mean and standard deviations. The categorical variables were shown as frequency and percentages. The independent t-test was used to compare the parametric variables between the genders. Pearson and Spearman correlations were used to determine the strength of the relationships between the examined variables. Binary logistic regression was used to estimate the odds ratios (ORs) and 95% confidence intervals (CIs) of the independent and interactive relationships of several prediction variables with depression, anxiety, and stress. The statistical significance was set at $p < 0.05$.

Results

Descriptive analyses and correlations

More than two thirds of the surveyed students (n = 432, 72%) and 258 (43%) of the students reported poor sleep quality or sub-threshold insomnia problems, respectively. Even 66 students (11%) reported moderate or severe insomnia (Table 1).

Above-threshold depression symptoms were reported by 168 students (28%) and anxiety symptoms by 180 of them (30%) (Table 2).

Depression was strongly associated with poor sleep quality (χ^2 = 20.35; df = 1; $p < 0.001$), and insomnia severity (χ^2 = 13.05.; df = 1; $p < 0.001$).

Above-threshold anxiety was associated only with insomnia severity (χ^2 = 16.42; df = 1; $p < 0.001$) (Table 2).

Table 1. The distribution of the Pittsburgh Sleep Quality Index (PSQI) scores

Components of the PSQI	PSQI sub-component	Percentage
PSQI component of sleep duration	≥ 7 h	30.3
	6 – 7 h	17.2
	5 – 6 h	9.3
	< 5 h	43.2
PSQI component of sleep disturbances	0	15.1
	1	83.9
	2	1.0
	3	0
PSQI component of sleep latency	0	11.9
	1	36.1
	2	38.2
	3	13.8
PSQI component of daytime dysfunction	0	92.4
	1	4.3
	2	2.1
	3	1.2
PSQI component of sleep efficiency	> 85%	34.4
	75 – 84%	8.7
	65 – 74%	7.6
	< 65%	49.3
PSQI component of sleep quality	Very good	32.8
	Fairly good	39.5
	Fairly bad	18.4
	Very bad	9.3
PSQI component of sleep medication	Not during the past month	82.0
	Less than once a week	8.0
	Once or twice a week	4.5
	Three or more times a week	1.5

Table 2. The distribution of Insomnia Severity Index (ISI) - each item response

Item ISI	0	1	2	3	4
1. falling asleep	19.6	24.5	22.8	21.8	11.3
2. staying asleep	0.5	4.4	18.5	53.8	22.8
3. early awakening	8.5	8.2	28.3	39.8	15.2
4. satisfaction	0.3	0.5	7.2	43.7	48.3
5. interference	1.6	8.5	42.0	36.1	11.8
6. noticeable	6.2	28.3	46.2	17.5	1.8
7. worry	0.8	2.7	39.2	41.1	16.2

Moderate, severe, or extremely severe levels of depression symptoms were reported by 137 students (22.8%) and "above mild" anxiety symptoms were reported by 217 (36.16%) students (Table 3).

Binary logistic regression was conducted in order to estimate the effects of gender, age, pocket money, physical activity, quality of sleep, and insomnia severity on the probability that the surveyed students would respond positively to questions about depression and anxiety.

The whole model, along with all the predictors, was statistically significant: for depression: χ^2 (8, N = 600) = 179.181, p< 0.001; for anxiety: χ^2 (8, N = 600) = 226.532, p< 0.001. This indicates that the model distinguishes those students who are from those who are not sorted, so that they have some of the symptoms. The assumptions of collinearity and singularity were satisfied, and non-typical points were also checked.

Table 3. The scores of depression, anxiety, and stress with respect to students' gender

Symptom Levels	Depression			Anxiety		
	Total	Male	Female	Total	Male	Female
		n %	n %		n %	n %
Normal	n = 384 64.0%	136 64.2	248 63.9	n = 331 55.2%	121 57.1	210 54.1
Mild	n = 79 13.2%	22 10.4	57 14.7	n = 52 8.7%	21 9.9	31 8.0
Moderate	n = 84 14.0%	30 14.2	54 13.9	n = 106 17.7%	25 11.8	81 20.9
Severe	n = 33 5.5%	15 7.1	18 4.6	n = 34 5.7%	17 8.0	17 4.4
Extremely severe	n = 20 3.3%	9 4.2	11 2.8	n = 77 12.8%	28 13.2	49 12.6

Table 4. Prediction of the levels of depression and anxiety in the surveyed students

Independent Variables	B	df	p	OR	95% CI for OR	
					Lower	Upper
Depression	Hosmer-Lemeshow test of goodness-of-fit (p = 0.101, for χ^2 = 13.34, df = 8)					
Gender (1)	0.358	1	0.098	1.431	0.936	2.187
Age	-0.036	1	0.466	0.964	0.875	1.063
Pocket money	0.000	1	0.103	1.000	1.000	1.000
Physical activity	-0.258	1	0.016	0.773	0.627	0.953
Quality of sleep (1)	0.299	1	0.011	2.748	1.192	2.038
Insomnia severity (1)	-0.708	1	0.026	0.493	0.264	0.918
Constant	-0.284	1	0.784	0.753	Correctly classified 77.3%	
Anxiety	Hosmer-Lemeshow test of goodness-of-fit (p = 0.102, for χ^2 = 13.30, df = 8)					
Gender (1)	-0.018	1	0.925	0.982	0.673	1.433
Age	-0.079	1	0.074	0.924	0.847	1.008
Pocket money	0.000	1	0.125	1.000	1.000	1.000
Physical activity	-0.312	1	0.001	0.732	0.610	0.878
Quality of sleep (1)	0.126	1	0.039	1.334	1.287	1.635
Insomnia severity (1)	-0.083	1	0.021	0.821	1.564	2.502
Constant	1.535	1	0.096	4.643	Correctly classified 64.2%	

As Table 4 shows, four independent variables (gender, age, pocket money, physical activity, quality of sleep, and insomnia severity) provided a unique statistically significant contribution to some of two presented models.

The strongest predictor of whether a surveyed student has high levels of depression symptoms was quality of sleep, where the odds ratio was OR = 2.75. This shows that respondents with higher scores of quality of sleep have symptoms of depression 2.75 times more often, with all other factors in the model being equal. Also, it has been observed that depression symptoms are more common in those students with higher insomnia severity indices.

Anxiety symptoms are the most present in those with higher insomnia severity indices. (Table 4).

Discussion

This sleep research has shown that anxiety and depression can uniquely predict poor sleep quality and insomnia symptoms in surveyed students.

Our findings are consistent with the view that exaggerated concerns about the real or presumed consequences of anxiety might affect the sleep variables through the magnification of contingent anxiety and depression states (3, 11-13).

Specifically, it looks like that the mental component is more strongly related to depression than the physical and social components which affect anxiety symptoms.

Poor sleep quality is a typical characteristic of depression, with a likely bidirectional relationship (11, 14, 15).

Poor sleepers often complain about intrusive and uncontrollable ruminative thinking during the pre-sleep period and report using ineffective mental control strategies to try to suppress unpleasant thoughts (16-22).

Despite methodological strengths, our study is not exempt from limitations. First, our findings are entirely based on cross-sectional data.

For instance, insomnia severity and poor sleep quality might precede the onset insurgence of depression or anxiety symptoms; it is still entirely possible that impaired sleep reinforces dysfunctional cognitive dispositions associated with psychological symptoms.

Causal inferences cannot properly be made without active control over the variables concerned.

Second, our study was based on a convenience sample. University students are not representative of the Serbian population, at least in terms of age, gender, education, and personality characteristics.

For instance, the prevalent female gender in our sample might have heightened the average anxiety and depression ratings.

Moreover, since university students have irregular sleep patterns, about 1/3 of the students were poor sleepers, and 40% reported subthreshold insomnia.

So, despite we admit that there might be a potential selection bias in our study, the sample characteristics increased the salience of our findings for a population at risk for sleep disturbances.

Anyway, it has been found that an anxiety pathway was strongly associated with insomnia severity, while a depression was more relevant for worsening the quality of sleep.

Non-depressed people with insomnia have a twofold higher risk of developing depression than people with no sleep difficulties.

Conclusion

It has been found that an anxiety pathway is strongly associated with insomnia severity, while the depression is more relevant for worsening the quality of sleep.

The further researches will provide more answers to public health professionals in which direction to take measures to lifestyle balance according to these issues.

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POVEZANOST KVALITETA SNA I NESANICE SA SIMPTOMIMA DEPRESIJE I ANKSIOZNOSTI KOD STUDENATA MEDICINE U SRBIJI

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Budući da su problemi sa spavanjem vrlo česti među studentima medicine, ova studija je istraživala povezanost depresije i anksioznosti sa kvalitetom sna i nesanicom.

Studija preseka izvedena je na Medicinskom fakultetu Univerziteta u Nišu 2016. godine i obuhvatila je 600 studenata oba pola. Studenti su ispunili upitnik, koji je sastavljen i razvijen iz skala „Depression Anxiety Stress Scale", „Pittsburgh Sleep Quality Index" i „Insomnia Severity Index".

Pokazalo se da su problemi sa spavanjem zaista česti među studentima: 432 (72%) studenta prijavila su loš kvalitet sna, dok 258 (43%) njih ima probleme sa nesanicom. Čak 66 studenata (11%) prijavilo je vrlo ozbiljnu ili ozbiljnu nesanicu. Simptome depresije iznad praga prijavilo je 168 studenata (28%), a simptome anksioznosti njih 180 (30%). Depresija je bila snažno povezana sa lošim kvalitetom sna (χ^2 = 20,35; df = 1; p < 0,001) i stepenom nesanice (χ^2 = 13,05; df = 1; p < 0,001). Anksioznost iznad praga bila je povezana samo sa ozbiljnošću nesanice (χ^2 = 16,42; df = 1; p < 0,001).

Nađeno je da je anksioznost bila snažno povezana sa nesanicom, dok je depresija bila povezanija sa pogoršanjem kvaliteta sna.

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Ključne reči: *kvalitet sna, nesanica, depresija, anksioznost*

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DISTRIBUTION OF PDGFRα+ CELLS AND INTERSTITIAL CELLS OF CAJAL IN THE HUMAN FETAL GUT

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Two types of interstitial cells, interstitial cells of Cajal (ICC) and "fibroblast-like" cells, recently named platelet-derived growth factors receptor positive (PDGFRα+) cells, are present within the muscular layer of gastrointestinal (GI) tract. ICC and PDGFRα+ cells represent different classes of cells with unique ultrastructure, molecular phenotype and function, and they occupy the same anatomical niches in the GI tract. It is considered that PDGFRα+ cells such as ICC, mediate enteric inhibitory neurotransmission. Platelet-derived growth factors (PDGFs) are major mitogens for many cell types of mesenchymal origin, like fibroblasts and smooth muscle cells, and during embryogenesis, PDGF signaling is important in organogenesis. In the available literature, there is no data on the presence and distribution of PDGFRα immunoreactive cells in the human intestine during fetal period.

The aim of this study was to identify PDGFRα immunoreactive cells in the gut of human fetuses, as well as to determine their distribution in relation to smooth muscle cells, ICC and enteric nerve structures.

The material consisted of 12 Human Fetuses, gestational age from 10 to 12 weeks. The immunohistochemical test was carried out with the PDGFR-α antibody, ICC were identified using the C-kit antibody, while the muscle structures were demonstrated by the Desmin antibody.

During the development of the human intestine, at week 11, PDGFRα immunoreactive cells are present within the circular muscle layer, while they are absent in the myenteric plexus region and in the parts below the serosa. Unlike them, ICC are present only around the inception of the myenteric plexus ganglia.

In the fetal period of the human development, PDGFRα immunoreactive cells are present in all parts of the intestines, they are localized within the circular muscle layer and do not coincide with the ICC.

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Key words: *PDGFRα, C-kit, interstitial cells of Cajal, fetal gastrointestinal tract*

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Introduction

The muscular layer of the gastrointestinal (GI) tract is a complex tissue that, in addition to smooth muscle cells, contains a variety of cellular phenotypes. Within the muscular layer of GI tract, there are at least two types of interstitial cells, interstitial cells of Cajal (ICC) and cells called "fibroblast-like" or "ICC-like cells" (1, 2). These interstitial cells of mesenchymal origin form gap junctions with each other and with surrounding smooth muscles thus conducting electrical signal and regulating muscle excitability (3). Interstitial cells of Cajal express c-kit (4), so immunohistochemical labeling of the Kit receptor has enabled the reliable identification and study of ICC function and pathophysiology (5) on human and animal material.

The studies of ICC development suggest that some types of ICC and smooth muscle cells have a common precursor that expresses the c-kit (6). Kit signaling is thought to be necessary for the separation of mesenchymal precursors during differentiatition toward ICC or smooth muscle cells, for maintaining the ICC phenotype, and for normal postnatal development (7). The pacemaker role of the ICC around the myenteric plexus (ICC-MY), which spontaneously generate slow waves necessary for peristaltic contraction, has recently been confirmed (3, 8). The ICC within the muscle layer (intramuscular ICC – ICC-IM) have a role as mediators of

cholinergic and nitrinergic neurotransmission (9-11), participate in afferent signaling and integration of sensory-motor function as an element of the afferent branch of the gastrointestinal reflex (12). ICC also have a sensory role in the transduction of mechanical stimuli, that is, they function as stretch receptors (13). Loss and dysfunction of ICC have been demonstrated in numerous motility disorders (14-18). Contrary to the studies of ICC, the study of „fibroblast-like" cells has only recently been made possible by immunohistochemical labeling of platelet derived growth factor receptor A (PDGFRα) (19). These cells are different from ICC, but they occupy the same anatomical niches in the GI tract in mice, primates, and humans (19-24). ICC and PDGFRα+ cells represent different classes of cells with unique ultrastructure, molecular phenotype and function. Smooth muscle cells are electrically connected to ICC and PDGFRα+ cells via gap junctions and form an integrated unit – smooth muscle cell, ICC and PDGFRα+ cells (SIP) syncytium (3). SIP cells express different receptors and ion channels, and changes in conductivity in any type of SIP cell affect the excitability and reactions of the syncytium. Interstitial cells are also found in various other smooth muscle organs; however, in most cases the physiological and pathophysiological role of these cells is not clearly defined (25, 26).

PDGFRα+ cells are also closely related to the motor neurons varices and are intertwined with ICC around neural processes (19, 27). Immunohistochemical studies have shown that PDGFRα+ cells in the musculature of the GI tract express small conductance Ca^{2+}-activated K^+ (SK_3) channels, and mediate enteric inhibitory responses to purines in GI muscles (28-30). This observation, together with the findings that PDGFRα+ cells are very closely related to enteric motor neurons and electrically paired with smooth muscle cells (31), suggests that they, such as ICC, may mediate enteric inhibitory neurotransmission. Major mitogens for many cell types of mesenchymal origin, like fibroblasts and smooth muscle cells, are platelet-derived growth factors (PDGFs) (32). During embryogenesis, PDGF signaling is important in organogenesis (33), while adult cells of multiple organs expressing PDGF ligands and receptors often play an important role in the pathophysiology of various disorders, including GI dysmotility (34).

In the available literature, there is no data on the presence and distribution of PDGFRα immunoreactive cells in the human intestine during fetal period.

Aim

The aim of this study was to identify PDGFRα immunoreactive cells in the gut of human fetuses, as well as to determine their distribution in relation to smooth muscle cells, ICC and enteric nerve structures.

Materials and methods

Material

The material consisted of 12 human fetuses, in the gestational age from 10 to 12 weeks. The tissue material was obtained from the Institute of Pathology, Clinical Center Niš, after legal abortions and premature births due to prepartal deaths. Gestational ages were estimated by anatomical criteria according to the crown-rump length, biparietal diameter, and foot length, as well as from the anamnestic data on pregnancy age. There were no gastrointestinal disorders in the specimens, and both sexes were represented in the sample. A macroscopic examination was performed in detail and only specimens that did not undergo post-mortem changes were selected. The study was approved by the Ethics Committee of the University of Niš Faculty of Medicine, and was performed within the internal project no. 22 of the University of Niš Faculty of Medicine.

Tissue preparation

Gut specimens were isolated and fixed in formaldehyde (10%), paraffin embedded, sequentially sectioned at 4 μm, and routinely H&E stained due to histological examination.

Immunohistochemistry

The specimens were exposed to PDGFRα antibodies, anti-c-kit antibodies to investigate ICC, and smooth muscle cells were immunohistochemically labeled with anti-desmin (DES) antibodies. Section deparaffinization was performed in xylol and descending series of alcohol rinses (less than 1 min each) followed by rehydration in distilled water. The tissue sections were incubated after blocking endogenous peroxidase (3% H_2O_2 for 10 min at room temperature) with the primary antibody in a humidified chamber at room temperature for one hour, followed by rinses in a phosphate-buffered solution (0.1 M PBS, pH 7.4). The primary antibodies were dissolved in Dako antibody diluent (EnVisionTM FLEX DM830 Code: K8006, Dako, Denmark). After secondary antibody administration (EnVisionTM FLEX SM802, Code: K8000, Dako, Denmark) for 45 min at room temperature, immune complexes were visualized by the Daco REAL EnVisionTM Detection System, Code: k5007 (Dako, Denmark). Mayer's haematoxylin was used for counterstaining of all immunolabeled sections, and immunoreactivity was absent in negative controls in which the primary antibody was omitted. The primary antibodies used in the research and their respective dilutions are listed in Table 1.

Table 1. Antibodies

Antigen	Clone	Supplier	Dilution
C-kit	CD-117	*Dako*	1 : 300
PDGFRα	Polyclonal	*Abcam*	1 : 50
Desmin	DE-R-11	*Dako*	1 : 100

Results

During the development of the human intestine, at week 11, immunoreactivity to desmin is present in all parts of the gut tissue. Desmin immunoreactivity is present in all parts of the intestine in the form of a concentric band of cells (Figure 1), which by their localization correspond to the circular muscle layer, while the longitudinal layer is not yet differentiated. Only in the terminal portions of the foregut and initial portions of the midgut are individual desmin immunopositive cells localized immediately below the serosa, representing the origin of the longitudinal muscle layer.

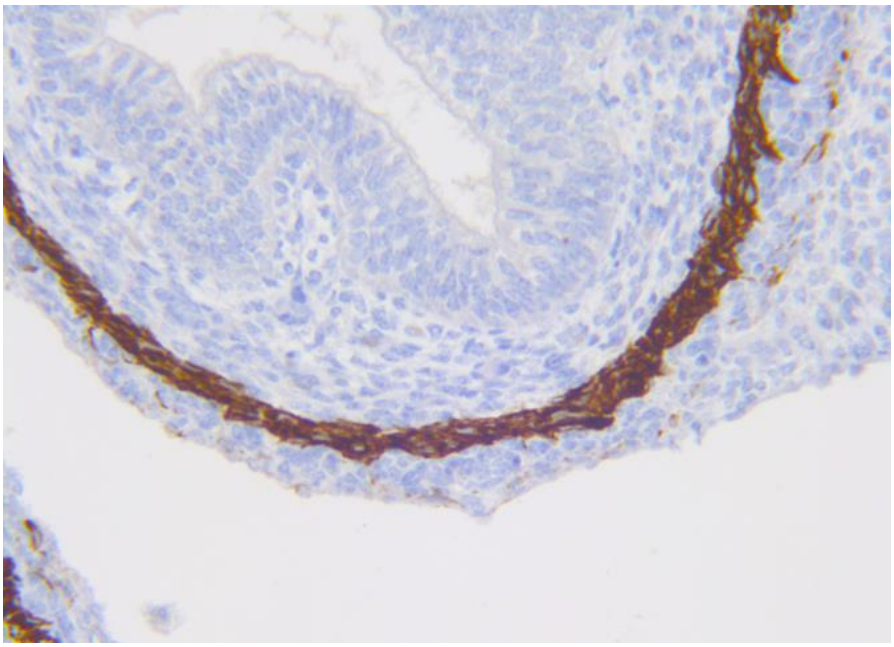

Figure 1. Desmin immunoreactive cells (dark brown stained) are present in the form of a concentric band and correspond to the circular muscle layer. Desmin immunohistochemistry, x200

PDGFRα immunoreactivity is present in all parts of the human primitive gut (Figure 2) at week 11. PDGFRα immunoreactive cells are present within the circular muscle layer, while they are not present in the myenteric plexus (MP) region, or in the parts below the serosa. PDGFRα+ cells are elongated, spindle-shaped cells, oriented in parallel to the longitudinal axis of the smooth muscle cells within the circular gut muscle layer. In the submucosal region, there are two PDGFRα low immunoreactive cells (Figure 2 arrowhead).

In week 11 of human development, c-kit immunoreactive cells are present in all parts of the gut in the MP region, as continuous rows and nets of cells present around the MP ganglia, at the outer border of the circular muscle layer (Figure 3). C-kit immunoreactive cells lie at the edges of the inception of the MP ganglia but they are not present within them. They are also absent within the circular muscle layer, as well as in the region below the serosa. In submucosa, in the area where submucosal ganglions develop, c-kit immunoreactivity is also absent. C-kit immunoreactive cells are multipolar with large round or oval nuclei, a small body, and numerous thin processes. Their processes form a network around the MP ganglia. In addition to ICC, a large number of c-kit immunoreactive mast cells are present, but they are easily distinguished from ICC on the basis of their shape and granular content.

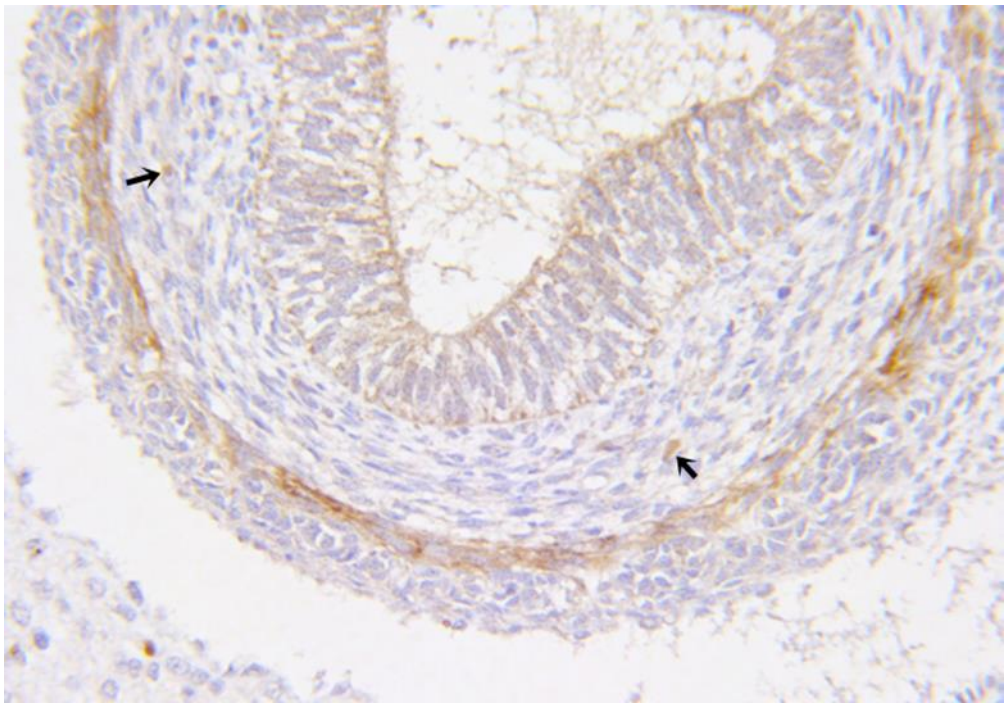

Figure 2. The distal portion of the midgut at 11th week of fetal development. PDGFRα immunoreactive cells (dark brown stained) are present within the circular muscle layer. Two PDGFRα low immunoreactive cells in the submucosal region (arrowhead). PDGFRα immunohistochemistry, x200

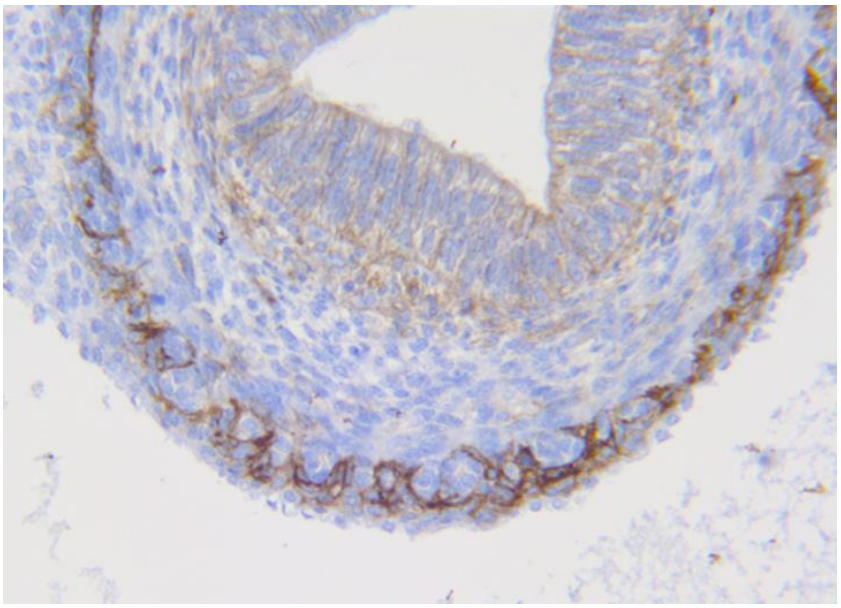

Figure 3. The distal portion of the midgut at 11th week of fetal development. C-kit immunoreactive cells (dark brown stained) are present in the MP region and clearly limit the onset of the MP ganglia (arrows). C-kit immunohistochemistry, x200

The comparison of desmin, C-kit and PDGFRα immunoreactivity in the distal midgut clearly shows that the localization of C-kit and PDGFRα immunoreactive cells is significantly different (Figure 4).

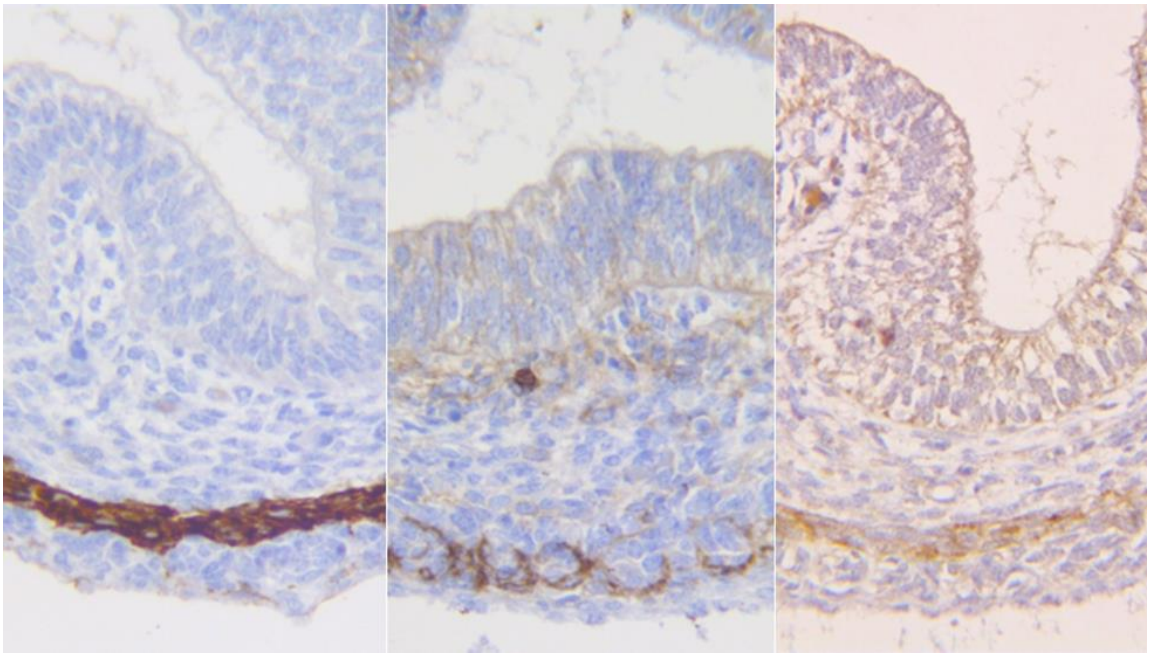

Figure 4. Comparison of desmin, C-kit and PDGFRα immunoreactivity in the distal midgut at 11th week of fetal development. PDGFRα immunoreactive cells are present only within the circular muscle layer, while ICGs do not exhibit PDGFRα immunoreactivity. x200

Discussion

Based on the desmin immunohistochemistry results of our research, we can observe that in all parts of the fetal intestine, the circular muscular layer develops first, and only later the longitudinal layer, following the principle of proximal-distal gradient. These results are consistent with the previous studies (35, 36). During 11th and 12th week of development, C-kit immunopositive cells, which correspond to ICC, are present in all parts of the human fetal intestine in the MP region and are surrounding the inception of the MP ganglia, but they are absent within the circular muscle layer. This finding is also consistent with the results of previous studies, which indicate that intramuscular ICC develop only in the late fetal period, while certain ICC subtypes develop after birth (37-39).

Platelet derived growth factor (PDGF) is a major mitogen for many cells of mesenchymal origin, including fibroblasts and smooth muscle cells, which is why PDGF signaling is especially important during embryogenesis (34, 40). PDGF receptor A (PDGFRα) is a receptor present on the surface of a large number of cell types, which binds one of the PDGF isoforms, causing cellular growth and differentiation during organ development and is responsible for the normal functioning of tissues and organs (41, 42). Previously considered mainly a developmental growth factor receptor in the GI tract, the discovery of receptor tyrosine kinase PDGFRα expression in "fibroblast-like" cells within tunica muscularis (19) has opened the door to new trials and definitions of the role of these cells (23, 27). The most significant finding of our study is that PDGFRα immunoreactive cells are present in all parts of the human intestine in the early fetal development period. These cells are localized within the circular muscle layer, on the contrary, no PDGFRα+ cells have been observed in the MP region as well as in parts just below the serosa. An identical localization of PDGFRα immunoreactive cells also exists in the gut of adults, with the difference that they are also present in the MP region in adults, where they form three-dimensional networks. This position of PDGFRα immunoreactive cells is consistent with the fact about their possible role in neurotransmission of signaling from ICC to smooth muscle cells (23, 43). It has been previously reported that PDGFRα+ cells have important roles in the morphogenesis of small intestinal mucosa villus of the mouse (44, 45). PDGFRα+ cells were also found in the subepithelial layer of the adult guinea pig GI tract (46). However, the details of the distribution and functions of PDGFRα+ cells in fetus GI tract have not been reported.

Kurahashi et al. have described a specific type of PDGFRα+ cells in the lamina propria of the human GI tract (47), and suggested that subepithelial PDGFRα+ cells have a role in sensory and secretomotor signaling, proliferation, differentiation, and apoptosis of epithelial cells, and in epithelial cellular pathology, including inflammatory responses and tumorigenesis. Subepithelial PDGFRα+ cells in adults form a sheath just beneath the epithelium and cover the crypts from their base to the luminal surface of the epithelium. It is suggested that these cells may have modulatory functions in immune and sensory responses and in the maintenance of mucosal homeostasis, but the roles of these cells in physiological and pathophysiological processes are still unknown. In our research, there was a low PDGFRα submucosal immunoreactivity during the early period of fetal development, and dominant PDGFRα were present within the circular muscle layer. As already mentioned, due to their close contact with enteric nerve endings and smooth muscle cells within the circular muscle layer, it is assumed that these cells primarily play a role in the neuromodulation of peristalsis.

Another important result of our study is that PDGFRα cells differ from ICC and that they are functionally close but still different cell types of interstitial cells. In adults, PDGFRα cells are widely distributed within the MP region and in circular and longitudinal muscle layers throughout the human colon (23). Blair et al. (21) have shown relationships between enteric neurons and interstitial cells in primates. They have shown that PDGFRα+ cells are closely associated with ICC and occupy the same anatomical niches as ICC-MY and ICC-IM. However, in contrast to the distribution of intestinal cells in adults, we showed in our study that, in the fetal period, PDGFRα activity was not observed in the ICC-MY domains around the ganglia. This finding indicates that PDGFRα cells develop later than the ICC within GI tract. Further, our results show that unlike the region of MP, PDGF cells are present in the circular muscle in the fetal period and there are still no differentiated ICC-IM.

Ultrastructural studies show that enteric neurons do not effectuate any direct contact with smooth muscle cells or synaptic specializations; on the other hand, ICC make close contacts with both cholinergic and nitrinergic neurons, forming synapse-like connections at one end and gap junctions with smooth muscle cells (3, 48). Calcium activated chloride channels (Ano1) are highly expressed and exclusively by ICC throughout the GI tract (21, 49), so the major excitatory neurotransmitter – acetylcholine induces depolarization by the activation of Ano1 currents (50). ICC-IM also respond to inhibitory neurotransmitters between neurons and smooth muscles (51). PDGFRα+ are very similar to ICC-IM in adults, and also form gap junctions with surrounding smooth muscle cells (3, 20, 21). PDGFRα+ cells express guanylate cyclase, purinergic P2Y1 receptors and small conductance Ca^{2+}- activated K^+ (SK_3K^+) channels (23, 27, 52) which indicates that PDGFRα+ cells mediate enteric inhibitory neurotransmision. It has been confirmed that PDGFRα+ cells, like ICC, generate inhibitory post junctional responses in GI muscles (27). Gap junctions provide electrical coupling between cells, such that induction of a K^+ current in PDGFRα+ cells results in hyperpolarization, first of these cells and immediately after that in net hyperpolarization of the SIP syncytium and reduce muscle contraction.

In contrast to our results, PDGFRα+ cells in adults form a network adjacent to ICC around the myenteric ganglia (23). ICC-MY have a pacemaker role to generate spontaneous electrical activity, while the function of myenteric PDGFRα+ is still unknown. Since they are interconnected with ICC-MP, they may have a role in the propagation and modulation of the electrical peristaltic waves.

At present, little is known about the involvement of PDGFRα+ cells in GI motor dysfunction. It is certain that the changes in purinergic neural inputs could have effects on colonic motility. Enhanced activation of PDGFRα in mice, contribute to the development of GI fibrosis and sarcoma (53). In a recent study (54), it has been concluded that colonic transit disorder may be due to the downregulation of the Kit and Ano1 channels and the upregulation of SK_3 channels in PDGFRα+ cells, suggesting that the imbalance between ICC and PDGFRα distribution might be a possible reason for gut dysmotility. Furthermore, some stromal tumors (GIST) are positive for PDGFRα (55, 56), so it is possible that these cells, like ICC, can be malignantly transformed.

Conclusion

In the fetal period of human development, PDGFRα immunoreactive cells are present in all parts of the intestine, localized within the circular muscle layer, and do not coincide with ICC.

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DISTRIBUCIJA PDGFRα+ ĆELIJA I INTERSTICIJALNIH ĆELIJA KAHALA U CREVU FETUSA ČOVEKA

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Unutar mišićnog sloja gastrointestinalnog (GI) trakta prisutna su dva tipa intersticijalnih ćelija: Intersticijalne ćelije Kahala (IĆK) i „fibroblastima-slične" ćelije, nedavno nazvane receptor za trombocitni faktora rasta pozitivne (PDGFRα+) ćelije. IĆK i PDGFRα+ ćelije predstavljaju različite ćelijske klase jedinstvene ultrastrukture i funkcije i zauzimaju iste anatomske niše u GI traktu. Smatra se da PDGFRα+ ćelije, kao i IĆK, učestvuju u modulaciji inhibitorne neurotransmisije. Trombocitni faktor rasta (PDGF) glavni je mitogen za mnoge ćelije mezenhimalnog porekla, kao što su fibroblasti i glatko mišićne ćelije, te je PDGF signalizacija neophodna tokom organogeneze.

Cilj rada je da identifikuje PDGFRα imunoreaktivne ćelije u crevima fetusa čoveka, kao i da odredi njihovu distribuciju u odnosu na glatko mišićne ćelije, IĆK i strukture enteričkog sistema.

Materijal je činilo 12 humanih fetusa, gestacione starosti od 10. do 12. nedelje. Imunohistohemijsko ispitivanje vršeno je PDGFRα antitelom, IĆK identifikovane su pomoću C-kit antitela, dok su mišićne strukture dokazane desmin antitelom.

PDGFRα imunoreaktivne ćelije prisutne su unutar kružnog mišićnog sloja, dok su odsutne u regionu mijenteričnog pleksusa i u delovima ispod seroze. Za razliku od njih, IĆK prisutne su samo oko začetaka gangliona mijenteričnog pleksusa.

U fetusnom periodu razvića čoveka, PDGFRα imunoreaktivne ćelije prisutne su u svim delovima creva, lokalizovane su unutar kružnog mišićnog sloja i ne podudaraju se sa IĆK.

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Ključne reči: *PDGFRα, C-kit, intersticijalne ćelije Kahala, gastrointestinalni trakt fetusa*

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EARLY DETECTION OF POTENTIAL CHRONIC ALCOHOLISM BY DETERMINING THE LEVEL OF IGA, MCV AND TRANSFERRIN

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The level of IgA, transferrin, and the MCV and the ratio of IgA/transferrin were used for the detection of parameters of chronic alcoholism and in parallel, the GGT activity was measured. Three groups of subjects were processed for this study: healthy subjects (N = 18), chronic alcoholics (N = 20) treated chronic alcoholics (N = 15), and finally, a group of workers who are employed at the plant spirits (N = 15). A nonparametric Mann-Whitney U test was used to process the obtained values. There were no significant differences between treated alcoholics and healthy subjects in all parameters tested (mean value with an AA: IgA = 2.9g/L, MCV = 93.8, transferrin = 3.1g/L, IgA/transferrin = 0.92, GGT = 11.4U/L; healthy subjects: IgA = 2.83g/L, MCV = 93.6, transferrin = 3.2g/L, IgA/transferrin = 0.9, GGT = 7.1U/L). In the group of untreated alcoholics, IgA values, MCV, and IgA/transferrin ratio were increased as well as the GGT activity (as expected) compared to healthy and treated alcoholics (alcoholics: IgA = 5.1g/L, MCV = 101, transferrin = 2.78g/L, IgA/transferrin = 1.74, GGT = 101.4U/L). Regarding the workers employed in the factory of alcoholic beverages, the level of MCV was significantly higher than in healthy individuals and that value was close to that in alcoholics (IgA = 1.95g/L, MCV = 99.6, transferrin = 2.8g/L, IgA/transferrin = 1.14, GGT = 18.7U/L). The concentration of transferrin was reduced in workers, so the ratio of IgA/transferrin is slightly higher than in healthy individuals, but without a significant difference. It can be seen that the results of the examined parameters may help not only to detect chronic alcoholics but also for monitoring the treatment, given the fact that, paradoxically, many alcoholics use alcohol while under treatment.

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Key words: *chronic disease, alcoholism, erythrocyte indices, MCV, transferrin, IgA, early detection*

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Introduction

Alcoholism is one of the most important problems of modern society because of its health and social consequences. Alcohol exerts harmful effects on the human body, causes psychopathological changes, neurological diseases, as well as organic changes in the cardiac muscle, especially in the stomach and liver. The social consequences of alcohol abuse are manifested in all aspects of life and work. One first starts in the family and the immediate social environment of alcoholics, and then expands to a broader, particularly work environment. Thus, alcoholism is not an isolated, personal problem or matter for the individual alcoholic, but a problem of the whole family and the social group in which the patient lives and works. Recent statistics show that alcoholism is the third most common disease in men.

The rich vineyard of the parish of Aleksandrovac is a significant producer and consumer of alcoholic beverages. The increased number of cases of chronic alcoholism in the municipality of Kruševac has led to the inclusion of a Clinic for mental health to provide treatment for people addicted to alcohol. The group family therapy was chosen as the most effective type of treatment, and the Club of Treated Alcoholics was formed, in the period from 1987 to 1990, and it included 142 patients. Of this number, 67 patients were cured without recurrence, 20 patients with recurrence, and 45 patients left the treatment. Since then, 48 other regular Club members have been cured. The success of the family group therapy was 45.53%, which encourages and promotes the further work of the Club, as a reputable

institution with success in the treatment of about 70%.

Somatic treatment of patients included a laboratory test to determine the general health of patients. Since it is often difficult to detect chronic alcoholism, and bearing in mind the fact that many alcoholics used alcohol while under treatment, we have included laboratory and processing parameters that are used for the detection of alcoholism: the activity of gamma-glutamyl transferase (GGT) levels, levels of immunoglobulin A (IgA) and serum transferrin, MCV and relationship IgA/transferrin. The aim was to determine which of these laboratory parameters could be most effectively used to detect chronic alcoholism (1, 2).

Materials and methods

Four groups of patients were determined for this study: chronic alcoholics (N = 20) treated chronic alcoholics, club members treated alcoholics (N = 15), a group of workers who are employed in the factory of alcoholic beverages (N = 15), and finally a group of healthy individuals which do not consume alcohol (N = 18), and the non-parametric Mann-Whitney U test was used to compare the obtained values.

The people who took part in the testing were chronic alcoholics who consumed alcohol for 15 years, treated alcoholics who consumed alcohol for 10 years, and workers who were exposed to ethanol for 11 years. In all subjects the following parameters were determined:

- GGT enzyme activity was determined by enzyme-kinetic color test from YUGOMEDIC by the method of Szasz's. GGT catalyzes the transfer of the gamma-glutamyl group of L-gamma-glutamyl-3-carboxy-4-nitro-anilide to glycylglycine. The color intensity was measured by creating a speed of 5-amino-2-nitrobenzoate at 405 nm at the temperature of 30 ^{O}C and it is proportional to the GGT activity in the sample. Reference values range from 4 to 28 IU/L.
- MCV was obtained from the processing of complete blood count on reading Culter JS. Reference values range from 82 to 97 fl.
- IgA (immunoglobulin A) and transferrin were determined by radial immunodiffusion on plates from Behring Werke Corporation, using the method of Mancini. Reference values for the IgA range from 0.90 to 4.50 g/L and transferrin from 2,00 to 4,00 g/L.

Results

Long-term use of alcohol causes dysfunction of the body which reflects the pathological findings of laboratory tests such as reducing the synthesis of proteins, changes in enzyme activity and so on (3, 4).

Table 1. Laboratory parameters: MCV, IgA, transferrin, IgA/transferrin and GGT in four groups of test subjects

Laboratory analysis	MCV FL	IgA g/L	Transferrin g/L	IgA/ transferrin	GGT IU/L
Mean healthy persons N = 18	93.6	2.83	3.20	0.90	7.1
Mean treated alcoholics N = 15	93.8	2.90	3.10	0.92	11.4
Mean alcoholics N = 20	101.0	5.10	2.78	1.74	101.0
Mean workers exposed to ethanol N = 15	99.6	1.95	2.80	1.14	18.7

Our findings confirm the experience of other authors that the detection and control of chronic alcoholism GGT proved to be a very good parameter. The alcohol stimulates the hepatic synthesis of GGT at the cellular membrane. In our analyses, GGT activity was very high in the group of chronic alcoholics compared to the control group. In the other two groups, there was no statistical significance, because the activity of GGT in treated alcoholics who abstained for more than a year was very close to the control group. What diminishes the value of the determination of GGT activity as a screening test for the detection of alcoholics is its non-specificity, prevalence in almost all body tissues and fluids, and the inductive effect of a number of drugs on its activity. Therefore, it is necessary to determine the level and other parameters that have been found to be influenced by changing the alcohol in the body (5, 6).

Discussion

Degradation of tissue in hepatocellular necrosis under the influence of alcohol causes the formation of antibodies whose presence is determined by the concentration of immunoglobulin. The tested amount of immunoglobulins A, G, and M (IgA, IgG, IgM) has significantly higher values in the group of chronic alcoholics than in the control group (as shown by IgA), which is also found in other authors. The mean value of IgA in untreated alcoholics was

significantly increased as compared to the control group, while the other groups showed no statistical difference compared to the control group. Accordingly, the IgA is the second parameter that would be used in screening assays for the detection and control of alcoholics.

In the group of chronic alcoholics, those with a reduced amount of transferrin were found in comparison with the control group, which is explained by alcohol-toxic liver damage which results in difficult protein synthesis and their transfer. The workers exposed to ethanol showed decreased transferrin concentration, while the mean transferrin in treated alcoholics was unchanged compared to the control group. Increased concentrations of IgA under the influence of alcohol and reduced levels of transferrin expressed by the quotient of IgA/transferrin result in an increase of the quotient which may be useful in the study of alcoholics are available as the third parameter. The ratio of IgA/transferrin in chronic alcoholics is increased with a statistical significance, as a group of workers in this ratio is slightly higher than in the control group, but without significant differences.

Finally, MCV in alcoholics showed an increase that reflects discrete anemia. MCV has increased in the group of untreated alcoholics compared to healthy persons and recovering alcoholics.

As for the workers employed in the factory of alcoholic beverages, MCV level was significantly increased compared to healthy subjects and reached a value close to that of an alcoholic.

Conclusion

The level of IgA, transferrin, and the MCV and the ratio of IgA/transferrin are parameters that can be used for the detection of alcohol in parallel with the determination of GGT activity.

There was no significant difference between the treated alcoholics and healthy controls in all tested parameters, which indicates a well-chosen way of treating alcoholics.

In the group of untreated alcoholics, the value of the alcoholic IgA, MCV, and the ratio of IgA/ transferrin were increased as well as the activity of GGT (as expected) compared to the healthy individuals and treated alcoholics.

As for the workers employed in the factory of alcoholic beverages, the MCV level was significantly higher than in healthy individuals, and this value is close to that of alcoholics as a reflection of anemia. In this group, the transferrin concentration is reduced, so that the ratio of IgA/ transferrin is slightly higher than that in healthy persons, but without significant differences. Both parameters indicate chronic ethanol intoxication of workers (7, 8).

Looking at the results of the examined parameters, one can see that they can help in the detection of chronic alcoholics and to monitor the treatment given the fact that many alcoholics use alcohol while under treatment. In chronic alcoholic's abstinence, the increase in IgA levels and the ratio of IgA/transferrin, as well as the increase in the activity of the enzyme GGT provide evidence of even minor or sporadic use of alcohol, and normal levels of these parameters in the serum during the treatment of abstinence were confirmed.

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RANO OTKRIVANJE POTENCIJALNOG HRONIČNOG ALKOHOLIZMA ODREĐIVANJEM NIVOA IGA, MCV I TRANSFERINA

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Nivo IgA, MCV i transferina, kao i odnos IgA i transferina, korišćeni su parametri za detekciju hroničnog alkoholizma. Paralelno sa time određivana je aktivnost GGT. Četiri grupe ispitanika obrađivano je za ovo istraživanje: zdrave osobe (N = 18), hronični alkoholičari (N = 20), lečeni hronični alkoholičari (N = 15) i najzad grupa radnika zaposlenih u fabrici alkoholnih pića (N = 15). Neparametrijski Mann-Whitney U-test korišćen je za procesuiranje dobijenih vrednosti. Nije bilo značajnije razlike između lečenih alkoholičara i zdravih osoba u svim ispitivanim parametrima (srednja vrednost kod lečenih alkoholičara: IgA = 2,9 g/L; MCV = 93,8; transferin = 3,1 g/L; IgA/transferin = 0,92; GGT = 11,4U /L; zdrave osobe: IgA = 2,83 g/L; MCV = 93,6; transferin = 3,2 g/L; IgA/transferin = 0,9; GGT = 7,1 U/L).U grupi nelečenih alkoholičara vrednosti IgA, MCV i odnos IgA i transferin povećane su kao što je povećana i aktivnost GGT (što je uobičajeno) u odnosu na aktivnost GGT kod zdravih osoba i lečenih alkoholičara (alkoholičari: IgA = 5,1 g/L; MCV = 101; transferin = 2,78 g/L. IgA/transferin = 1,74; GGT = 101,4 U/L). Što se tiče radnika zaposlenih u fabrici alkoholnih pića, nivo MCV značajno je povećan u odnosu na nivo kod zdravih osoba i ta je vrednost bliska onoj kod alkoholičara (radnici: IgA = 1,95 g/L; MCV = 99,6; transferin = 2,8 g/L; IgA/transferin = 1,14; GGT = 18,7 U/L). Koncentracija transferina smanjena je kod radnika, pa je i odnos IgA/ transferin neznatno viši nego kod zdravih osoba, ali bez značajne razlike. Može se videti da dobijeni rezultati ispitivanih parametara mogu pomoći ne samo za otkrivanje hroničnog alkoholizma, već i za praćenje lečenja, imajući u vidu činjenicu da mnogi alkoholičari koriste alkohol dok su podvrgnuti tretmanu.

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Ključne reči: *hronična bolest, alkoholizam, eritrocitni indeksi, MCV, transferin IgA, rano otkrivanje*

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RELATIONSHIP BETWEEN BODY COMPOSITION AND VERTICAL JUMP PERFORMANCE AMONG ADOLESCENTS

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With the aim to investigate the relationship between the body composition of adolescents and their vertical jump performance, this research was carried out on a sample of seventh grade elementary school students (47 male students). The sample of measuring instruments for body composition assessment included: body height, body mass, sum of five skinfolds thicknesses (biceps, triceps, subscapularis, suprailiac, and calf), body fat percentage, and muscle mass percentage. The SJ and CMJ tests were used for the assessment of vertical jump performance. At the multivariate level the results showed that body composition, as a predictor system, explained 44% (p = .000) of the variance of SJ and 41% (p = .000) of the variance of CMJ. At the univariate level of it was noted that the sum of five skinfolds had a high influence on the predictor system for SJ (t = -3.77; p = .001) and also a high influence on CMJ (t = -2.98; p = .005). The sum of five skinfolds had a negative impact on SJ and CMJ tests for vertical jump performance assessment. It could be concluded that the relationship between body composition components and vertical jump performance was clearly demonstrated in adolescents.

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Key words: *relationship, body composition, vertical jump, adolescents*

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Introduction

Vertical jump performance is an important factor in many sports, and the increasing vertical displacement of an athlete can positively affect achievement in sports (1). Also, vertical jump performance is very often used for talent identification and prediction of future success in sports, especially among adolescents in sensitive periods, when growth and development occur very dynamically (2, 3).

Beside physiological and biomechanical factors, vertical jump performance is affected by various anthropometric characteristics (4, 5). Most of the studies report that well-developed muscle mass and low percentage of subcutaneous adipose tissue are predictors of good sport performance (6-8).

Body composition has been described as a confounding factor in vertical jump performance, and several studies have attempted to categorize those body composition variables which better explain jump ability during childhood and adolescence (9), and tried to determine the nature of the relationship between anthropometric factors and vertical jump performance (4, 5, 10-14).

Two studies have identified a significant negative relationship between the sum of skinfold thickness and vertical jump among karate athletes (12) and hockey and cycling athletes (5). Researchers have also suggested lower skinfold thickness is preferable as it indicates lesser fat mass which generates no power but has to be carried vertically while jumping. Similar results were found among recreational athletes (10), where body fat percentage is negatively related to vertical jump height. Marković and Jarić (11) investigate the relationship between vertical jump height and body mass, and the results show that body mass is independent of vertical jump height. Previous studies found no significant relationship between body height relative to vertical jump performance (10, 13, 15).

Anthropometry is considered a simple, inexpensive and easy-to-use method in epidemiological studies. Skinfold measurements provide excess

weight information, and the sum of skinfolds indicates body fat distribution (16).

A reliable and valid approach for estimation of human body composition parameters is a technique known as bioelectrical impedance (BIA). This method is noninvasive, safe, provides rapid measurements, requires little operator skills and subject cooperation, and is portable (17).

The most commonly used tests for assessing explosive strength of the legs and jumping ability are the squad jump (SJ) and countermovement jump (CMJ). The SJ and CMJ tests are a simple, practical, valid, and very reliable measure of the lower body power compared to other jumping ability tests. The SJ is commonly used to test the concentric strength of the leg extensors, whereas the CMJ is used to measure the reactive strength of the lower body (18).

The aim

The aim of this study was to investigate the associations between body composition, the vertical jump performance of adolescents, and ability to predict SJ and CMJ from body composition variables.

Materials and methods

The sample of this study consisted of 47 7th grade elementary school male students, aged 13 years ± 6 months (BH: 165.44 ± 8.69 cm; BM: 56.62 ± 10.29 kg). The sample included every student who volunteered to participate in the research with the consent of their parents. An additional requirement was that students were clinically healthy during the testing.

All experimental procedures and possible risks and benefits were explained to each student. Signed consent was obtained from their parents prior to the onset of participation and approval to conduct the study was granted by school principals. The study protects the children's privacy by allowing for anonymity and was designed in compliance with the recommendations for clinical research of the Declaration of Helsinki (2013) of the World Medical Association. This study was also reviewed and approved by the Ethics Committee of the Faculty of Sport and Physical Education, University of Niš.

Body height (BH) was measured using the Martin anthropometer GPM 101 (GPM GmbH Switzerland), following standard procedures (19). Values of BH were measured and recorded in millimeters (mm). Body mass (BM) was measured with an accuracy of 0.1 kg with an electronic body weight scale Omron BF511 (Omron Healthcare Co, Kyoto, Japan). Skinfold thickness was measured using GPM 6100 (GPM GmbH Switzerland), with an accuracy of 0.2 mm at the biceps, triceps, subscapular, suprailiac and calf sites, according to the methodology recommended by the International Biological Program (20). A GPM caliper provides a constant pressure of 10g/mm^2. The measurement results were evaluated 2 seconds after the grip was caught on the skin. All five sites of skinfold thicknesses were summed up to provide the sum of skinfolds (SUM5). Body composition components, body fat percent (BF%), and muscle mass percent (MM%) were assessed using the BIA electronic scale Omron BF511. The participants were asked to avoid the following procedures before body composition measurement, as described by Rech et al. (21), not to perform any physical exercises 12 hours before testing, not to eat or drink anything during the four hours before the evaluation, to urinate at least 30 minutes before the evaluation, not to take any diuretics during the seven days prior to the test, and not to consume alcohol during 48 hours preceding the test.

Optojump (Microgate, Italy) is used for SJ and CMJ assessment. The Optojump system consists of 2 transmitting and receiving bars equipped with 33 optical Light-Emitting Diods (LEDs) fitted in the transmitting bar at 3,125 cm intervals. The LEDs on the transmitting bar communicate continuously with those on the receiving bar. The system detects any interruptions in communication between the bars and calculates their duration. This makes it possible to measure flight and contact times during the performance of a series of jumps with an accuracy of 1/1,000 of a second.

For the Sqaut Jump (SJ), the participants started from an upright standing position with their hands on their hips; they were then instructed to flex their knees and hold a predetermined knee position (~90^O) for a count of 3 s. At that point, the participants were instructed to jump as high as possible without performing any countermovement phase. For the Countermovement Jump (CMJ), the participants started from an upright standing position with their hands on their hips (i.e., without an arm swing); they were then instructed to flex their knees (~90^O) as quickly as possible, and then jump as high as possible in the ensuing concentric phase (22).

Statistical analyses

Descriptive statistics, the Kolmogorov–Smirnov (normality of the distribution), and Levene's (homogeneity of variance) tests were calculated for all experimental data before inferential testing. Pearson simple and partial correlation coefficients were used to determine the correlation between the predictor variables (BH, BM, SUM5, BF%, MM%) and criterion variables (SJ, CMJ). For the further investigation of the association and explanatory power of the predictor variables for criterion variable, a multiple linear regression analysis was used. Statistical procedures were performed using STATISTICA 10 (StatSoft Inc, Tulsa OK, USA) and the level of significance was set at $p \leq 0.05$.

Results

The Kolmogorov-Smirnov test showed that the data were normally distributed and no violation of homogeneity of variance was found using Levene's test. With the normal distribution of the results of BC, SJ, and CMJ assessment, it is possible

to apply parametric statistical procedures. For determining the explanatory power of the predictor variables on the criterion variable, multiple linear regression analyses were applied. Selected BC predictor variables (BH, BM, SUM5, BF%, MM%) were included for building the initial regression models for prediction of SJ and CMJ as the criterion variables. The initial models were tested for assumptions for multicollinearity among predictor variables. The first step was the observation of the correlation matrix between the predictor variables. The correlation matrix showed that the variable BF% was in a high significant correlation with most of the predictor variables (BH, r =- 0.46; SUM5, r = 0.87; MM%, r = -0.96). The next step in the confirmation of the presence of multicollinearity was the observation of the variance inflation factor (VIF). After observation, the VIF value was high for BF% (VIF = 30.11); therefore, the presence of multicollinearity was confirmed for BF%. In the final step, BF% was excluded from the initial regression models due to multicollinearity with other variables from the predictor system. The remaining variables were included in the final regression models for further analysis. The results of this analysis are given in Table 1 for SJ, and Table 2 for CMJ.

Table 1 presents the results from the final regression model for SJ as a criterion. A statistically significant simple negative correlation was found between SUM5 and SJ, and a positive correlation between MM% and SJ. Furthermore, the analysis of partial correlation coefficients showed a statistically significant negative correlation between SUM5 and SJ. The multiple linear regression identified that BC components provided good explanatory power for SJ 44% (p = .000). The BC component with the highest explanatory power in the regression model for SJ was SUM5 (p = 0.001).

Table 1. Descriptive statistics, correlation coefficients and multiple regression model results for SJ

	Mean	SD	Min.	Max.	Beta	Part r	r	t(33)	p-level
BH (cm)	165.44	8.69	147.20	190.60	0.04	0.02	0.27	0.15	0.882
BM (kg)	56.62	10.29	38.70	78.00	0.31	0.18	-0.06	1.21	0.234
SUM5 (mm)	57.44	17.57	29.60	104.60	**-0.92**	**-0.50**	**-0.61**	**-3.77**	**0.001**
MM% (k%)	38.50	2.92	32.80	43.60	-0.23	-0.14	**0.50**	-0.89	0.378
SJ [cm]	22.70	4.86	8.90	30.90					
R = .66 R^2 = .44 F(4;42) = 8.26 SEE = 3.807 p = **.000***									

Legend: Mean - arithmetic mean; SD - standard deviation; Beta – standardized regression coefficient; Part r - partial correlation coefficient; r – simple correlation coefficient; R - coefficient of multiple correlation; R^2 - coefficient of multiple determination; F – F-test of the relationship between the dependent variable and the set of independent variables; SEE - standard error of estimate; p – coefficient of statistical significance of multiple regression.

Table 2. Descriptive statistics, correlation coefficients and multiple regression model results for CMJ

	Mean	SD	Min.	Max.	Beta	Part r	r	t(33)	p-level
BH (cm)	165.44	8.69	147.20	190.60	-0.07	-0.04	**0.30**	-0.25	0.804
BM (kg)	56.62	10.29	38.70	78.00	0.36	0.21	-0.02	1.38	0.176
SUM5 (mm)	57.44	17.57	29.60	104.60	**-0.75**	**-0.42**	**-0.58**	**-2.98**	**0.005**
MM% (k%)	38.50	2.92	32.80	43.60	0.01	0.00	**0.53**	0.03	0.976
CMJ [cm]	24.03	4.73	11.30	32.80					
R = .64 R^2 = .41 F(4;42) = 7.29 SEE = 3.806 p = **.000***									

Legend: Mean - arithmetic mean; SD - standard deviation; Beta – standardized regression coefficient; Part r - partial correlation coefficient; r – simple correlation coefficient; R - coefficient of multiple correlation; R^2 - coefficient of multiple determination; F – F-test of the relationship between the dependent variable and the set of independent variables; SEE - standard error of estimate; p – coefficient of statistical significance of multiple regression.

Similar results are presented in Table 2, where CMJ was the criterion variable. Statistically significant simple positive correlations were found between BH and MM% from the predictor system and CMJ, and a negative correlation between SUM5 and CMJ. Partial correlation analysis coefficients showed

a statistically significant negative correlation only between SUM5 and CMJ. The multiple linear regression identified that BC components provided good explanatory power for CMJ 41% (p = .000). The BC component with the highest explanatory power in the regression model for CMJ was SUM5 (p = 0.005).

Discussion

The results of anthropometric measures obtained from our sample showed that the average age, BH, and BM were similar to the values obtained in similar studies (3, 5, 9, 23).

The purpose of this study was to examine the relationship between body composition components and the vertical jump performance of 13-year-old boys. The SJ and CMJ have widely been accepted as a criterion for the assessment of vertical jump performance.

The results obtained from simple correlation coefficients for SJ indicate a significant negative correlation between SUM5 and SJ (r = -0.61), and a significant positive correlation between MM% and SJ (r = 0.50). The results from the simple correlation coefficients for CMJ are very similar with the previously outlined results for SJ in Table 1. A significant negative correlation was found between SUM5 and CMJ (r = -0.58), and significant positive correlations were found between MM% and CMJ (r = 0.53), and BH and CMJ (r = 0.30). These results were expected and support the findings of many previous studies which found similar correlation coefficients between body fat, muscle mass, and vertical jump performance (3-5, 9, 10, 12, 15, 23-26).

After the exclusion of BF% due to multicollinearity, all other body composition components were analyzed in order to explain the SJ and CMJ variation of adolescents included in this study.

At the multivariate level, the results showed that body composition, as a predictor system, explained 44% (p = .000) of the variance of SJ and 41% (p = .000) of the variance of CMJ. At the univariate level it was noticed that the sum of five skinfolds (biceps, triceps, subscapular, suprailiac and calf), with a significant negative partial correlation (SJ: Part. r = -0.50; CMJ: Part. r = -0.42) and a strong beta coefficient (SJ: Beta = -0.92; CMJ: Beta = -0.75), had a high influence on the predictor system for SJ (Table 1: t = -3.77; p = .001), and also a high influence on CMJ (Table 2: t = -2.98; p = .005). The sum of five skinfolds had a negative impact on SJ and CMJ tests for vertical jump performance assessment and represents a body composition marker that had the highest predictive power for SJ and CMJ.

The best explanatory power of SUM5 for predicting SJ and CMJ may indicate that using the sum of five sites of the skinfolds is more accurate for estimating the influence of total body fat on vertical jump performance, as fat distribution does not occur in a similar way for individuals. These results can be explained by the fact that body fat percentage is a good indicator of the total amount of adipose tissue in the body, but not the distribution of adipose tissue, while anthropometric measures of skinfolds are good indicators of body fat distribution in the body (27). When using the sum of these skinfolds, it is possible to clearly see the trend of global accumulation of body fat (16). Our data indicate that the participants have much more subcutaneous adipose tissue on their abdominal region (suprailiac skinfold) than the others skinfold sites.

The participants with lower body fat demonstrated the highest vertical jump performance, especially in SJ, in accordance with higher negative simple and partial correlation coefficients between SUM5 and SJ, as compared to SUM5 and CMJ. That can be explained by the difference in vertical jump techniques that are not biomechanically similar for SJ and CMJ (28). Hip extension velocity and trunk angular displacement are essential for jump height during the propulsive phase of the CMJ test (29). According to Piucco and Santos (25), excess body fat induces an increase in body mass which results in a loss of athletic performance in movements that involve speed and explosive power, such as jumps, since acceleration is equal to force divided by mass (30). It can be stated that lower subcutaneous adipose tissue, especially in the trunk region, is a desirable body composition component, since it means less ballast mass (fat) which generates no power. Thus it provides a lighter body mass to be carried and allows a higher vertical velocity to be achieved during jump performance (12).

The results of this study suggest that there is no significant relationship between body mass and vertical jump performance among adolescents. Our findings are similar to those of previous studies (4, 9, 10), which also showed that body mass has no significant bearing on the vertical jump. This is supported by the findings of Marković and Jarić (11), who studied the relationship between vertical jump height and body mass, where the result showed that body mass is independent of vertical jump height.

Our results indicate no significant relationship between body height relative to vertical jump performance on the SJ and CMJ tests, in accordance with previous studies (10, 13, 15), except a weak but still significant simple correlation that was found between BH and CMJ. Based on the conclusions of Maćkala et al. (31), that the highest correlation exists between body height and trunk length (r = 0.93), our assumption leads us again to propulsive phase of the CMJ test where trunk angular displacement is crucial for jump height (29).

Conclusion

From the obtained results it could be concluded that the relationship between body composition components and vertical jump performance was clearly demonstrated among the adolescents. With the exception of BH, BM, and MM%, the remaining predictor, SUM5, was able to explain the SJ and CMJ variation among the adolescents in this study. The sum of biceps, triceps, subscapularis, suprailiac, and calf skinfolds showed the best power to explain vertical jump performance with a negative impact on SJ and CMJ values among the adolescents. However, this study has some limitations which have to be pointed out. The sample included

only seventh grade students, and only field tests were used to estimate body composition. Future studies should be conducted including the same and other samples by using direct measures of body composition, such as computed tomography and magnetic resonance imaging, in order to develop more accurate regression models. Also, we must consider that the participants in this study were adolescents in sensitive periods, when growth and motor skill development occur very dynamically, so the obtained results are valid only for the same age groups. Future studies can be done to evaluate further influence of each skinfold site on vertical jump performance, to obtain more accurate information.

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RELACIJE IZMEĐU TELESNE KOMPOZICIJE I VISINE VERTIKALNOG SKOKA KOD ADOLESCENATA

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Sa ciljem da se utvrde relacije između telesne kompozicije i visine vertikalnog skoka kod adolescenata, sprovedeno je istraživanje na uzorku učenika sedmog razreda (47 dečaka). Uzorak mernih instrumenata za procenu telesne kompozicije je bio sačinjen od: telesne visine, telesne mase, sume pet kožnih nabora (tricepsa, bicepsa, leđa, trbuha i potkolenice), procenta masnog tkiva i procenta mišićnog tkiva, kao sistem prediktorskih varijabli. Kao kriterijumske varijable, visina skoka iz čučnja (SJ) i visina skoka iz čučnja sa pripremom (CMJ) primenjeni su za procenu visine vertikalnog skoka. Na multivarijantnom nivou, rezultati su pokazali da komponente telesne kompozicije, kao prediktorski sistem, objašnjavaju 44% (p = ,000) varijanse SJ i 41% (p = ,000) varijanse CMJ, kao kriterijuma. Na univarijantnom nivou uočeno je da suma kožnih nabora ima najveći uticaj u prediktorskom sistemu za kriterijum SJ (t = -3,77; p = ,001), kao i za kriterijum CMJ (t = -2,98; p = ,005). Suma kožnih nabora ima negativan uticaj na visinu vertikalnog skoka SJ i CMJ. Može se zaključiti da postoje značajne relacije između komponenti telesne kompozicije, kao prediktora, i visine vertikalnog skoka, kao kriterijuma, kod adolescenata.

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Ključne reči: *relacije, telesna kompozicija, vertikalni skok, adolescenti*

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CHARACTERISTICS OF CHRONIC VENOUS ULCERATION RELATED TO AGE

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Venous ulceration occurs in 1% of the total human population, and occurs more often in people older than 65 years. The aim of the study was to determine the characteristics of chronic venous ulceration (CVU) related to the patients age. The study included 102 ambulatory patients with CVU, treated at the Clinic of Skin Diseases of the Clinical Center Niš. The study group consisted of patients ≥ 65 years, while the control group consisted of patients < 65 years. The study used patients' data such as age, gender, history of deep vein thrombosis, previous episodes of ulceration, previous operation on the veins, body mass index, calf circumference (CC), number of ulcerations, ulceration localization, ulcer size, duration of the ulcer and ulcer locoregional characteristics. Patients aged ≥ 65 years had longer incidence of ulceration and a larger number of previous episodes of ulceration. In patients aged ≥ 65 years, calf circumference was statistically higher, which can significantly affect the speed and healing rate. Larger surface area were more commonly reported in patients aged < 65 years with statistically significant $p < 0.05$, while elderly patients had statistically ($p < 0.01$) prolonged ulceration. Concerning the locoregional characteristics of CVU, dermatitis was more commonly seen in patients aged ≥ 65 years, while lipodermatosclerosis was more prevalent in patients aged < 65 years. In relation to the wound infection, patients aged ≥ 65 years had a statistically higher prevalence of ulcer infections. Elderly patients with CVU represent a distinct group in terms of aetiology, natural history and prognosis.

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Key words: *chronic leg ulceration, venous ulceration, the elderly*

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Introduction

Venous ulceration is one of the most common vascular diseases in the human population. It arises as a complication of chronic venous insufficiency. Chronic venous insufficiency is responsible for the occurrence of about 70% of chronic ulcerations of the lower extremities, especially those that do not heal within 6 weeks (1).

Venous ulceration occurs in 1% of the total human population, and occurs more often in people older than 65 years of age. The ratio of presentation shows a significantly higher proportion of women in the ratio of 3:1 (women: men) (2-5).

Chronic venous ulceration (CVU) occurs in 0.6-3% of the population over 60 years and in patients over 80 years the percentage increases by another 5%. Chronic venous ulceration is a common cause of morbidity and their prevalence in human population range from 1.9% -13.1% (6-10). The incidence of ulceration is thought to increase as a result of aging and increased factors for atherosclerosis, such as smoking, obesity, and diabetes are also responsible. During the life, almost 10% of the population can develop a chronic wound, with a mortality of 2.5% (10).

The aim of the study was to determine the characteristics of CVU related to the patient's age.

Materials and methods

The study included 102 ambulatory patients aged over 18 years with a CVU, treated at the Clinic for Skin Diseases of the Clinical Center Niš. The study lasted from April 2015 to February 2016. According to the age, patients were divided into two groups. The study group consisted of patients ≥65 years of age, while the control group consisted of patients < 65 years of age.

Before joining the study, all patients were subjected to Doppler ultrasound (DUS) examination in order to evaluate the venous and arterial leg system. The ankle-brachial pressure index (ABPI) was measured in all patients. Venous aetiology of

ulceration was verified by DUS. Patients with venous ulcerations over 3 cm^2 and duration of illness of over 3 months were involved in the examination.

Patients with ABPI < 0.8, with cardiac insufficiency (ejection fraction < 35), pregnant women, patients with malignant diseases, diabetes, patients on immunosuppressive and cortical therapy were excluded from the study.

Patients who had ulcerations associated with skin vasculitis, pyoderma gangraenosum and other neutrophilic dermatoses were excluded from this study.

The study used patients' data such as age, gender, history of deep vein thrombosis, previous episodes of ulceration, previous operation on the veins, body mass index (BMI), calf circumference (CC), number of ulcerations, ulceration localization, ulcer size (US), duration of the ulcer. The study also used information regarding dermatitis, lipodermatosclerosis and ulcer infection as locoregional characteristics of ulcerations.

Determination of the dimensions of the ulceration was performed by measuring the maximum width and length of ulceration, as well as by computerized process, which consists of mapping the two-dimensional digital image onto the polygonal mesh.

The calf and ankle circumference were measured in the recumbent position with the maximal (calf) and the minimal (ankle) point determined visually.

Doppler scan was used to standardize the superficial, perforated and deep venous system of the lower extremities. Venous reflux greater than 1s was considered significant.

The reflux was categorized into three groups: presence of superficial reflux only; additional presence of one or more insufficient perforating calf veins, but no deep venous reflux; or the presence of deep venous reflux at any level of the cru-femoral axis, with or without varicose veins.

Data analysis

Values are expressed as the mean ± standard deviations (SD). Differences among groups were examined by Mann-Whitney Rank Sum Test, Chi-square test and by Fisher exact test (Jandel Sigma Stat, version 2).

Table 1. Comparison of patients developing chronic venous ulcerations in relation to age

Variable	Age < 65 (n = 51)	Age ≥ 65 (n = 51)	p – value
Gender			
Male; n (%)	24 (47.05%)	23 (45.1%)	n.s.
Female; n (%)	27 (52.95%)	28 (55.9%)	n.s.
Age			
(mean± SD)	52.81 ± 6.60	71.88 ± 5.76	n.a
Medijana (rank)	53 (42-64)	70 (65-85)	n.a
History of deep vein thrombosis			
n (%)	21 (41.17%)	22 (43.14%)	n.s.
Previous episodes of ulceration			
n (%)	31 (60.78%)	45 (88.23%)	< 0.01
Previous operations			
n (%)	18 (35.29%)	16 (31.37%)	n.s.
Body mass index			
(mean ± SD)	32.40 ± 1.34	29.99 ± 1.68	n.s.
Calf circumference (cm)			
(mean ± SD)	34.67 ± 6.23	38.56 ± 5.34	< 0.05
Number of the ulcers			
n (%)	44 (88%)a; 7 (12%)b	40 (76%)a; 11 (24%)b	n.s.
Localisation of the ulcers			
(right leg) n (%)	24 (47.06%)	24 (47.06%)	n.s.
Size of the ulcer (cm2)			
(mean ± SD)	16.99 ± 10.31	12.1 ± 5.86	< 0.05
Duration of the ulcer			
(yrs ± SD)	6.54 ± 3.68	12.16 ± 6.23	< 0.01
Superficial reflux only			
n (%)	2 (3.92%)	1 (1.96%)	n.s.
Perforating vein incompetence			
n (%)	30 (58.82%)	30 (58.82%)	n.s.
Deep venous reflux			
n (%)	22 (43.14%)	21 (41.17%)	n.s.
Dermatitis			
n (%)	23 (45.1%)	29 (56.86%)	< 0.05
Lipodermatosclerosis			
n (%)	30 (58.82%)	16 (31.37%)	< 0.01
Infection			
n (%)	30 (58.82%)	37 (72.55%)	< 0.05

Abbreviations: BMI – body mass index; a – one ulcer; b – two ulcers

*Mann-Whitney Rank Sum Test; †Chi-square test; n.s. - not significant, n.a. - not applicable

Results

Both the investigated group, age ≥ 65, and the control group, age < 65, had 51 patients. The average age of the age < 65 group was 53 years, while the average age of the age ≥ 65 group was 70 years. There was no statistically significant difference between sex and history of deep vein thrombosis. Somewhat higher representation of CVU was in the female population. Previous episodes of ulceration were statistically more common in the age ≥ 65 group ($p > 0.01$). Concerning previous operations on the venous system and body mass index values, the number of ulceration and localization of ulceration did not have a statistically significant difference between the examined and the control group of patients. Calf circumference was statistically higher $p < 0.05$ in the age ≥ 65 group. Larger surface area were more commonly reported in patients aged < 65 years with statistically significant $p < 0.05$, while elderly patients aged ≥ 65 years had statistically ($p < 0.01$) longer prolonged ulcerations. There was no statistically significant difference in surface reflux only, perforating vein incompetence and deep venous reflux among examined groups. Concerning the locoregional characteristics of CVU, dermatitis was more prevalent in patients aged ≥ 65 years ($p < 0.05$), while lipodermatosclerosis was more prevalent in patients aged < 65 years ($p < 0.01$). In relation to the infection of ulcers, statistically higher prevalence was found in the age ≥ 65 group ($p < 0.05$) (Table 1).

Discussion

Venous leg ulcers are one of the most common vascular diseases in human populations. Venous ulcer represents the clinical expression of decompensated chronic venous insufficiency. The average age of patients with chronic venous ulcers is between 50 and 60 years, out of which 55% of patients are female and 45% male (11). Venous ulceration occurs most commonly in patients older than 65 years, although the incidence and prevalence of chronic venous ulceration in the elderly population is not well established. The study of Margolis et al. (12) aimed to highlight the prevalence and incidence of chronic venous ulceration in the population of patients over 65 years of age. Data are obtained from the primary health care register. The annual prevalence of venous ulceration among the older population was 1.69. The overall incidence of venous ulceration was 0.76 for men and 1.42 for women. The study showed that chronic venous ulceration is a significant health problem in elderly patients.

In our study, the female gender was somewhat more represented. The average age of patients in the age < 65 group was 53 years, while in the age > 65 group it was 70 years.

A study by MacKenzie et al. (13) showed that patients who developed CVU before their 50th birthday were more likely to be male, obese, had a worse disease that appeared less responsive to treatment and had a history of deep vein thrombosis (DVT) and/or long bone fracture suggesting a post-thrombotic aetiology. The proportion of CVUs that are post-thrombotic in aetiology remains controversial. However, recent data suggesting that up to 50% of all CVU patients have at least one thrombophilia suggests that previous, often unrecognized DVT probably plays a significant role in the development of a larger proportion of ulcers than previously appreciated.

There was no statistically significant difference between sex groups and history of deep vein thrombosis. Higher representation of CVU was in the female population. Previous episodes of ulceration were statistically more common in the age ≥ 65 group ($p > 0.01$). Concerning previous operations on the venous system and body mass index values, the number of ulceration and localization of ulceration did not have a statistically significant difference between the examined and the control group of patients. Calf circumference was statistically higher ($p < 0.05$) in the age ≥ 65 group of patients. Several studies (11, 14) showed the importance of calf circumference in healing CVU. Calf circumference < 33cm represents a positive predictor for successful healing of venous ulceration, and patients with a larger calf circumference represent a risk group and require more intensive and prolonged therapy.

A number of studies were concerned with the presence of chronic venous disorders, surgery on the venous system and their effect on the healing effect of chronic venous ulceration in patients with the use of a multiplex compressive bandage. Also, dermatological local characteristics of venous ulceration were monitored for their presence and influence on the effectiveness of healing chronic venous ulceration in patients with the applied multilayer compressive bandage. (1, 11, 14)

Large-area ulceration was more common in age < 65 with statistically significant $p < 0.05$, while elderly patients age ≥ 65 had statistically ($p < 0.01$) prolonged ulceration.

In summary, patients aged ≥ 65 years had longer incidence of ulceration and a larger number of previous episodes of ulceration. In patients aged ≥ 65 years, calf circumference was statistically higher, which can significantly affect the speed and effectiveness of healing of CVU. Larger surface area were more commonly reported in patients aged < 65 years with statistically significant $p < 0.05$, while elderly patients had statistically ($p < 0.01$) prolonged ulceration. Concerning the locoregional characteristics of CVU, dermatitis was more commonly seen in patients aged ≥ 65 years, while lipodermatosclerosis was more prevalent in patients aged < 65 years. In relation to the wound infection, patients aged ≥ 65 years had a statistically higher prevalence of infections.

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KARAKTERISTIKE HRONIČNE VENSKE ULCERACIJE POVEZANE SA STAROŠĆU

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Venska ulceracija javlja se kod 1% ukupne humane populacije, a češće se javlja kod osoba starijih od 65 godina. Cilj studije bio je da se odrede karakteristike hroničnih venskih ulceracija (CVU) u odnosu na starosnu dob bolesnika. Studijom su obuhvaćena 102 ambulantna bolesnika sa CVU, lečena na Klinici za kožne bolesti Kliničkog centra Niš. Studijska grupa sastojala se od bolesnika koji su imali 65 ili više godina, dok su kontrolnu grupu činili bolesnici mlađi od 65 godina. U studiji su korišćeni podaci bolesnika kao što su starost, pol, istorija duboke venske tromboze, prethodne epizode ulceracije, prethodna operacija na venama, indeks telesne mase, obim gležnja (CC), broj ulceracija, lokalizacija ulceracije, veličina ulceracija, trajanje ulceracija i lokalne karakteristike ulceracija. Bolesnici od 65 i više godina imali su dužu istoriju ulceracija, kao i veći broj prethodnih epizoda ulceracija.

Kod bolesnika starijih od 65 godina obim gležnja bio je statistički veći, što može znatno uticati na brzinu i efikasnost lečenja. Veća površina javlja se kod bolesnika starosne dobi manje od 65 godina sa statističkom značajnošću ($p < 0,05$), dok su stariji bolesnici imali dužu istoriju ulceracija ($p < 0,01$). Što se tiče lokalno-regionalnih karakteristika CVU, dermatitis se češće javlja kod bolesnika starijih od 65 godina, dok je lipodermatoskleroza bila preovlađujuća kod bolesnika starijih od 65 godina. U odnosu na infekciju rane, bolesnici koji su imali 65 ili više godina imali su statistički veću prevalenciju infekcija ulceracija. Stariji bolesnici sa CVU predstavljaju posebnu grupu u smislu etiologije, istorije i prognoze bolesti.

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Ključne reči: *hronične ulceracije nogu, venska ulceracija, starije osobe*

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SIGNIFICANCE OF GENOTYPIC ALPHA GALACTOSIDASE A MUTATIONS IN FABRY DISEASE TREATMENT

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Fabry disease (FD) is a rare inherited X-linked lysosomal storage disorder caused by a deficiency in alfa-galactosidase A (α-GAL). It is resulting in the accumulation of glycosphingolipids that leads to multiple organ dysfunction and ultimately signs and symptoms of the disease. The aim of this study was to examine the significance of genotypic α-GAL mutations in the treatment of FD. The disease can be divided into a severe, classical phenotype, and a milder nonclassical phenotype. Numerous α-GAL mutations are described in gene mutation databases. Missense, nonsense, consensus splice site, cryptic splicing, and frameshift mutations are reported. Enzyme replacement therapy (ERT) can lead to a significant clinical improvement. Depending on the α-GAL mutation, there are various recommendations for initiation of ERT in adult male and female patients with classic Fabry mutations, later-onset Fabry mutations or α-GAL variants of unknown significance. ERT with recombinant human agalsidase alfa and agalsidase beta is currently available therapy. Although there are no uniform guidelines, development of signs or symptoms related to FD should be an indication to start ERT. Treatment with ERT should be combined with adjuvant treatments for specific disease manifestations. Migalastat is a new oral pharmacological molecule developed as an alternative treatment to intravenous ERT for patients with FD and amenable mutations. Migalastat and ERT have similar effects on renal function in patients with FD. Long-term treatment of adult Fabry patients should involve timely ERT, regular assessment of disease progression in all patients, use of appropriate adjunctive therapies and multidisciplinary team approach.

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Introduction

Alfa-galactosidase A (α-GAL) is an enzyme that cleaves the terminal α-galactosyl unit of the α-gal epitopes and converts this epitope into a disaccharide called N-acetyllactosamine. This enzyme predominantly hydrolyzes ceramide trihexoside, and it can catalyze the hydrolysis of melibiose into galactose and glucose. The enzyme is encoded by the α-GAL gene. Defects in human α-GAL result in Fabry disease (FD), because of failure to catabolize α-D-galactosyl glycolipid moieties (1, 2). FD is a rare inherited X-linked lysosomal storage disorder caused by a deficiency in alfa-galactosidase A (α-GAL), which removes terminal galactose from various galacto-glycolipids (3). It is resulting in accumulation of glycosphingolipids with terminal a-D-galactosyl residue, particularly globotriaosylceramide and globotriaosylsphingosine, in plasma, vascular endothelial cells, podocytes, cardiomyocytes, and arterial smooth muscle cells (4–6).

Although the exact pathophysiology of FD is still only partly understood, this accumulation of glycosphingolipids leads to multiple organ involvement and ultimately signs and symptoms of the disease (7). This multisystem disorder is manifested by developing progressive proteinuric kidney disease, a fibrotic cardiac disease resulting in rhythm and conduction disturbances, progressive hypertrophic cardiomyopathy, small-fiber neuropathy, and mostly ischemic cerebrovascular stroke (4). Although FD is X–linked disorder, women often have less severe signs and symptoms of FD, compared with men (8). Cardiac involvement contributes to considerable morbidity and early death due to heart failure or ventricular arrhythmias. The incidence of FD has been estimated to be approximately 1 in 40,000 to 1

in 117,000 male (9, 10). Prevalence of FD may be much higher than previously believed. High incidence of later-onset FD was revealed by newborn screening (1 in 1,250 to 1 in 3,100 male newborns). Data in the literature shows that 1.2% of young patients with the unexplained acute cerebrovascular disease have FD (11–13).

The disease can be divided into a severe, classical phenotype, and a milder nonclassical phenotype. Patients with classical FD had a history of more events than patients with nonclassical disease and they are more likely to develop complications. Patients with classical FD most often have symptoms such as neuropathic pain, cornea verticillata, and angiokeratoma. Clinical manifestations include hypertrophic cardiomyopathy, arrhythmias, progressive renal failure, and stroke. Furthermore, patients with classical FD have lower glomerular filtration rate (GFR), higher left ventricular mass and higher plasma globotriaosylsphingosine concentrations than patients with nonclassical FD. Nonclassical FD is characterized by a more variable disease course, patients are generally moderately and mildly affected and disease manifestations may be limited to a single organ (4, 14, 15). There are described various manifestations of FD, as a cardiac variant with manifestations limited to the heart (with left ventricular hypertrophy of unknown etiology) and a renal variant among hemodialysis patients with unknown etiology. Lung manifestations, as dyspnea, wheezing and a dry cough are frequent symptoms in many Fabry patients (16). Thus, the great importance for the treatment of FD is accurate and early diagnosis (13, 17, 18).

There is a wide phenotypic variability among patients with FD. Patients with missense α-GAL mutations and variable residual enzyme activity have milder disease manifestations. For example, the N215S mutation may have residual enzyme activity in plasma. In heterozygous females, random X-inactivation may result in the normal level of α-GAL activity in the plasma or white blood cells in up to 60% of women (19). FD in early adulthood is characterized by more extensive angiokeratomas, high albuminuria (> 1g/24 hours), edema or lymphedema, fever, hypohidrosis or anhidrosis, lymphadenopathy, heat sensitivity, diarrhea, abdominal pain, and cardiac problems. FD in later adulthood is manifested by heart disease (usually fibrotic, left and right ventricular hypertrophy, heart valve abnormalities and dysrhythmias, angina, diastolic heart failure), chronic kidney disease including end-stage renal disease, stroke or transient ischemic attacks and deafness (19–22).

The aim of this study was to examine the significance of genotypic α-GAL mutations in the treatment of FD. The data used in the research are obtained from the books and relevant literature by means of PubMed browser.

Genetics

The complete genomic sequences of the human α-GAL gene have been determined and to date, several disease-causing α-GAL mutations have been identified, including missense mutations, small deletions, insertions, splice mutations, and large gene rearrangements (23).

More than 500 pathologic variants of α-GAL have already been described; most of them are family-specific. Numerous α-GAL mutations are described in gene mutation databases (24, 25). FD is caused by missense, nonsense, consensus splice site, cryptic splicing, and frameshift mutations (small and large deletions and insertions). Mentioned mutations are associated with the classic or later-onset FD phenotype, α-GAL variants of unclear significance, and benign variants. Patients with missense and nonsense mutations have nearly absent GLA activities and increased lyso- globotriaosylceramide levels (20, 26).

Patients with mutations within intronic or regulatory α-GAL regions have nonclassical FD phenotypes accompanied by late-onset organ manifestations such as cardiomyopathy, kidney failure, stroke, or neuropathic pain. The clinical impact of the α-GAL p.A143T variation in adult Fabry patients was determined in the recent study and patients with this mutation have clinical symptoms and manifestations compared with other classical missense mutations. Most male p.A143T patients had only slightly decreased residual α-GAL activities and normal lyso-Gb3 levels, while α-GAL activity in heterozygous females was normal. Female and male p.A143T patients had a less renal and cardiac involvement in comparison to FD patients with other missense mutations and these patients showed less severe FD-typical symptoms. Both gender p.A143T patients suffering from stroke or transient ischemic attack showed no further FD-typical organ manifestations. The risk for albuminuria and increased disease severity scores was significantly lower in p.A143T patients in comparison to groups with classical FD missense mutations. There was no accumulation of neurologic events in family members of p.A143T patients with stroke or transient ischemic attack. Female p.A143T patients with stroke or TRANSIENT ISCHEMIC ATTACK did not show skewed X chromosome inactivation. This study suggested the p.A143T variation to be more likely a neutral variant or a genetic modifier than a disease causing mutation. p.A143T could be a genetic variant of unknown significance. Thus, recommendation is that p.A143T patients with stroke or TRANSIENT ISCHEMIC ATTACK of unknown etiology should be further evaluated, since the diagnosis of FD is not probable and enzyme replacement therapy (ERT) or chaperone treatment should not be an unreflected option (27).

The multiplicity of mutations may contribute to variations in the residual enzyme activity and the different clinical presentations. Most of α-GAL gene variants is unique for each family. In study on FD screening in a predominantly hypertensive population with left ventricular hypertrophy (LVH) was found a mutation of unknown significance in α-GAL gene not previously described in the literature – GLA c.785G>T; p.W262L, whose significance could not be defined. This clinical investigation was able to establish the association between the mutation and the clinical presentation. It is also documented that the clinical management required defining the role of the mutation on the development of the clinical

presentation. This study allowed the definition of a novel causal mutation for Fabry disease – GLA c.785G > T; p.W262L (28).

Two new mutations were identified in Turkey. The female patient with M11V mutation had rheumatologic symptoms and microalbuminuria. The male patient with R190X mutation had a classical phenotype. R190X mutation causes premature termination, and probably leads to degradation of the protein (29).

Benign and probably benign variants have a high frequency. Medical specialists should be aware that, due to this high frequency, such mutations may be seen in screening studies. This benign polymorphism may not be related to actual Fabry-related manifestations, as there is no published evidence of lysosomal substrate accumulation in the tissues expressing them (30). Most often, nonsense, consensus splice site, and most frameshift mutations are associated with the classic phenotype. They result in low or no α-GAL enzyme activity. On the other side, missense mutations and rare cryptic splicing mutations are associated with the later-onset phenotypes and they result in enzymes with residual α-GAL activity. Current clinical studies have not stratified Fabry patients by genotype (26, 31).

Women with heterozygous α-GAL mutations suffer from significant multisystemic disease and reduced quality of life and must be monitored and treated accordingly. The asymptomatic female carrier of FD is the exception, not the rule (32). In female heterozygous Fabry patients who express the normally functioning α-GAL allele, symptoms are mild and rarely occur, while female patients who express the mutant α-GAL allele have a disease course which may be similar to the male disease phenotype (either classic or later-onset). It depends on the underlying α-GAL mutation in their family. At least 43% of obligate carrier women have severe clinical manifestations (33). While the onset of first Fabry-associated symptoms in affected hemizygous males with low or absent enzymatic α-GAL activity starts in early childhood, females with FD present with a heterogeneous clinical picture and variable disease progression, independent of the presence of a nonsense or a missense mutation. Recent study showed that although females with missense mutations seem to have a lower risk to suffer from severe FD manifestations, affected females showed a similar disease burden compared to females with nonsense mutations. The variation and the missing genotype-phenotype correlation might be due to the controversially discussed X-chromosomal inactivation.

Individuals with the c.196G > C nucleotide change which leads to the E66Q enzyme having low α-GAL activity and they have been suspected to have the later-onset Fabry disease phenotype leading to renal and cardiac disease. Biochemical, pathological and structural studies strongly suggest that the c.196G > C is not a pathogenic mutation but is a functional polymorphism (34).

Analysis of genotype-phenotype correlations in FD is complicated by a number of factors, such as the high proportion of private mutations and the large phenotypic heterogeneity (35). Some genotype-phenotype correlations, as the missense mutation p.N215S is established in patients with cardiac manifestations (hypertrophic cardiomyopathy) (36). Determining genotype-phenotype relationships is important for the assessment of whether affected patients may benefit from ERT. The atypical or late-onset type phenotypes present a therapeutic dilemma.

The genetic background of the patient, concomitant diseases, environmental modifiers and the presence of additional deleterious α-GAL variants or variants of unknown significance may alter the impact of a given gene mutation (30).

Management recommendations

Enzyme replacement therapy (ERT) can lead to significant clinical improvement. ERT with recombinant human agalsidase alfa and agalsidase beta is currently available therapy aimed at the etiology of FD. There is no scientific evidence when the optimal moment of ERT initiation is. Although there are no uniform guidelines, development of signs or symptoms related to FD should be an indication to start ERT. If ERT has not already been started earlier for nonrenal manifestations such as pain, the development of chronic kidney disease (CKD), pathological albuminuria, decreased GFR or progressive decrease in GFR may be an indication to start (37). Research recommendations for initiation of therapy include:

1) determination of the beginning treatment in asymptomatic patients, females, patients with nonclassic disease;

2) obtaining expanded information on the natural history of FD in classic female patients and nonclassic FD patients, and the effects of ERT in these groups and

3) undertaking X-linked inactivation studies and early initiation of therapy in females (19).

Depending on the mutation there are various recommendations for initiation of ERT in adult male and female patients with classic Fabry mutations, later-onset Fabry mutations or α-GAL variants of unclear significance. Depending on the approach to treatment, patients with classic Fabry mutation can be divided into three groups: symptomatic or asymptomatic male patients, symptomatic female patients, and asymptomatic female patients. In the first group, ERT should be considered and is appropriate in all patients at any age of presentation. Treatment decisions may be influenced by the advanced elderly age of the patient and severe comorbidity. In the second group, initiation of ERT is warranted when signs and symptoms suggesting major organ involvement occur. In the third group, ERT should be considered if there is a laboratory, histological, or imaging evidence of kidney, heart, or the cerebral injury. ERT should also be considered if a skewed X chromosome inactivation pattern with predominant expression of the mutant α-GAL allele with or without very low α-GAL activity has been demonstrated in the presence of signs and symptoms of the disease. Male and female patients with later-onset Fabry mutation or missense α-GAL variants of unclear significance should start ERT if there is a

laboratory, histological, or imaging evidence of injury to the kidney, heart, or the central nervous system, even in the absence of typical Fabry symptoms. The abnormalities should be attributable to FD; this may require histological assessment or biochemical evidence of globotriaosylceramide accumulation. The advice of an expert in genetics and management of FD should be sought for interpretation of the pathogenicity of any α-GAL variants of unclear significance. Individuals with well-characterized benign α-GAL polymorphisms should not be treated with ERT. In the absence of demonstrable FD-related tissue pathology or clinical symptoms, ERT may not be appropriate, particularly in heterozygous female patients. These patients should be monitored regularly by a multidisciplinary care team (19, 37–39). Current FD guidelines and recommendations suggest ERT initiation in females with FD after the onset of first FD-typical renal, cardiac, and cerebral complications, or in rapidly progressive disease (39, 40). Fifty-seven percent of female FD patients were under ERT, nearly all presented with different organ manifestations that justify ERT initiation according to current European guidelines. One third of females were untreated although indications (organ manifestations). Some of them refused ERT, or can't receive ERT due to pregnancy or future family planning, or they were refusing ERT because of possible benefit from future chaperone treatment (41).

Periodic monitoring of anti-agalsidase antibodies in patients receiving ERT is also recommended, considering these natural defense mechanisms may block the effectiveness of ERT treatment and lead to dose adjustment. Early treatment of FD may improve clinical outcome, initiation of ERT at a younger age in patients with classical FD results in a better biochemical response (42). ERT should not be restricted to hemizygous men but should be considered for both heterozygous females and children (20).

ERT should be combined with adjunctive therapies for adult patients with FD. Renal, cardiac, neurological, gastrointestinal, cerebrovascular and other complications of FD that cause chronic tissue injury should be treated with symptomatic specific therapy (43–45).

Migalastat is an oral pharmacological chaperone developed as an alternative treatment to intravenous ERT for patients with FD and amenable mutations. This new oral small-molecule stabilizes specific mutant (amenable) forms of α-Gal to facilitate normal lysosomal trafficking. In this way, this drug may increase endogenous enzymatic activity in patients with specific missense α-Gal mutations. Migalastat and ERT have similar effects on renal function. There is no significant change in left ventricular mass index in patients treated with Migalastat compared to patients treated by ERT. In randomized, active-controlled study renal, cardiac or cerebrovascular events occurred in 29% and 44% of patients in the migalastat and ERT groups, respectively. Plasma globotriaosylsphingosine was low and stable in migalastat treated patient with FD. Migalastat was generally safe and well tolerated (46–48).

Conclusion

FD affects very emotionally and physically on patients and their families. Long-term treatment of adult patients with FD should include ERT, regular assessment of disease progression, and the use of appropriate adjunctive therapies (38, 40).

Numerous α-Gal mutations complicate therapeutic management of this rare chronic disease. FD requires multidisciplinary care in the treatment of organ-specific complications. Active participation of medical specialists experienced in treating this disorder is necessary, as well as communication between the neurologist, nephrologist, cardiologist, medical geneticist, genetic counselor, psychologist, and nurse. Significance of genetic polymorphisms in FD pathology remains unknown (49) and the response to therapy may depend on α-GAL gene mutations.

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ZNAČAJ GENOTIPSKIH MUTACIJA ALFA GALAKTOZIDAZE A U TERAPIJI FABRIJEVE BOLESTI

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Fabrijeva bolest (FB) je retko nasledno „X vezano" lizozomalno oboljenje izazvano deficijencijom alfa galaktozidaze A (α-GAL). To dovodi do nagomilavanja glikosfingolipida, što dalje vodi do disfunkcije mnogih organa i na kraju do znakova i simptoma bolesti. Cilj ovog rada bio je da se ispita značaj genotipskih mutacija α-GAL u terapiji FB. Bolest se može manifestovati u vidu teškog, klasičnog fenotipa i blagog, neklasičnog fenotipa. Opisane su brojne α-GAL mutacije u bazi podataka o genskim mutacijama, kao što su mutacije pogrešnog smisla (missense), besmislene mutacije (nonsense), mutacije obrade (splice site), kriptičke mutacije (cryptic splicing) i mutacije pomeranja okvira čitanja (frameshift). Terapija zamene enzima (TZE) može doprineti značajnom kliničkom poboljšanju. U zavisnosti od vrste mutacije α-GAL, postoje različite preporuke o započinjanju TZE kod odraslih muškaraca i žena sa klasičnim mutacijama, mutacijama koje dovode do kasnog početka Fabrijeve bolesti i varijantama α-GAL nepoznatog značaja. Trenutno dostupna TZE je primena rekombinantne agalzidaze alfa i agalzidaze beta. Iako nema jedinstvenih vodiča, razvoj znakova i simptoma bolesti trebalo bi da bude indikacija za početak lečenja TZE. Ovu terapiju treba kombinovati sa adjuvantnom terapijom za specifične manifestacije bolesti. Migalastat je novi farmakološki oralni molekul, razvijen kao alternativa intravenskoj TZE za bolesnike sa FB. Migalastat i TZE imaju slične efekte na bubrežnu funkciju kod bolesnika sa ovom bolešću. Dugoročni tretman odraslih bolesnika sa FB treba da podrazumeva pravovremenu TZE, redovno praćenje progresije bolesti svih obolelih, primenu odgovarajuće adjuvantne terapije i multidisciplinarni pristup bolesti.

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Ključne reči: *Fabrijeva bolest, mutacije alfa galaktozidaze A, terapija*

Case report

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GLOBE-PRESERVING SURGERY FOR TREATMENT OF ADVANCED EYELID CARCINOMA INFILTRATING ANTERIOR PERIORBITAL FAT TISSUE: CASE REPORT

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Basal cell carcinoma (BCC) is the commonest eyelid malignancy followed by squamous cell carcinoma and other rare tumors. Often, periocular skin BCC attacks deeply into tissues and infiltrates the orbit. Generally, periorbital malignancies behave more aggressively than malignancies at other cutaneous sites. In case of orbital infiltration, the surgical management remains challenging.

A 64-year-old male patient with basal cell skin cancer of periorbital skin invading the orbit is presented. The patient refused orbital exenteration. A globe-preserving tumor excision with primary reconstruction was performed. During a two-year follow up there was no sign of tumor relapse, but the orbital movements were slightly reduced.

Although in most cases of orbital invasion the exenteration remains the treatment of choice, a globe-sparing treatment may be appropriate in selected patients. The gold standard of treatment is a wide surgical excision which provides the lowest recurrence rate. The primary reconstruction yields acceptable aesthetic and functional results.

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Key words: *basal cell carcinoma, exenteration, globe-sparing excision*

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Introduction

The periocular/periorbital skin involves the skin of the upper lid, lower lid, lid margins, medial canthus with lacrimal system, and lateral canthus. In that region, different benign and malignant neoplasms occur. Periocular malignancies show aggressive behavior, and the treatment and repair of defect are challenging compared to tumors at other cutaneous sites. Five to ten percent of cutaneous malignancies occur in the periorbital region, with basal cell carcinoma (BCC) reported as the commonest periocular tumor (up to 90%) (1-3), followed by squamous cell carcinoma, sebaceous gland carcinoma, cutaneous melanoma, Merkel cell carcinoma, microcystic adnexal carcinoma, and other malignant neoplasms. General statement is that early diagnosis and proper radical surgery obtains best chance for eradication, while uncontrolled tumor growth could invade the anatomical structures in their proximity such as the orbit. Orbital invasion by periorbital skin BCC is reported in 0.8–5.5% cases (1, 4-6), but local invasion can rarely spread to intracranial structures and be the cause of death (3).

Orbital invasion is more often seen in male gender, in cases of multiple recurrences, large tumors, aggressive histologic subtypes, perineural invasion, medial canthal location and older patients (2, 5). The most common primary location for BCCs inclined to invasion is the medial canthal region (53.6–56.2%), followed by the lower eyelid (20.3–35.7%), the upper eyelid (4.7–7.1%) and the lateral canthus (3.6–18.7%) (2,4,5). Tumor can infiltrate the periosteum of the orbital wall especially in medial and lateral canthus where the skin is in close proximity to periosteum (7).

Aggressive subtypes of BCC (infiltrative, morpheaform/sclerosing and basosquamous) make more than 80% of cases of orbital invasion, with the infiltrative one being the most commonly reported subtype encountered (51.6–78.6%) (1, 8).

Orbital invasion may be entirely asymptomatic. It means that the diagnosis requires a high index of suspicion (3, 9).

Signs of orbital invasion include an orbital mass fixed to bone, strabismus, limitation of ocular

motility, and globe displacement or destruction. Recurrent tumors are in advanced stages, graded by TMN staging system, suspicious at huge subclinical extension, leaving larger operative defects. It is important to obtain tumor excision with margin control (2).

The delicate anatomy and specific functional rule of the periocular zone requires adequate surgical skills and care related to radicality (free margins have to be respected), meeting also aesthetic demands (10).

The management of periocular BCC with orbital invasion represents challenges and often requires an oncological board decision. The board includes plastic surgeon, ophthalmologist and radiation oncologist. Treatment should be individualized, patient-related, taking into account different factors such as general health of the patient, patient's wish, as well as the tumor extent and visual function. Exenteration alone or with radiotherapy is the treatment of choice for patients with bulbar or extensive orbital invasion with an estimated recurrence rate of up to 28.5% (5, 11, 12).

Total orbital exenteration is a surgical complete removal of the globe and all orbital contents including periorbita (periosteum). Subtotal orbital exenteration is classified as the removal of the globe and partial removal of orbital tissues (13).

In selected cases, globe-sparing local tumor excision alone or with radiotherapy is an alternative option in surgical treatment (5). Globe-sparing excision could be performed in patients with anterior orbital involvement only, in patients with a single eye, or when a patient declines exenteration (2, 14).

Margin control should be strongly considered for cases treated with globe sparing local excision. The control of choice for resection borders is paraffin-embedded tissue in any case of BCC with orbital invasion. High-quality tissue samples are illustrative in cases involving orbital fat (1).

Complications of globe-preserving tumor excision are usually late functional disturbance of restricted ocular motility, epiphora secondary to nasolacrimal obstruction and abnormal lid position (1).

The close follow-up imaging should be performed in all patients, (regular MRI is preferable). It can detect early posterior recurrences, which may sometimes be hidden and undiagnosed due to the presence of scar tissue (2).

Case report

A 64-year-old man was referred to the Clinic for exulcerated periorbital skin tumor affecting the lateral third of both eyelids and lateral canthal region. Tumor was infiltrating the orbit, being fixed to the lateral orbital wall periosteum. The tumor had appeared four years before, but the patient was not referred to any surgeon till that moment. The oculomotor functions and visual acuity were in reference ranges. Computed tomography scans presented a tumor mass infiltrating extraconal orbital fat and lateral orbital wall periosteum. Multidisciplinary oncology board strongly suggested an orbital exenteration, but the patient refused to underwent this type of surgery. A conservative approach was discussed with the patient and a globe-sparing surgery was advocated. The patient was informed about the risks of this treatment. After tumor excision affecting the lateral third of eyelids and lateral cantus, the periorbital fat and broad area of lateral orbital wall periosteum was removed.

The defect was reconstructed primarily. A partial thickness skin graft was applied for conjunctival reconstruction, and a transposition flap was used for palpebral reconstruction. Donor region was closed using a tension free technique. After the removal of stiches, an acceptable aesthetic result was obtained. Pathological analysis indicated the diagnosis of BCC and clear resection margins on all samples. During a two-year follow-up, there was neither tumor recurrence nor visual impairment.

Results are presented in figures 1-7.

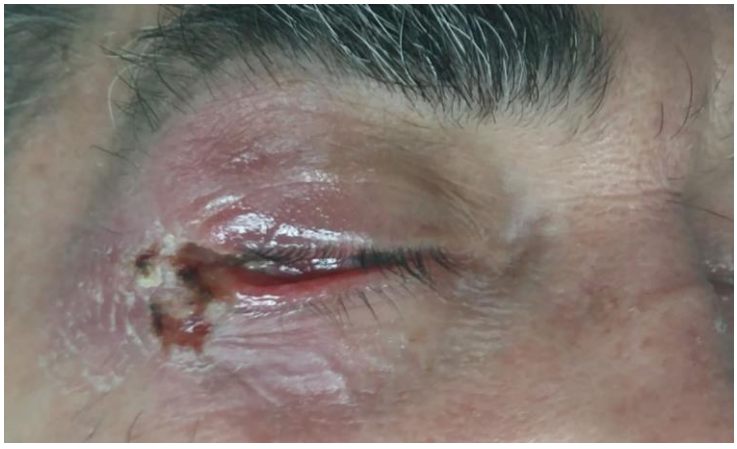

Figure 1. Right lateral canthal infiltrative basal cell carcinoma with lateral orbital invasion

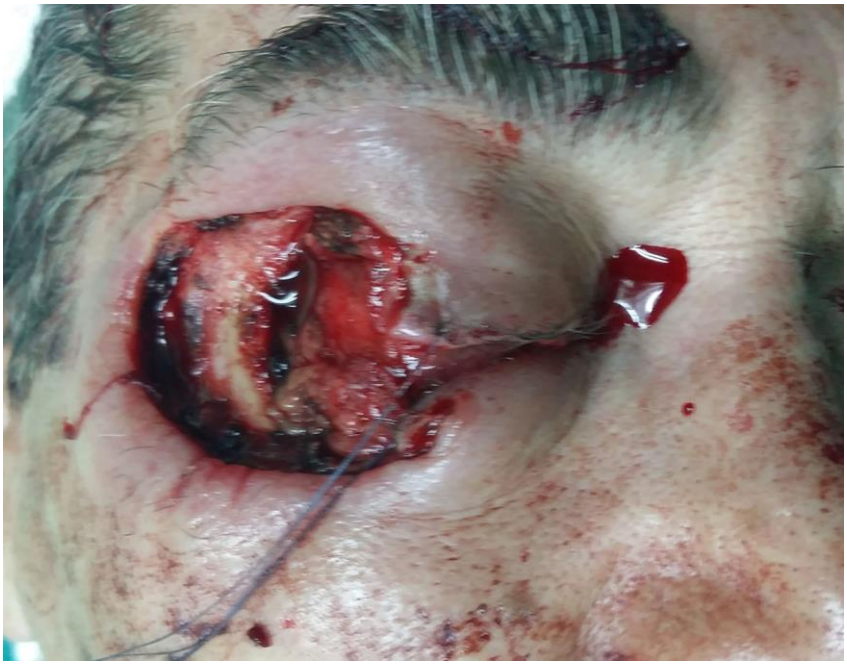

Figure 2. A globe-sparing resection of the lateral canthus and lateral orbital rim was performed

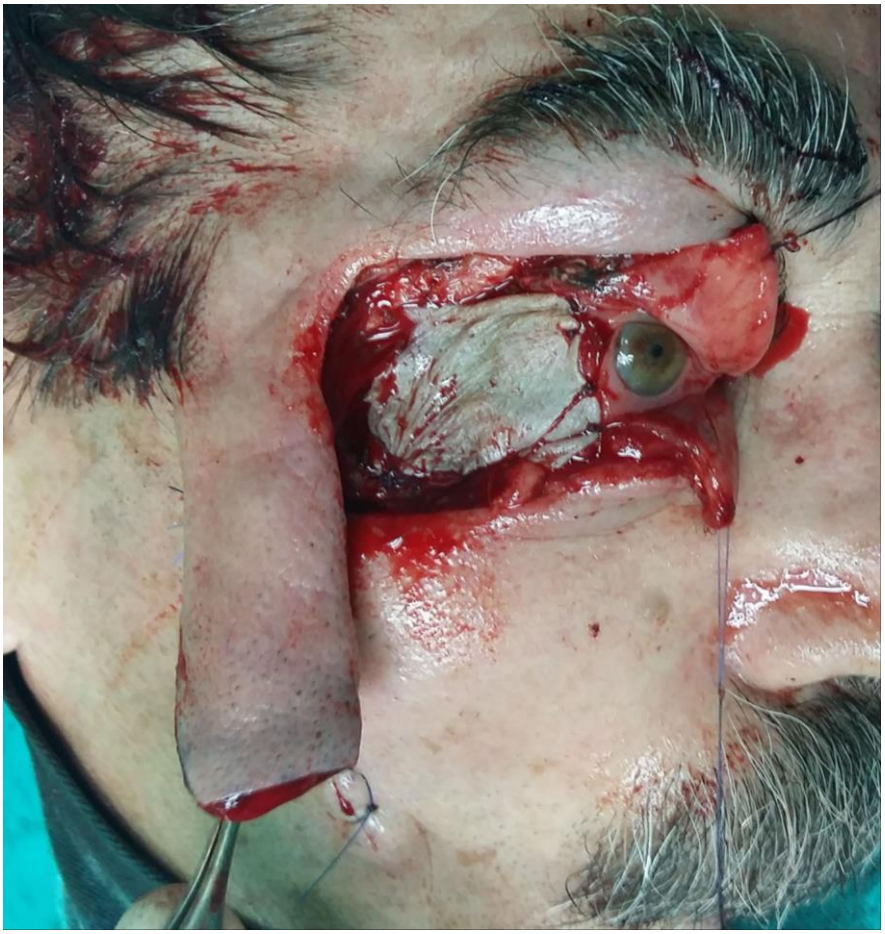

Figure 3. Bulbar conjunctiva was reconstructed with buccal mucosal graft

Figure 4. Transposition cheek flap was used as a skin graft for palpebral conjunctival reconstruction

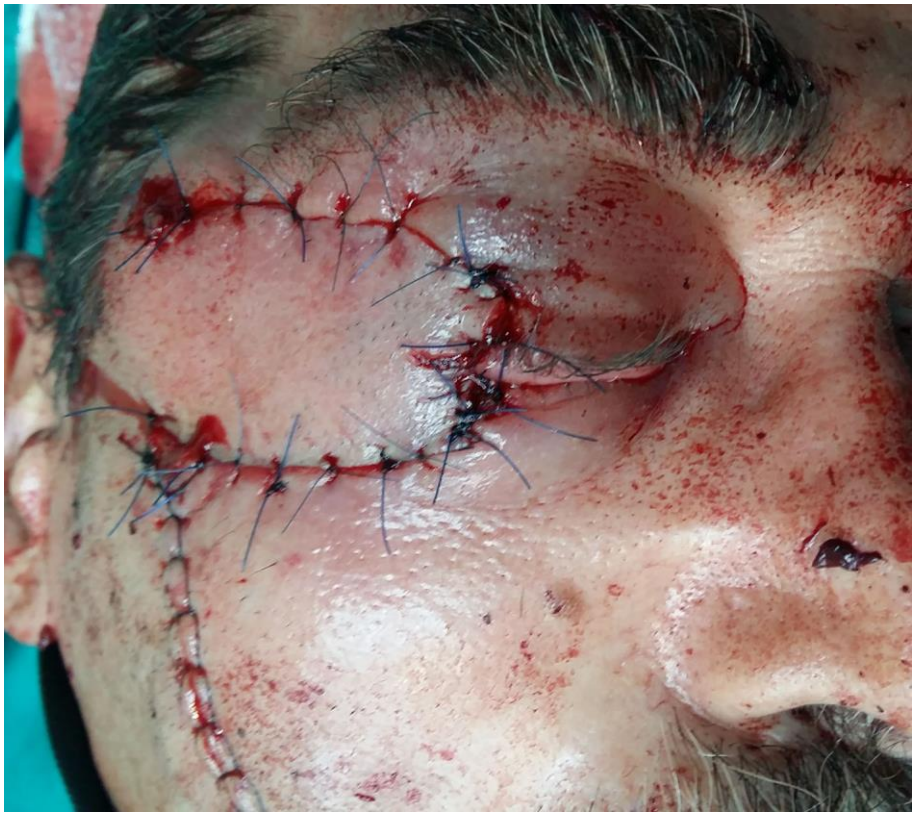

Figure 5. End of surgery

Figure 6. Postoperative results 24 months after surgery -*open eyes*-

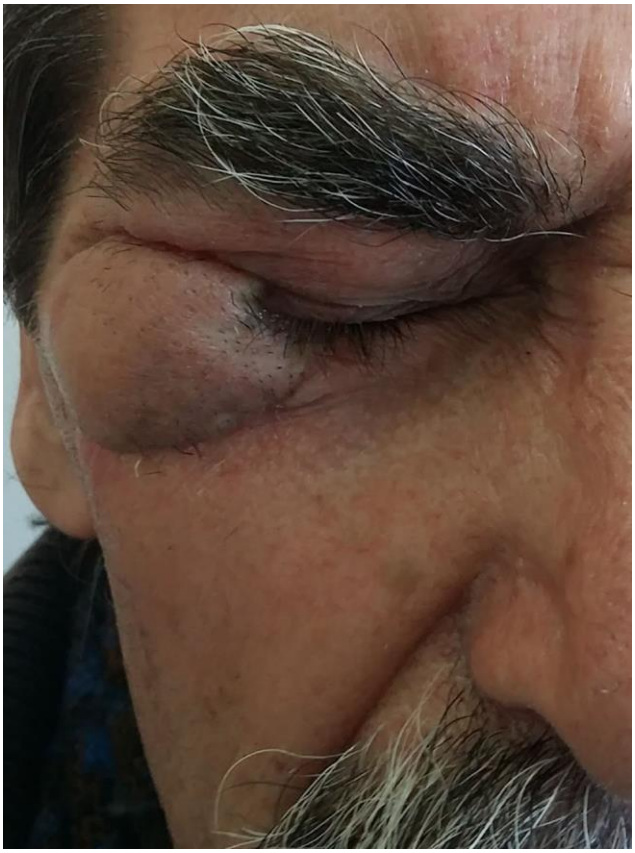

Figure 7. Postoperative results 24 months after surgery -*closed eyes*-

Discussion

The above presented patient represents a periorbital skin BCC spread into the anterior orbit with peribulbar fat and periorbita invasion. The patient was managed using surgical techniques of globe-sparing (preserving) surgical tumor excision. There is only scant literature on the topic (2). BCC is typically a slow-growing malignancy demonstrating little local invasion; however, BCC arising in the periocular region has been associated with a more aggressive phenotype (11).

Local invasion into orbital tissues is associated with a worse prognosis (3).

Many risk factors have been identified for orbital invasion by periocular BCC (11).

Because our patients refused exenteration, the only possible surgical technique was globe-sparing surgery. The signs suggestive of orbital invasion including restricted ocular motility, malposed eye globe, palpable mass fixed to orbital periosteum were present.

A higher incidence of orbital invasion by periocular BCC in male patients is well known and reported in about 77% of patients (5).

This could be discussed according to higher aggressiveness of BCC in males and because male patients apply for medical treatment in more advanced tumor stages than females (11).

Periorbital BCC is commonly diagnosed from 5^{th} to 7^{th} decade of life, but orbital invasion is mostly registered in patients in their late sixties (68–70 years) (2,5).

In 71.4–84.4 % of patients with orbital invasion by BCC, the tumor is recurrent or previously incompletely excised. The remainder are advanced primary tumors found in the first visit (7).

An alternative approach in selected cases with early anterior orbital invasion is conservative globe-sparing (non-exenterating) excision with or without adjunctive radiotherapy (2,15).

Although there is significant literature concerning orbital invasion by eyelid malignancies, conservative surgery and its outcomes are not well-described. In patients after globe sparing surgery, an annual postoperative MRI for at least 5 years is mandatory as part of the follow-up (1).

We report a patient with histologically confirmed, anterior orbital invasion by BCC who was managed with conservative (non-exenterating) surgery. Indication for surgery, clinical presentation, surgical technique, and outcome are discussed.

Some authors report the recurrence rate after globe sparing surgery for medial canthal BCC with orbital invasion about 5% during a mean follow-up of 3.2 years (1). There is no published data regarding recurrence rate with longer follow-up, but it is likely to be higher.

The decision of radical (exenteration) or conservative- globe sparing surgery in case of orbital invasion in periorbital BCC must be made after detailed discussion with the patient. The same principle applies to lateral canthal BCC with limited anterior orbital invasion and the globe-sparing approach may be considered. This type of surgery brings the risk of postoperative complications and possible revision procedures. When the tumor is highly aggressive and has higher risk of recurrence, or perineural invasion, exenteration may be suggested as the preferred treatment choice (1,10).

Conclusion

Orbital invasion by periocular BCC is rare, but the treatment is difficult and the complication rate is high. Radical tumor removal must be confirmed by paraffin margin control. Besides orbital exenteration in selected cases, conservative surgery with globe sparing excision is one of the recommended approaches. Globe-sparing surgical excision with mandatory yearly performed MR imaging as a regular follow-up may be appropriate in case of anterior orbital involvement only, in patients with a single eye or with poor vision in the opposite eye. Also, globe-sparing procedure could be performed in cases when patient refuses exenteration.

In case of orbital infiltration by periorbital skin BCC, the orbital extenteration remains the gold standard, but globe-sparing surgery can be considered in select cases.

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Prikaz bolesnika

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HIRURŠKO LEČENJE UZNAPREDOVALOG KARCINOMA KAPKA KOJI INFILTRIRA PREDNJE PERIBULBARNO MASNO TKIVO UZ OČUVANJE OČNE JABUČICE – PRIKAZ SLUČAJA

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Karcinom bazalnih ćelija (KBĆ) najčešći je malignitet kapka, koji prate karcinom skvamoznih ćelija i drugi retki tumori. KBĆ periokularne kože često se širi u dublje slojeve i infiltrira orbitu. Periorbitalni maligniteti uglavnom se ponašaju mnogo agresivnije nego maligniteti na ostalim mestima na koži. U slučaju infiltracije orbite, hiruško lečenje ostaje izazov.

Prikazan je šezdesetčetvorogodišnji bolesnik muškog pola sa karcinomom bazalnih ćelija kože periorbite koji invadira u orbitu. Bolesnik je odbio egzenteraciju orbite. Izvedena je ekscizija tumora sa primarnom rekonstrukcijom i očuvanjem očne jabučice. Za vreme dvogodišnjeg praćenja, nije bilo znakova recidiva tumora, ali su pokreti očne jabučice bili blago redukovani.

Iako u većini slučajeva orbitalne invazije egzenteracija ostaje izborna metoda lečenja, poštedna operacija očne jabučice može biti odgovarajuća kod određenih bolesnika. Zlatni standard u lečenju predstavlja ekstenzivna hirurgija, kod koje se očekuje najniža stopa recidiva. Primarna rekonstrukcija omogućava zadovoljavajuće estetske i funkcionalne rezultate.

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Ključne reči: *karcinom bazalnih ćelija, egzenteracija, poštedna ekscizija očne jabučice*

Case report

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INVASIVE DUCTAL BREAST CANCER METASTASIS INTO THE TEMPOROMANDIBULAR JOINT TWO YEARS AFTER THE INITIAL TREATMENT: A CASE REPORT

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A tumor metastatic to the oral cavity and jaws is a relatively rare finding. These types of tumor represent a serious challenge for surgical oncologists, because of difficult diagnosis, unspecific symptomatology and atypical radiographic findings. The symptoms that occur with tumors metastatic to the temporomandibular joint and ramus of the lower jaw are usually unspecific, and usually include painful sensations, tumescence, difficulty opening mouth, which often mislead the clinician towards a temporomandibular joint disease, such as arthritis, joint subluxation, degenerative changes and infection.

In this case, the patient described is a 62-year-old woman, with a metastatic tumor in the temporomandi-bular joint and lower jaw region, and a very uncertain prognosis. In this paper, we will discuss surgical and oncological therapeutic options recommendable in this case, based on radiographic findings and histopathological cancer type.

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Key words: *breast cancer metastasis, temporomandibular joint, histopathological type and subtype, surgical treatment*

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Introduction

Metastatic tumors represent a rare pathological entity in the head and neck region, constituting about 1% of the total number of all malignant tumors affecting the region (HNR) (1).

Lymphatic and hematogenous dissemination of infraclavicular malignancies which metastasize to the HNR much more frequently occurs via the ductus thoracicus, compared to the spread via the Batson paraspinal venous plexus (2).

In the available literature, the occurrence of mtastatic tumors is described in all of the anatomical sections of the NHR, i.e. in the lymph nodes, salivary glands, and thyroid, while most of the studies reported so far have presented the data about the oral cavity and jaw soft tissue involvement (3).

The most frequent site of the primary tumor metastasizing to the NHR are lungs in men and breasts in women (4).

Clinical manifestations of the tumors metastatic to the region are rather diverse. These vary from the changes such as anamnestically longterm painless tumor formations involving the salivary gland, bulbous protrusions caused by retrobulbar processes, severe headaches, neck lymphadenopathy in the area covering the tumescences and pain in the region of jaws, exophytic and ulcerous changes in the oral cavity, followed by paresthesias and progressive tooth loss.

The occurrence of metastases in distant organs indicates the onset of the terminal disease stage, with the average patient survival from 3.7 to 32 months after the histopathological verification of metastatic disease (5).

We will here present the case of a patient with completed diagnostic and therapeutic procedures conducted in order to treat breast cancer metastasis to the lower jaw region.

Case report

A female patient aged 62 years was referred to the Clinic of Dentistry, Department for Maxillofacial Surgery in Niš by her oncologist, in order to have

the tumefaction in the preauricular region on the right side treated (Figure 1).

Examining the medical documentation, we found the information about a surgical intervention on her right breast performed two years ago, which consisted of axillary dissection with radical mastectomy, along with adequate oncological therapy in order to treat the invasive ductal carcinoma.

Anamnestically, the patient had noticed the mentioned tumefaction in the right preauricular region three months before the presentation. The appearance and spontaneous enlargement of the tumefaction did not result in any subjective complaints. The sudden enlargement of the lesion, followed by reduced mouth opening and spontaneous episodes of bleeding from the tumor were the reasons for visiting her oncologist who, after the examination, referred the patient to the Maxillofacial Surgery Clinic.

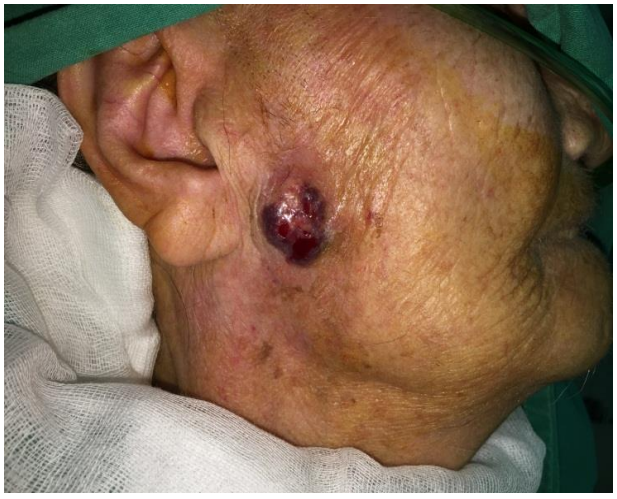

Figure 1. Exulcerated tumor formation in the right parotid region

As a part of the preoperative preparation, exciseonal biopsy of the tumefaction was performed, which indicated the presence of an invasive ductal carcinoma, as well as a CXR (chest x-ray) and AXR (abdominal x-ray), which did not indicate the presence of any secon-dary deposits.

Multi-slice CT of the head and neck region was performed as well, indicating the presence of an expansive tumor formation in the neck and lower jaw ramus area, with an extension into the bone and soft-tissue structures of the upper jaw joint (TMJ) and into the infratemporal space (Figure 2). Along with that, the presence of neck lymphadenopathy, levels I, II and III, was established as well.

On the account of clinical findings such as these and histopathological verification of disseminated primary malignant disease, modified radical neck dissection type III was performed (MRND, Type III) in general endotracheal anesthesia (GETA), followed by hemimandibulectomy with disarticulation of the TMJ (temporomandibular joint), tumor extirpation in the infratemporal fossa, excision of the tumor in the preauriacular region, with an adequate reconstruction of the created soft tissue defect with a cervicofacial flap. The post-operative course was uneventful, and the patient was released from the clinic on the tenth postoperative day.

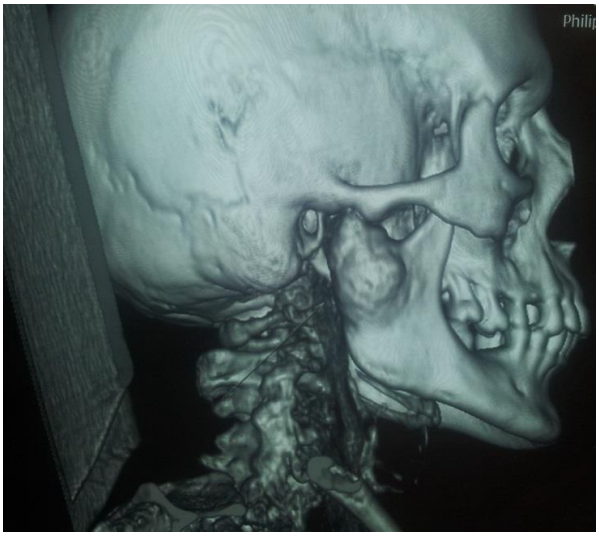

Figure 2. MSCT of the head and neck: an expansive tumor formation in the lower jaw

Histopathological analysis revealed the presence of a moderately to poorly differentiated tumor process in the tissue sample sent for analysis, composed of polygonal cells with prominent nuclei and nucleoli, containing a large number of pathological mitoses. Tumor cells for the most part demonstrated a solid tumor growth pattern, creating focal glandular arrangements (Figure 3). Moreover, vast fields of necrosis were present as well. Immunohistochemical analysis confirmed the presence of a secondary deposit originating from a malignancy in the breast. The observed cells expressed CK7, while the results were negative for Er, Pr and HER2. The steroid receptor status matched the initial, primary tumor status.

Lymphonodal findings in the neck indicated the presence of a metastatic process (12/27).

Histopathological verification of an invasive ductal carcinoma with positive lymph nodes in the neck necessitated the approach with chemoradiation therapy. After the completion of oncological treatment, the management continued with regular post-discharge monthly follow-up examinations, during which the absence of tumor recurrence 18 months after the surgery was confirmed.

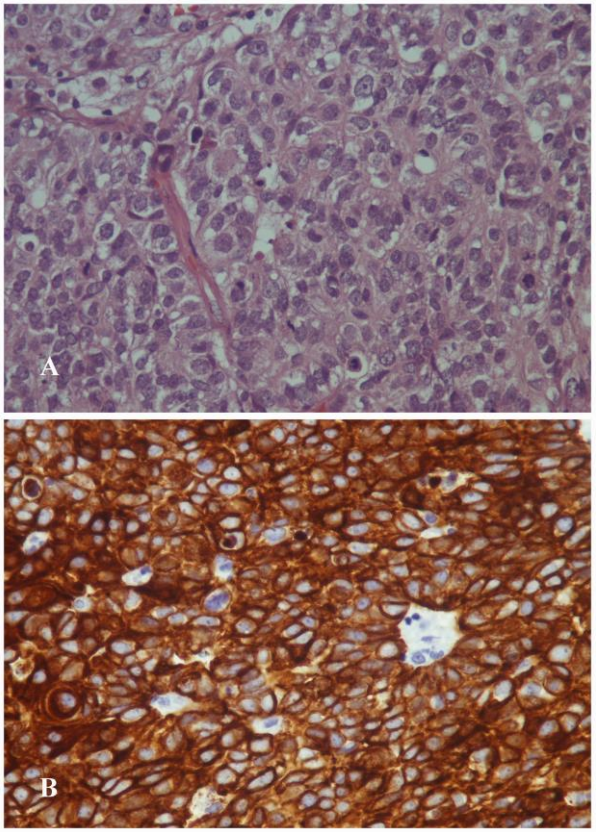

Figure 3. Tumor cells arranged predominantly in solid focal fields, with the formation of glandular structures
A-H&Ex40
B-CK7x40

Discussion

Breast cancer represents a malignant proliferation of epithelial cells. Breast cancers represent the most frequent tumor type among women (excluding skin tumors). Long-term studies have shown that the risk of developing breast cancer grows with age; however, a decreased incidence of breast tumors from the onset of menopause has been reported. In the life of every woman we can recognize the three stages that have the greatest impact on the development of breast cancer; these are the age at menarche, the age at first pregnancy, and the age at the onset of menopause. According to the data from 2007, there were 180,510 new breast cancer cases in the USA, with 40,910 reported deaths from breast cancer (6).

Metastatic tumors in the oral cavity are rare, and comprise about 1% of all oral cavity malignancies (7).

The lower jaw is more frequently affected than the maxilla, and the predilection area for the development of metastases is the region of the lower jaw body and ramus (8).

Out of all metastatic tumors affecting the oral cavity and jaws, about 61% occur in the mandible, 24% in maxilla, and 15% in soft tissues. Primary tumors that metastasize to the mandible area are most frequently those affecting the breast (31%), lungs (18%), kidney (15%), thyroid, prostate and colon (6%), abdomen and skin (5%), testicle (3%), urinary bladder, liver, uterus and ovary (1%) (9).

The study conducted by Babatunde O. Akinbami, during the 30-year period, involved 1.537 patients with malignant tumors in the oral cavity, 24 of which were metastatic. Among these, 14 were located in the mandible, 4 in maxilla, and only 1 affected both the maxilla and mandible. Out of these 24 cases, 12 were females. In 4 women of these, the primary tumor was localized in the breast and had metastasized to the lower jaw (8).

Examining the 114 cases of metastatic jaw tumors by D'Silva, it was found that the most frequent symptom of these tumors was pain. The remaining signs and symptoms included the presence of a swelling, intraoral tumor mass, tooth loss, loose teeth, regional lymphadenopathy, gingival irritation, ulceration, exophytic growth, bad breath, trismus, lower lip paresthesia (10). Reviewing the bone x-ray findings in such cases, it can be noticed that the occurrence of metastatic tumors may vary from distinctly limited to vaguely limited, which we often describe as a "moth eaten" appearance. In the study by Hirshberg in 2008, which included 673 patients, it was concluded that 5% of the cases did not have any radiographic changes. It is known that a familial history plays an important role in the development of breast cancer. Around 10% of breast cancers are directly asso-ciated with embryonic mutations. The Li Fraumani syndrome is characterized by an inherited mutation of the p53 tumor suppressor gene, which produces an increased incidence of breast tumors, osteosarcoma and other malignancies. Inherited mutations of the PTEN gene are also associated with the development of breast tumors. A tumor suppressor gene, BRCA-1, could be held responsible for the activation of a zinc finger protein, after which it functions as a transcription factor. This gene is also involved in the action of "repair genes". A woman who inherits the allele mutation of this gene, regardless of her parent's status, has a 60-80% greater chance to develop a breast cancer, and 33 % greater chance to develop an ovarian cancer. In men, this mutation increases the risk for prostate cancer. BRCA-2 also affects the growth of incidence of breast tumors. A p53 mutation is present in about 40% of patients with breast tumors. An acquired mutation of the PTEN gene occurs in around 10% of those affected with breast tumors. With some breast tumors, a decreased expression of BRCA-1 gene and an abnormal location of BRCA-1 protein has been recognized. It can be concluded that a weaker BRCA-1 and BRCA-2 tumor suppressor gene expression has a sporadic influence on the occurrence of breast cancer. With around 25% of the patients, an enhanced dominant oncogene expression has an important role in the development of breast cancer. The product of this gene belongs to the family of epidermal growth factor receptors; it is called erbB2 (HER2), and its hyperexpression has been reported in breast tumors (11).

ErbB2 (HER2) is a "growth signal molecule" which can be found on the surface of normal breast epithelial cells, the values of which may increase to

an extreme level in 20% of breast cancers, indicating an autonomous growth and genetic instability, and consequentially, its increased expression indicates a greater chance for tumor recurrence, and hence a poor prognosis (12).

An extreme increase of HER2 values has been found in 20 % of those diagnosed with breast cancer; it thus indicates a shorter survival time and more aggressive clinical course of the disease, compared to the tumors with normal HER2 values (13).

Breast tumors with negative ER/PR receptor status have poorer prognosis than those with positive ER/ PR receptor status, the reason for that being that with positive ER/PR receptors there is a possibility for initia-ting a hormone therapy (14).

The prognosis in patients having condylar metastases is very poor, since out of these 70% are estimated to already have distant metastases (15). Considering the above data related to clinical presentation and prognostic parameters, we may conclude that the prognosis for the patient described in this paper is rather uncertain.

Another important prognosis factor for breast cancer is the proliferative index or Ki67 index (16). The tumors showing a higher value of this index generally have poorer prognosis. Moreover, the values of Ki67 protein have an impact on the choice of therapy, so that with hormonedependent tumors with high Ki67 values adjuvant chemotherapy is usually advised because of the tumor tendency to recur. It is worth mentioning that in 2000, a molecular classification of breast carcinoma was published, describing these 4 subtypes:

1. Luminal,
2. Her2-expressing,
3. Basal-like,
4. Normal breast look-alike cancer (17).

A reclassification of this division followed soon, by the International Breast Cancer Study Group, which was published in St. Gallen in 2011, and which was related to immunohistochemical expression of hormone receptors of Her2, ER/PR and proliferative index of Ki67, according to which all breast cancers were divided into 4 subtypes as follows:

1. Luminal A,
2. Luminal B,
3. Her2-positive,
4. Triple negative/basal-like (Table 1).

Table 1. Molecular classification of invasive breast carcinoma st. Gallen, 2011 (18)

Molecular subtype	Surrogate subtype	ER		PgR	HER2	PI (Ki-67)
Luminal A	Luminal A-like	+	or	+	_	< 14%
Luminal B	Luminal B-like (HER2-negative)	+	or	+	_	≥ 14%
	Luminal B-like (HER2-positive)	+	or	+	+	Any
HER2-overexpression	HER2-positive	_		_	+	Any
Basal-like	Triple negative	_		_	_	Any

ER: estrogen receptor; PgR: Progesterone receptor; HER2: Human epidermal growth factor receptor 2;
PI: Proliferation index; +: Positive; -: Negative

In the case of the patient described in this paper, the values of erbB2 (Her2), PR/ER were as follows:

- Her2-negative;
- ER- negative;
- PR- negative;
- Ki67- positive (26%).

Taking into account the measured hormone receptor values and according to the reclassification by the International Breast Cancer Study Group in St. Gallen in 2011, the breast cancer described in this patient belonged to the forth group – basal-like cancer type. In the cases of a basal-like cancer type with negative Her2 and ER/PR values, hormonal therapy was not indicated, and the patient underwent chemoradiation therapy following the surgical treatment.

Conclusion

Despite their being relatively rare clinical entities, tumors metastatic to the head and neck region represent a specific type of challenge in the practice of a surgical oncologist. Most of the patients are definitively diagnos-ed in more advanced disease stages, which makes the prognosis of their disease very uncertain. A detailed patient history with complete clinical examination and biopsy with supple-

mental radiological findings, are vital for making the definitive diagnosis and for a therapy to be successful. Based on the presented findings, it can be concluded that an adequate surgical therapy, with proper immunohistochemistry and determination of the type and subtype of these carcinomas, represents a promising, individualized oncological approach in the treat-ment of affected patients, increasing the percentage of therapeutic success and healing rates.

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METASTAZA INVAZIVNOG DUKTALNOG KARCINOMA DOJKE U PREDELU TEMPOROMANDIBULARNOG ZGLOBA DVE GODINE NAKON INICIJALNOG TRETMANA: PRIKAZ SLUČAJA

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Metastaze tumora u predelu usne duplje i vilice predstavljaju redak patološki entitet. Ovakvi tipovi tumora predstavljaju težak izazov za onkološke hirurge zbog otežane dijagnostike, nespecifične simptomatologije i netipičnog radiografskog nalaza. Simptomi koji se javljaju kod metastatskih tumora temporomandibularnog zgloba i ramusa donje vilice najčešće su nespecifični i manifestuju se u vidu bolnih senzacija, otoka, otežanog otvaranja usta, što kliničara često odvodi na oboljenja temporomandibularnog zgloba kao što su artritis, subluksacija zgloba, degenerativne promene i infekcije.

U našem slučaju prikazana je pacijentkinja stara 62 godine sa postojanjem metastatskog tumora u predelu temporomandibularnog zgloba i donje vilice sa veoma neizvesnom prognozom. U daljem tekstu diskutovaće se o izobru hirurške i onkološke terapije na osnovu radiografskih nalaza i histopatološkog tipa karcinoma

Acta Medica Medianae 2020;59(1):90-95.

Ključne reči: *metastaza karcinoma dojke, temporomandibularni zglob, histopatološki tip i podtip, hirurški tretman*

Case report

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INTERNAL HERNIA ASSOCIATED WITH MECKEL'S DIVERTICULUM IN GERIATRIC PATIENT

Aleksandar Karanikolić, Miodrag Djordjević, Ivan Pešić, Lidija Djordjević, Nebojša Ignjatović, Aleksandar Zlatić, Toplica Bojić

This report describes an unusual geriatric case of acute small bowel obstruction (SBO) due to Meckel diverticulum (MD).

We present a 65 year old male with 90 cm portion of necrotic ileum. Another bowel segment portion with a strong giant MD adherence to the mesentery created a bridge. The mentioned bowel loops went through it, giving rise to an internal hernia.

We want to highlight that internal hernias are not easy to diagnose clinically and abdominal CT images are strongly recommended when suspecting any case of internal hernia in geriatric population.

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Key words: *small bowel obstruction, internal hernia, the elderly*

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Introduction

Meckel's diverticulum (MD) occurs in about 1-3% of general population and the majority of them are asymptomatic (1). Small bowel obstruction (SBO) caused by MD is very rare and can be rarely seen in everyday clinical practice (2). Giant MD can be defined as MD with increased dimensions compared to those commonly found (3). This report describes the phenomenon of small bowel obstruction in geriatric patient secondary to an adhesion from the distal end of a giant Meckel's diverticulum. The adhesion formed a band that trapped a loop of the ileum.

Case report

We present a 65 year old male patient with a 2-day history of repeated bile stained vomiting, obstipation, and abdominal distension. He underwent surgery for coronary artery bypass seven years ago. He had been on a regular antihypertensive treatment for 20 years. He described the abdominal pain as crampy, initiated in the epigastrium, passing to the lower abdomen on the second day. Physical exam showed blood pressure to be 160/80 mmHg, heart rate 84 bpm, respiratory rate 24 rpm, temperature 37.6 ^{O}C. Abdominal examination revealed a soft but distended abdomen and decreased bowel sounds with tenderness in the lower abdominal part. Laboratory tests revealed a high white blood cell count (19.4 × 103 per cubic millimeter) with 85% segmented leukocytes, an elevated C-reactive protein level (231.2 mg/L). Plain abdominal radiograph showed gaseous distension of the small bowel. Abdominal sonography showed fluid-filled dilated small bowel loops. The abdominal cavity was explored finding serohematic fluid. Ischemic and necrotic signs in an ileum segment were detected. A 90 cm portion of the ileum with ischemic and necrotic features was identified at 1.5 m from the ileocecal valve. Moreover, we found that another bowel segment portion with a strong giant MD adherence to the mesentery created a bridge. The mentioned bowel loops went through it, giving rise to an internal hernia. Hence, the strangulation of the bowel segment leading to an ischemic process (Figure 1). The mesenteric adherence was partially liberated afterwards, allowing the intestinal hernia reduction (Figure 2). The ischemic and necrotic bowel portions were resected and intestinal anastomosis was done afterwards. At the end of the procedure the necrotic bowel samples were sent to the pathology department. We performed the resection of MD in order to prevent the possibility of further complications.

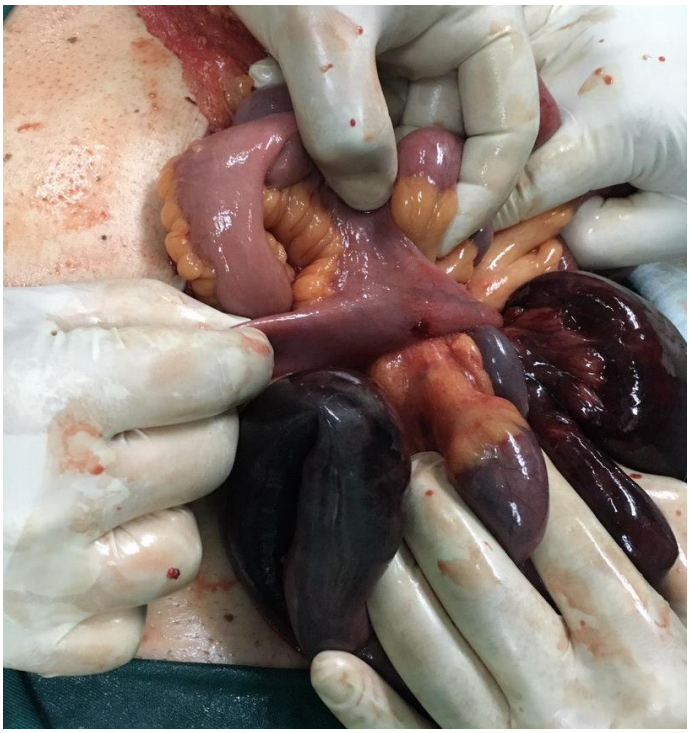

Figure 1. Strangulation of the bowel segment leading to an ischemic process in the internal hernia

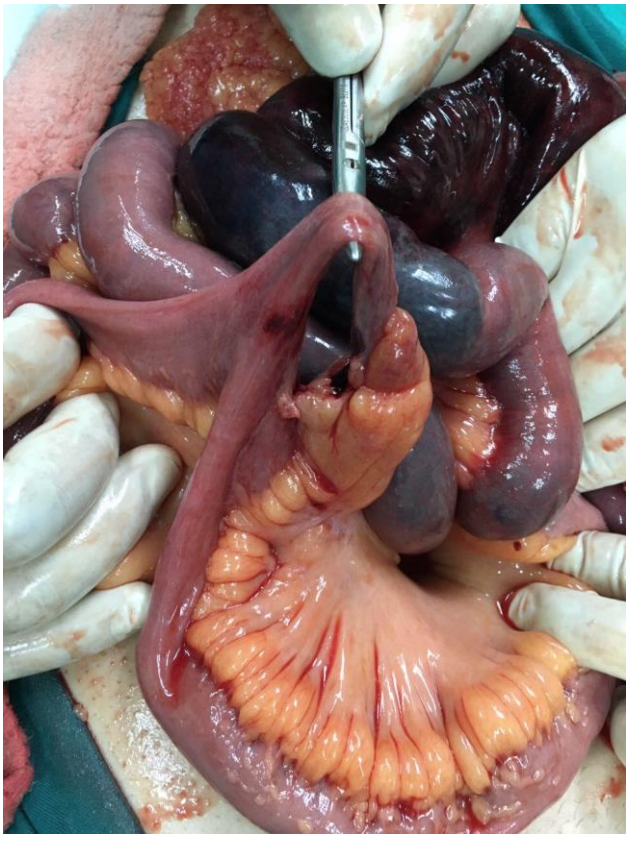

Figure 2. Giant Meckel's diverticulum formed the opening of internal hernia

Discussion

Patients with Meckel's diverticulum have the lifetime risk of complications of around 4% (4). Complications due to Meckel's diverticulum include hemorrhage, intestinal obstruction, diverticulitis, Littre's hernia, umbilicus sinus, and tumours (5). Intestinal obstruction is the most common complication in adults and gastrointestinal haemorrhage is the most common complication in children. Intestinal obstruction caused by Meckel's diverticulum can occur for several reasons: by entangling a loop of small bowel around a fibrous cord or within a mesodiverticular band, intussusception, volvulus, incarceration within a hernia sac (Littre's hernia), chronic Meckel's diverticulitis, foreign body, or neoplasm (4).

Clinically, intestinal obstruction can be asymptomatic or cause significant discomfort ranging from constant vague epigastric pain to intermittent colicky periumbilical pain. Additional symptoms include nausea, vomiting and recurrent intestinal obstruction. Symptom severity relates to the duration and reducibility of the internal hernia and the presence or absence of incarceration and strangulation (1).

Imaging studies often play an important role in the diagnosis of internal hernias caused by various etiological factors, because they are often difficult to identify clinically. Native abdominal radiography and ultrasound had no interest in setting up adequate diagnosis. These diagnostic procedures showed a picture of acute intestinal obstruction. However, abdominal CT has become the first-line imaging technique in these patients because of its availability, speed, and multiplanar reformatting capabilities. Unfortunately, in this case, we did not use the abdominal CT in the diagnosis, which proved to be a bad decision. However, one should take into account that in our hospital abdominal CT is not used as a standard tool in the diagnosis of intestinal obstruction.

Meckel's diverticulum is traditionally considered a pediatric disease that is associated with intestinal hemorrhage or perforation. Meckel's diverticulum complicated with internal hernia presenting as an intestinal obstruction is exceedingly rare (5).

Jain and Viswanath (2013) (6) reported case of small bowel obstruction due to a Meckel's diverticulum complicated with internal hernia in a 60 year old male patient.

Fuentes-Diaz et al. (2015) (3) presented a 19 year old male with acute abdominal pain suggestive of appendicitis. During appendectomy, they discovered ischemic and necrotic signs in a bowel segment, leading them to perform a laparotomy that revealed a portion of ischemic and necrotic jejunum, and another bowel segment with a strong adherence to the mesentery root that created an internal hernia. The internal hernia was reduced and the injured bowel portions were resected.

Devanaboyna et al. (2008) (7) presented MD causing mechanical small bowel obstruction in a 33 year old gentlman. The MD and adhesion were excised with translinear cutting stapling device and the no ischemic small bowel was decompressed.

Meckel's diverticulum as a cause of SBO in elderly patients is very rare. The specificity of this case is that MD was not part of gangrenous or inflammatory affected small bowel loops. For this reason MD should be considered as the cause of SBO in the geriatric population. Whenever we have a dilemma regarding the etiology of SBO, CT examination of the abdomen should be done.

A timely and accurate diagnosis of intestinal obstruction in elderly patients caused by giant MD in clinical practice is not simple. Abdominal CT images are strongly recommended when suspecting any case of internal hernia in geriatric population in order to prevent intestinal gangrene.

Conflict of Interest

The authors declare no conflicts of interest

Statement of Ethics

The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. Guardian of the subject gave the informed written consent to participate in this research.

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Prikaz bolesnika

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UNUTRAŠNJA INKARCERACIJA UZROKOVANA MECKELOVIM DIVERTIKULUMOM KOD BOLESNIKA STARIJEG ŽIVOTNOG DOBA

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Predstavljamo redak slučaj akutne obstrukcije tankog creva uzrokovane Meckelovim divertikulumom (MD) kod bolesnik starijeg životnog doba.

Prikazan je bolesnik star 65 godina, kod kojeg je verifikovan nekrotični ileum u dužini od 90 cm. Dugačak MD slepljen za mezenterijum tankog creva formirao je otvor kroz koji je prošao deo tankog creva dovodeći do unutrašnje inkarceracije, ishemije i nekroze.

Želimo istaći da unutrašnju inkarceraciju nije lako dijagnostifikovati, te je potrebno uraditi MSCT snimanje abdomena kada postoji sumnja na unutrašnju inkarceraciju, naročito kod bolesnika starijeg životnog doba.

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Ključne reči: *obstrukcija tankog creva, unutrašnja kila, gerijatrijski bolesnici*

Review article

UDC: 616-092:[669.71:615.9
doi:10.5633/amm.2020.0115

PATHOPHYSIOLOGICAL MECHANISMS OF ALUMINIUM TOXICITY

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Aluminium forms about 8% of the earth's crust. It is most commonly found as bauxite ore, which is used for extraction of this metal. Aluminium has a high reactivity and forms compounds such as aluminium oxide, aluminium hydroxide, and potassium aluminium sulfate. Exposure of these compounds to oxidants leads to the formation of a superficial coating of aluminium oxide, which is highly resistant to corrosion and insoluble in water. However, acid rains have allowed the dissolution of these compounds and the entry of aluminium into biological systems. It can enter in human body through water, food, drugs, and inhalation of polluted air.

Once when accumulates in the body aluminium exhibits toxic effects on different organ systems: central nervous, respiratory, skeletal, hematopoietic, reproductive, digestive (liver), and integumentary system. Toxic systemic effects of aluminium are first observed in patients with kidney failure treated with medicines containing aluminium compounds which manifest as: dialysis encephalopathy syndrome, osteomalacia with osteodystrophy and microcytic anaemia.

Aluminium is on the top of a surprisingly short list of neurotoxic inorganic elements and their compounds. It is linked with development of neurodegenerative diseases, including autism, attention deficit disorders, amyotrophic lateral sclerosis, Alzheimer's disease, dementia, Gulf war syndrome, and Parkinsonism. Clinical and experimental studies suggest several possible mechanisms of toxic aluminium action on cells. Those are: increased production of oxidative stress, alteration of membrane function, disruption of intracellular signaling, and alteration or inhibition of enzyme functions.

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Key words: *aluminium, toxicity, oxidative stress, neurodegenerative diseases, pathogenesis*

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Introduction

Aluminium (Al) is a silvery-white, lightweight, ductile, malleable and non-magnetic metal. It has atomic number 13 and belongs to boron group of the periodic table of the elements. Al forms about 8% of the earth's crust. Native aluminium is extremely rarely found in nature. It is most commonly found as bauxite ore, which is used for extraction of this metal. Aluminium has a high reactivity and forms compounds such as aluminium oxide, aluminium hydroxide, and potassium aluminium sulfate. Exposure of Al to water, oxygen or other oxidants leads to the formation of a superficial coating of aluminium oxide, which is highly resistant to corrosion. Aluminium oxide is insoluble in water, but it is soluble in mineral acids and strong alkalis. The concentration of dissolved Al^{3+} is low in surface and subsoil waters, because Al minerals are insoluble at neutral pH. Rain-borne acidification and the use of acidifying fertilizers increase the concentration of soluble Al^{3+} in soil and waters (1-3). The result of soil pollution is an increase in concentration of aluminium in cultivated plants. This is one of the ways how aluminium may enter in food chain. It was believed that aluminium is harmless to environment, but it is shown that it is toxic to plants and animals (1-3).

Aluminium is widely used in metal alloy production, as a construction material in automotive and aviation industry, electrical industry, as solid fuel rocket propellent, for manufacture of explosives and fireworks. It is also used for the production of cooking utensils and dishes, for food packaging (cans, containers, foils), and as food additives (4, 5).

Mineral compounds of aluminium of natural origin (beonite and zeolite) are used to purify drinking water as coagulants in order to reduce the level of organic matter, color, cloudiness and microorganisms (4-6). Aluminium is also used in pharmacy, medicine, cosmetics and dentistry (antacids, astringents, antiperspirants, dental crowns and dentures) (5).

Aluminium can enter in human body through water, food, drugs, and inhalation of polluted air (5-7). Aluminium is a non-essential element which is not found in large quantities in human body. The total human body content of aluminium may range from 50-150 mg, with an average of about 65 mg. Daily intake of aluminium may range from 10-110 mg (8). It is characterized by low intestinal absorption, slow tissue uptake and rapid urinary excretion. Absorption of Al in the digestive tract is estimated to be less than 1%. It is influenced by solubility of Al compounds and enhanced with increased gastric acidity, presence of organic acids (ascorbic, citric) and lack of Fe and Ca in the diet. The accumulation of Al is reported in patients treated with medicines containing Al compounds (antacids, aluminium in dialysis fluid) in renal failure (8). About half of body content is found in the skeleton, one quarter in the lungs and the rest is in brain, kidneys, liver, spleen and thyroid. Aluminium may pass both blood-brain and placental barrier. It is not recognized that Al^{3+} has some function in living organisms and it is regarded as biologically inert (9, 10). Aluminium when accumulate in human body exhibits toxic effects on different organ systems: central nervous, respiratory, skeletal, hematopoietic, reproductive, digestive (liver), and integumentary system.

Effects of aluminium on central nervous system

Aluminium is on the top of a surprisingly short list of neurotoxic inorganic elements and their compounds. It is linked with development of neurodegenerative diseases, including autism, attention deficit disorder, amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), dementia, Gulf war syndrome and parkinsonism (7, 11-17).

Aluminium is accused to develop neurotoxicity through oxidative stress, cell mediated toxicity, apoptosis, inflammatory events in the brain, glutamate toxicity, effects on calcium homeostasis, gene expression, aluminium induced neurofibrillary tangle (NFT) formation and irreversible blockade of ion channels by beta-amyloid (11, 18). Neurotoxicity of aluminium salts has been reported in numerous animal studies. Exposition to excessive intake of aluminium causes learning and memory disorders in rats due to deposition of Aβ in the hippocampus and cortex. Al-induced neurophysical and neurobehavioral pathological changes similar to AD were registered in animals (19, 20). Chronic exposition of rats to high-dose $AlCl_3$ injections over a prolonged period can reduce locomotor and cognitive functions in rats as well as reduced body weight gain which may be a sign of systemic toxicity (21).

A number of clinical studies have shown that exposure to aluminium in dialysis fluid in patients with kidney failure resulted with encephalopathy. Aluminium induced encephalopathy due to bladder irrigation with 1% alum in patient with kidney failure is also reported (22). People in Camelford (south west of England) were exposed to the drinking water contaminated with 20 tonnes of aluminium sulphate in July 1988. They had considerable damages of cerebral function, which were not related to anxiety (23). A mild form of encephalopathy was registered in 64 former aluminium dust-exposed foundry workers in Italy. It was characterized by mild intellectual deficit, loss of muscle control, tremor, and spinocerebellar degeneration (24).

Aluminium levels were assessed in 118 patients with neurodegenerative diseases: multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), and Alzheimer's disease (AD) as well as in 73 healthy subjects. MS was diagnosed in 85.6% of total neurodegenerative diseases (ND). Al was present in 44.8% of cases comprehensive of ND and healthy patients. Al level was significantly higher in patients with neurodegenerative diseases than in healthy subjects. Calcium disodium ethylene diaminetetra acetic acid (EDTA) chelation treatment reduced Al burden in patients with neurodegenerative diseases and ameliorated their clinical conditions (25).

Macrophagic myofasciitis was registered after intramuscular injections of Al-containing vaccines in patients (5). It was characterized by persistence of aluminium hydroxide within macrophages at the site of immunization, muscle lesions, arthromyalgias, chronic fatigue, and cognitive impairment (both visual and verbal memory deficit; including attention deficit) (26).

Aluminium salts (aluminium hydroxide, aluminium phosphate, and aluminium potassium sulfate) have been used in vaccines over eight decades. Even though it has been reported that these salts used as adjuvants can cause severe local reactions (erythema, subcutaneous nodules and contact hypersensitivity) they are still in use, because they enhance antigenicity of some vaccines (as diphtheria and tetanus vaccines) (27).

It was recorded that vaccinated children were exposed more to aluminium (11-26%) than undervaccinated children. "Power analyses demonstrated that safety studies of aluminium could detect relative risks ranging from 1.1 to 5.8 for a range of adverse event incidence" (28). It has been hypothesized that aluminium hydroxide, which was used as adjuvant in multiple vaccines that soldiers underwent, is associated with Gulf War syndrome (29, 30). The Global Advisory Committee on Vaccine Safety of WHO does not find that there is basis at present to change recommendations for vaccination practices (31).

Though, aluminium neurotoxicity is documented, there is common opinion that healthy adults may tolerate repeated oral exposures to aluminium (up to 3500-7200 mg/day from antacids and buffered aspirin) without any toxic effect. It is explained by low absorption and rapid and primarily urinary excretion of Al compounds. But it is clear

that even low daily doses of aluminium can cause systemic intoxication in patients with kidney failure, preterm infants and young children (5).

Effects of aluminium on respiratory system

Aluminium dust has hazardous effect on respiratory system of aluminium production workers. It is shown that they suffer from respiratory symptoms such as cough, wheezing, dyspnea, and chest tightness. Potroom workers who breathe large amounts of aluminium dusts can get wide range of lung diseases: chronic bronchitis, chronic obstructive pulmonary disease, potroom asthma, alveolitis, pneumoconiosis, and even oncological respiratory diseases. All these toxic effects cannot be attributed solely to aluminium. Aluminium potroom workers are exposed not only to alumina dust, but also to particles of fluorides and traces of different elements (vanadium, chromium, nickel), polycyclic aromatic hydrocarbon, mineral dusts (as silica and asbestos), coal tar pitch volatiles, fumes and gases (hydrogen fluoride, carbon oxides, sulfur dioxide, and oxides of nitrogen), extreme heat, and high static magnetic fields (5, 24, 32-34).

Effects of aluminium on bone and hematopoietic tissue

Most of the aluminium that accumulates in body is deposited in the bones (8). Aluminium deposits are found also in the hydroxyapatite of the bone matrix in patients with coeliac disease (due to an increased intestinal permeability) and in the case of long-term use of aluminium anti-acid drugs (35, 36).

Patients with kidney failure due to the toxic effect of aluminium in dialysis fluid developed osteomalacia, osteodystrophy and microcytic anemia and dialysis encephalopathy. Aluminium bone disease is characterized by low serum parathyroid hormone (PTH) levels. Removal of aluminium from the dialysate has resulted in disappearance of the bone disease and in an increase in plasma PTH levels (35). The administration of aluminium in rats with chronic renal failure has resulted in reduction of both synthesis and release of PTH (37).

Although very low doses of Al have mitogenic effect in bones of experimental animals, high doses of Al inhibit remodeling of bone by slowing osteoblast and osteoclast activities (35, 36). In neonatal rat osteoblast tissue culture $AlCl_3$ has been shown to destroy calcium homeostasis, which activates the Ca^{2+}/calmodulin-dependent protein kinase II signaling pathway and thus promotes osteoblast apoptosis (38). Al occupies the unmineralized type I collagen, which is freshly laid down at the mineralization front of the bone surface instead of calcium. The result of impaired bone calcification is development of osteomalacia associated with hypercalcemia, and hypercalciuria (25).

Aluminium chronic intoxication causes a microcytic hypochromic anemia in patients with compromised kidney function (39-41). This anemia is not reversible by iron and it is characterized by decreased red cell count as well as hematocrit and hemoglobin concentration. Pathogenesis factors responsible for Al induced anemia are: shortened erythrocyte lifespan due to reduced erythrocyte membrane integrity; inhibition of δ-aminolevulinic acid dehydratase (42), reduced Fe uptake by transferrin due to competitive interaction between iron and aluminium (43).

Downregulation of transferrin receptor expression and impaired intracellular delivery of Fe from transferrin is also recognized as pathogenesis factor for this type of anemia (39, 40).

Similar values of affinity constant of aluminium and iron for the binding of transferrin receptors are recorded in cultured Human erythroleukemic K562 cells. Opposite to previous findings, it is reported that Al modified Fe uptake without affecting the expression of transferrin receptors. It is concluded that Al induced upregulation of nontransferrin bound iron uptake as an adaptation aimed to enable incorporation of iron which is essential for cellular metabolism (43).

Effects of aluminium on reproductive system

The decline in male fertility, observed in the twentieth century, is a very current issue in contemporary science. Male fertility has declined by 50% over several decades of the industrial revolution. It was not known for a long time about harmful effects of aluminium on male fertility (44-47). Exposure to Al has been reported to affect testicular development and testosterone synthesis in experimental animals (48-51). Although it was shown that Al is capable of compromising male fertility by inducing a state of oxidative stress in the testes (48-52), other mechanisms such as inhibition of microtubule assembly could also be involved in Al-induced testicular damage. Aluminium showed negative impact on reproductive abilities of adult bank voles by causing morphologically abnormal development of the gonads and by decreasing the quality and quantity of sperm (53). Intraperitoneal administration of $AlCl_3$ induced dose dependent decrease of testosterone levels in the testes and plasma of mice (48). In male Swiss albino mice treated intraperitoneally with $AlCl_3$ were observed "deformations of the Sertoli cells, epithelial sloughing, tubular atrophy, and abnormal germ cells" (50). In Wistar rats treated with aluminium sulphate in drinking water is recorded significant decrease of sexual accessory glands: seminal vesicles, prostates, bulbourethral glands and of seminiferous tubules (54). Chronic oral exposure to aluminium at low levels is reported to have as negative impact as high levels on reproductive parameters in Wistar rats. These findings are suggesting adverse impact of aluminium on male fertility (55).

Decreased pregnancy rate was observed in untreated females mated with males treated intraperitoneally with 100 or 200 mg/kg/day of aluminium nitrate for 4 weeks. Treated male mice showed significantly decreased body weight, as well as testicular and epididymal weights. Also, significant decreases in testicular and spermatid counts and epididymal sperm counts were recorded. Sperm moti-

lity and morphology were unaffected. Histological changes manifested as necrosis of spermatocytes/spermatids in the testes, whereas the tubular diameters were unaffected by aluminium administration (56). A negative impact of aluminium on rabbit sperm cell motility and viability has been shown *in vitro* (51). Rabbits orally treated with $AlCl_3$ for 16 weeks had significantly decreased libido, ejaculate volume, sperm concentration, total sperm output, sperm motility (%), total motile sperm per ejaculate, packed sperm volume and total functional sperm fraction. Relative weights of testes and epididymis were also significantly decreased (49). High concentrations of Al in human semen, seminal plasma, spermatozoa, blood, and urine have been linked to poor sperm quality and viability in men (47).

The effects of aluminium on the female reproductive system are not enough elucidated yet. Histopathological changes in the mice ovaries and decreased fertility, after 12 weeks of aluminium chloride administration (dose range 1000-1400 mg/kg) were showed. Both the number of pregnant females and the number of absorbed fetuses were decreased (53). Female mice were exposed by nasal drip during whole pregnancy to Alumina nanoparticles. Aluminium content in hippocampus of newborns was significantly increased. Neurodevelopmental toxicity was registered in the offspring at first month of age as significantly increased anxiety-like behavior with impaired learning and memory performance (57). Subchronic oral exposure to $AlCl_3$ caused the damage of the ovarian structure in rats. Metabolism of Fe, Zn and Cu was disturbed. Activities of Na^+-K^+-ATPase, Mg^{2+}-ATPase and Ca^{2+}-ATPase in ovaries decreased, and expression of follicle stimulating hormone (FSH) and luteinizing hormone (LH) receptors were suppressed (58, 59). The significant decrease of litter size, modification of sex ratio (increase of female pups number), and significant delay of vaginal opening compared to control group were registered in female Wistar rats exposed to aluminium sulphate by drinking water (60). Oral application of $AlCl_3$ (200 mg daily) during 30 days resulted in a significant decline in uterine and ovarian protein levels and in 3β- and 17β-hydroxysteroid dehydrogenase activities. Estradiol levels also declined. Hypercholesterolemia and significant accumulation of cholesterol in the ovaries of treated mice as well as accumulation of glycogen in uterus were reported too. Toxic effect in female mice due administration of aluminium chloride has affected steroidogenesis in ovary, and carbohydrate metabolism in uterus. These effects were reversible upon withdrawal of the treatments (61). In adult female Wistar rats treated with aluminium nitrate, the presence of electron-dense material in lysosomes of myometrium and endometrium cells as well as in the cells of the internal theca and granulosa cells was showed by transmission electron microscopy. Also, impaired endoplasmic reticulum, mitochondria and vacuolation were registered. It was concluded that lysosomes of uterus and ovary cells had defense function and extract aluminium and deposit it in an insoluble form (62).

Effects of aluminium on liver

Aluminium is accumulated in the liver, less than in bones, but the manifestations of Al toxicity in liver have been described. Morphological and morphometric changes highly suggestive of toxic hepatitis were registered in Albino Wistar rats treated with aluminium chloride solved in distilled water intragastrically for 21 days. Architectural derangement was observed as well as degenerative changes at cellular level: nuclear variations such as karyorrhexis and pyknosis (63). In of $AlCl_3$-treated rats a significant rises in plasma levels of AST, ALT, ALP, and LDH in $AlCl_3$ were recorded as well as a significant reduction in total protein level. A significant level of oxidative stress in liver tissue was also registered (64). High doses of Al induce toxic effects and damage the lysosomes in the liver and the extent of lysosomal damage depended of dose and duration of Al loading (65). Accumulation of bile acids in serum in rats and piglets (66), and increased transferrin excretion in the bile were also found (67). Reduction of some cytochrome P450 isoenzymes, nicotine adenine dinucleotide phosphate (NADPH), cytochrome c reductase, and a 4-fold increase in glucuronyltransferase activity were registered in rats treated parenterally with Al. These findings indicate increased conjugating activity (68) and changes in cytochrome P450 isoenzymes may alter metabolism of drugs.

Effects of aluminium on skin

Although absorbed through the skin, aluminium exposure via intact skin is rather mild, confirming this is an effective barrier (69). Aluminium salts, particularly aluminium chlorohydrate, are used in various antiperspirant cosmetics products, as they block secretion of sweat (70).

Following the dermal application of aluminium chlorohydrate penetration rate was very low (around 0.01%) (71). Only insignificant transdermal absorption of Al was shown after application of three different antiperspirant formulations on intact skin. Also, there were low cutaneous quantities of Al ranging 0.5-1.8 μg/cm^2. On the other hand, Al uptake after topical antiperspirant application, through the pre-damaged skin (stripped skin) was significantly higher (0.06% or 11.50 μg/cm^2) (70). In this respect, there was a discussion of whether the breast cancer was associated with the use of Al containing antiperspirants, as a form of aluminium-related chronic diseases (69). However, no significant link between antiperspirants or deodorants use and increased risk for breast cancer was found. A population based case-control study involving 813 breast cancer patients revealed no significant association of regular antiperspirant use or following hair removal 1h after shaving (72).

Intact skin is an effective barrier for Al exposure thus its effects on human keratinocytes are supposed minimal. However, the study of Al nanoparticle interactions with human epidermal keratinocytes showed the particles localization within the cytoplasmic vacuoles of the cells and there was indication of their interactions with cytokine assays

(73). Detrimental effects of Al were shown on human skin fibroblast cultures and were governed by lipid peroxidation as a pathway of Al cytotoxicity. The experiment showed significant malonodialdehyde (MDA) production after 24 h incubation with Al (74). In the perspective, the epidemiological study on internal exposure after antiperspirants use should be performed, and the association with hair removal products or shaving.

Mechanisms of aluminium toxic effects

Clinical and experimental studies suggest several mechanisms of toxic aluminium action on cells. Those are: increased production of oxidative stress, alteration of membrane function, disruption of intracellular signaling, and alteration or inhibition of enzyme functions. All of them may eventually cause tissue damage.

1. Association of aluminium and increased oxidative stress

Large number of studies demonstrated there is a link between increased Al concentrations and oxidative stress. Generally, there are several mechanisms that produce imbalance between free radical production and antioxidant defense system. Aluminium was shown to take part in disruption of metal ion homeostasis and potential oxidative stress through the reactive oxygen species (ROS) and reactive nitrogen species (RNS) generation (75, 76).

Oxidative stress has multiple effects on molecules structure and function, and leads to lipid peroxidation, protein modifications and DNA damage. Aluminium driven oxidative stress was shown to lead to the germ cell apoptosis and decrease in intracellular ATP level (hypoenergosis and motility) (77). Also, its redox state is implicated in a variety of neurological disorders. Another example is Al mediated testicular damage through depletion of antioxidative enzymes protection, and an induction of NO byproducts and consequent inhibition of steroidogenesis (79). Although Al does not undergo redox change, it may enhance iron driven biological oxidation by formation of Al superoxide and by catalyzing H_2O_2 formation while reducing Fe^{3+} (78).

By creating a labile iron pool, a redox-active iron, Al interferes with cellular pathways of iron metabolism. This pool has a capacity to promote ROS generation. It is regulated by cytosolic iron regulatory proteins and dependent expression of iron import and storage machineries, or ferritin degradation or synthesis (80).This relationship was proven in animal model study where Al serum levels were found inversely correlated with Fe serum levels, implying on Al intoxication intervene in Fe metabolism (48).

Al can also displace other biological cations, such as calcium, zinc, copper and magnesium from their binding sites. Thus released ions can catalyze hydrogen peroxide transformation to the highly reactive hydroxyl radical, and further initiate lipid peroxidation (81).

Another mechanism of detrimental Al effect is impairment of mitochondrial bioenergetics, also associated with ROS generation (79). Dysfunction of mitochondrial bioenergetics progressively leads to myocardial failure, because energy insufficiency plays a key role in systolic heart failure (82).

Aluminium was also linked to the production of RNS by induction of NO byproducts. Also, increased ROS, through other mechanisms, reduce the amount of bioactive NO by formation of toxic peroxynitrite. Al administration significantly increased NO production and decreased adenosine 3, 5-cyclic monophosphate (cAMP) and testosterone (83).

Several studies demonstrated that Al may cause changes in antioxidants activity, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase/reductase (84). $AlCl_3$ was suggested to inhibit the activity of superoxide dismutase (SOD). It is demonstrated that one month administration of $AlCl_3$ significantly decreased the activities of SOD, suggesting that Al have catalytic activity for ROS production. Reduced SOD and glutathione peroxidase (GPX) activities might be attributed to the elevated level of protein and lipioxidative products (85).

By decreasing the activity of glutathione synthetase, Al might slow the glutathione (GSH) synthesis, one of the most important antioxidants in cells. It is hypothesized that this process occurs through the depletion of ATP. Particularly, Al forms a strong complex with ATP, as it has high affinity toward phosphate ions, and lowers its availability in the cell (79). Another suggested mechanism of decreased GSH is the insufficient supply of NADPH, due to the Al mediated inhibition of NADPH generation. Al inhibits NADP-isocitrate dehydrogenase, the only enzyme supplying NADPH in mitochondria (86). Al, at doses above 120 mg/kg bw in Wistar rats (orally), produced significant reduction of GSH content and an increase of oxidized/reduced glutathione ratio, in the small intestine mucosa. Also, activities of both GSH synthase and GSSG reductase were significantly reduced. This change in epithelial cells redox state contributed to alteration in GSH-dependent absorptive functions (87).

Al induced oxidative unbalances are associated with lipid peroxidation process. Malondialdehyde (MDA), a lipid peroxidation indicator, is found increased in testis and epididymis after Al exposure (88). Also, lipid peroxidation was found significantly increased in the small intestine when higher Al doses were used (>120 mg/kg) in animal model (89).

2. Aluminium caused alteration of membrane function

The plasma membrane seems to be the target for Al related toxicity, as these trivalent cations readily engaged in interactions with the membrane components thereby affecting associated processes. Al may form electrostatic bonds with oxygen donor ligands or interact with the membrane lipids (79). Also, it may directly alter electrical potential and membrane surface potential (89). Interestingly, binding of Al to the membrane lipids causes the membrane to become more rigid, which ultimately affects the cell motility and viability (90). Production of ROS and lipid peroxidation of membrane lipids has profound and progressive negative consequences. It hinders membrane fluidity (to become rigid), increase permeability, alter r

eceptor function, etcetera (79). All these changes further influence intracellular processes such as enzyme inactivation, DNA damage and cell death. Chronic aluminium-in-duced neurotoxicity has been related to lipid per-oxidation of the brain cells that probably arises from altered lipoprotein metabolism (91).

3. The channel hypothesis of Alzheimer's disease (AD)

There are controversies about ion channel hypothesis for Alzheimer amyloid peptide neurotoxicity. According to some reports beta-amyloid (Aβ) peptides which accumulate in plaques in the brain in Alzheimer's disease can form ion channels in lipid bilayers. liposomes, neurons, oocyctes, and endothelial cells. These channels are heterogeneous in size, voltage-independent, and poorly selective for ions and they can allow influx of (Ca^{2+}), Na^+, K^+, Cs^+, Li^+, and possibly Cl^- (18). Overload with positive ions may damage and/or kill neurons.

There is no doubt that Aβ is capable to induce transmembrane ion fluctuations in living cells. But in more recent report are presented data which suggest "that Aβ is capable of self-assembling into structures that either form a pore through membranes or generate transient defects in membranes". It is concluded that Ca^{2+} influx through Aβ-induced pores or membrane defects and disruption of Ca^{2+} homeostasis could contribute to development of Alzheimer's disease. These authors left open the possibility that Aβ activates intrinsic ion channels or ion pumps in cells (92).

4. Aluminium and disruption of intracellular signaling

Several intracellular signaling pathways are reported to be modified by Al ions. Al was found to disturb secondary messenger signaling, including cAMP, Ca^{2+}, and phosphoinositide and inositol-1, 4, 5-triphosphate (IP_3), all of which are involved in a variety of processes, ranging from cell growth, differentiation to apoptosis (93).

Aluminium alters cyclic AMP and cyclic GMP levels (less sensitive than AMP). Al has elevated cyclic AMP levels in rat cortex following oral administration for 4 weeks. Supposed mechanism is a G-protein stimulation of adenylate cyclase as Al may replace the Mg^{2+} that confers the structure to the triphosphate of GMP (94).

Al is reported as an effective voltage sensitive calcium channel blocker. It blocks Ca entry into the cell and inhibits Ca^{2+}/Mg^{2+}-ATPase (on endoplasmic reticulum) dependent sequestration of Ca^{2+} from the cytosol. Al action in this process is complex as it increases the activity of the Ca^{2+} ATPase, similarly to Mg^{2+}, but also displaces these two molecules and thus disrupts calcium transport (95). When applied extracellularly, Al reduces voltage-activated calcium channel currents in a concentration and pH dependent manner and irreversibly (96).

Al interference with IP_3 and its intracellular depletion is based on Al^{3+} much higher affinity for the phosphatidylcholine surface than Ca^{2+} (additionally disturbing Ca homeostasis) (97).

5. Aluminium caused alteration of enzyme functions Nikotinamide Adenine Dinucleotide Phosphate

Taking into account previously described mechanisms of Al action it can be concluded that there is substantial influence on cellular protein function and metabolism. Its effects on proteins are varied, ranging from alteration in their expression due to the Al binding to deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), to direct inhibition of several enzymes such as hexokinase, phosphatases, phosphodiesterase and phosphooxydase, glucose-6-phosphate dehydrogenase and NADP isocitrate dehydrogenase. For example, Al inhibits hexokinase because it changes Mg ion in Mg-ATPase, a hexokinase substrate (97). Or, by perturbation of intracellular Fe metabolism and Fe-S cluster Al promotes the inhibition of aconitase activity, enzyme involved in metabolism of citrate, in Pseudomonas fluorescens (98).

Al exposure ($AlCl_3$) induced significant change of intestinal enzymes, as well as expression of the multidrug resistance-associated protein 2 which was nearly 3-fold increased. Gamma-glutamyltranspeptidase activity was also increased, while glutathione (GSH) synthase and glutathione disulfide (GSSG)-reductase were decreased (87).

By changing phosphoinositides metabolism Al causes cytoskeletal rearrangements and abnormalities, which alter cellular motility and viability (78). Already mentioned increased NO production by Al may react with superoxide or hydrogen peroxide to generate more reactive compounds which cause oxidation of thiol proteins (79).

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PATOFIZIOLOŠKI MEHANIZMI ALUMINIJUMSKE TOKSIČNOSTI

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Aluminijum čini oko 8% minerala zemljine kore. Najčešće se nalazi u obliku boksita rude koja se koristi za dobijanje tog metala. Aluminijum je visoko reaktivan i formira jedinjenja kao što su aluminijum-oksid, aluminijum-hidroksid i natrijum-aluminijum-sulfat. U kontaktu sa oksidansima, ta jedinjenja stvaraju površni pasivizirajući sloj aluminijum-oksida, koji sprečava koroziju i čini ga nerastvorljivim u vodi. Međutim, kisele kiše omogućavaju rastvaranje tih jedinjenja i ulazak aluminijuma u biološke sisteme. Aluminijum može ući u ljudski organizam preko vode, hrane, lekova i udisanjem zagađenog vazduha. Nakon što se akumulira u telu, on ispoljava toksične efekte na: centralni nervni, respiratorni, hematopoetski, reproduktivni, digestivni (jetru) i koštani sistem. Toksični sistemski efekti aluminijuma najpre su uočeni kod bolesnika sa bubrežnom insuficijencijom, lečenih lekovima koji sadrže aluminijumska jedinjenja (dijalizna encefalopatija, osteomalacija sa osteodistrofijom i mikrocitna anemija).

Aluminijum je u vrhu kratke liste neurotoksičkih neorganskih elemenata i njihovih jedinjenja. Povezuje se sa razvojem neurodegenerativnih bolesti, uključujući autizam, poremećaje pažnje, amiotrofičnu lateralnu sklerozu, Alchajmerovu bolest, demenciju, sindrom Zalivskog rata i parkinsonizam. Kliničke i eksperimentalne studije ukazale su na više mogućih mehanizama kojima aluminijum toksično utiče na ćelije. Tu spadaju: povećana produkcija oksidativnog stresa, promena funkcije membrana, poremećaj intracelularne signalizacije i promena ili inhibicija funkcije enzima.

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Ključne reči: *aluminijum, toksičnost, oksidativni stres, neurodegenerativne bolesti, patogeneza*

Review article

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USE OF BOTULINUM TOXIN A AND SUBSEQUENT REHABILITATION IN AMBULATORY CHILDREN WITH SPASTIC CEREBRAL PALSY – EFFECTS AND DILEMMAS

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The definition of cerebral palsy (CP) includes a group of permanent developmental disorders of movement and posture that cause activity limitation due to non-progressive lesions in the developing fetal or newborn infant brain. The most common type of CP is spastic cerebral palsy (SCP). One of the primary aims in treating children with SCP is to enable them to perform functional activities, to stimulate effective movement, to prevent deformities of bone and joint system, and to reduce pain. Limb deformities are the most prominent manifestation in children with SCP, greatly preventing them from performing activities of daily living. The reduction of spasticity and prevention of contractures, the development of performing functional activities in the full potential, as well as delays in performing surgical intervention, have dramatically increased the use of botulinum toxin type A (BTA) in treating children with CP lately. It is known as the most potent neurotoxin found in nature that reduces spasticity after application and results in irreversible denervation at the neuromuscular junction, while functional recovery is time limited. In that time period it is necessary to evaluate the functional motor status of a child, clearly and realistically define aims in cooperation with parents, and apply adequate rehabilitation protocol. The international consensus statement in 2010 defined the protocol, dosage, the site of application, and rehabilitation protocol. After being used for two and a half decades, certain dilemmas arose, particularly those regarding rehabilitation effects after BTA application.

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Key words: *cerebral palsy, children, botulinum toxin type A, spasticity, rehabilitation*

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Introduction

Cerebral palsy (CP) is a movement disorder produced by an injury to the immature brain. CP may be regarded as a static brain lesion causing a permanent motor impairment with evolving musculoskeletal manifestations. A broad spectrum of etiologies mediates neonatal brain injury in preterm and term infants. The most common cause can be cerebrovascular injury. Other etiologies includes: trauma, infections, metabolic dysfunction, etc. CP is the most common movement disorder in children. It occurs in about 1-3 per 1,000 live births (1). Signs and symptoms appear during infancy or preschool years and can vary greatly. In general, CP causes impaired movement associated with abnormal reflexes, floppiness or rigidity of the limbs and trunk, abnormal posture, involuntary movements, unsteady walking, or some combination of these. Children with CP also may suffer reduced range of motion at various joints of their bodies due to muscle stiffness (2). Spastic CP (SCP) is the most common type of CP. Up to 80% of all individuals with CP suffer from some degree of spasticity. The degree of spasticity can vary from mild muscle stiffness to severe, painful, and uncontrollable muscle spasms (3, 4). In a person with CP there is the damage in the brain which is usually in the area of the brain that controls muscle tone and movement of limbs.

The neural control of movement

The only action a human can bring about is muscular contraction. This muscular contraction results in movements that may in turn produce walking, writing or speech. But, the first step in performing any action is a thought which emerges in certain areas of the brain, which stimulate cortical motor centers via their connections. So, before an action is made it has been recorded as an electrical potential known as the "readiness potential", and it is located in the supplementary motor area, just anterior to the motor strip. This readiness potential occurs up to one second prior to a voluntary movement, whether the movement occurs in the hand, toe, mouth, tongue or eye, and irrespective of whether the movements are complex and programmed, or simple. Large, fast-firing neurons in the cortex, which are known as Betz cells, contribute axons to motor tracts which descend from the cortex to the brainstem (corticobulbar fibres) and spinal cord (corticospinal tracts) to connect with motor nerves that innervate muscles. The muscles, when stimulated by these motor tracts, then contract to bring about the desired purposeful movement and action (5).

Spasicity and musculoskeletal problems

Spasticity is one of the major problems in patients who have an upper motor lesion in the brain or spinal cord. The clinical feature of spasticity is increased muscle tone evidenced by a velocity-dependent increase in resistance to passive movement. Due to spasticity, the muscle growth in a child with CP will be abnormal for the following reasons: A spastic muscle will not allow stretch to the same degree as one with normal tone, as a result a muscle that initially has dynamic contracture, will soon develop static contracture. Bone growth in CP child is likely to be abnormal. The best example is femoral anteversion. A child with CP typically stands and walks with hips and knees in some flexion. Deformities of the feet are the most common musculoskeletal problem in children with CP. The most common deformity is equinus, which is present in the large majority of children with CP at the beginning of standing and walking. Children with SCP and equinus gait have longer-than-normal Achilles tendons and shorter-than-normal muscle bellies (6). Spasticity can affect the entire body, but it is generally worse in the lower limbs of children with bilateral involvement and in the upper limbs of children with unilateral involvement (7). Spasticity of the trunk muscles can cause postural problems while spasticity of bulbar origin can result in difficulty in feeding and communication (8). The most commonly affected lower limb muscles in children with SCP are gastroc-soleus, hamstrings, rectus femoris, adductors, and psoas. In the upper limb, spasticity is most frequently found in the shoulder external rotators, elbow, wrist and finger flexors, and the elbow pronators (9). Spasticity is thought to interfere with voluntary control and to increase energy consumption during movement (10). A child who is trying to walk with impaired motor control as well as dynamic and structural musculoskeletal deformities does not have an easy task, and he or she must learn to cope with the resultant problems. Pathological gait is a mixture of many abnormalities.

Management of spasticity in cp

The treatment includes functional therapies (physiotherapy, occupational therapy, speech therapy, constraint-induced movement therapy, robotic-assisted therapy, etc.); injections of botulinum toxin A (BTA); orthoses, casting and splinting; pharmacotherapies; intrathecal baclofen; selective dorsal rhizotomy (SDR); and single-event multi-level orthopaedic surgery, including the minimal invasive and other surgical reconstructive techniques (11). CP rehabilitation programs for children utilize a multidisciplinary approach where members of a team are selected with respect to a child's age, developmental level, severity of impairment, and availability of services. Management of spasticity in CP involves multidisciplinary intervention intended to increase functionality, sustain health, and improve quality of life for children and their carers. Several methods have been developed to assess the degree of spasticity and success of the treatment. The most commonly used tests in clinical practice are the MAS-Modified Ashworth scale and Modified Tardieu scale (12).

A priority in selecting rehabilitation protocol should be arranged as individual therapy approach. By analyzing up-to-date literature data it can be concluded that the most common and comprehensive parts of each treatment are physiotherapy and occupational therapy, with greatly expanding BTA application aiming at avoiding or delaying surgical intervention.

Botulinum Toxin Type A (BTA) and Rehabilitation

BTA is a neurotoxic protein produced by the bacterium Clostridium botulinum and related species. It prevents the release of the neurotransmitter acetylcholine from axon endings at the neuromuscular junction and thus causes flaccid paralysis. There are eight types of botulinum toxin, named type A–H. Injections of BTA are recommended for isolated (focal) spasticity. The effects of BTA last for approximately three-four months as the muscle will recover via proximal axonal sprouting, the formation of new neuromuscular junctions, and the regeneration of the original neuromuscular junctions. BTA is considered a safe and effective therapy for children with CP, especially in the hands of experienced injectors and for the majority of children. Over the past two decades BTA has been established as an important treatment modality for spastic movement disorders in children with CP. In most countries worldwide, it is licensed for children older than two years. Recommendations for treatment with BTA have been published since 1993, with continuous optimization and development of new treatment concepts. This leads to modifications in the clinical decision making process, indications, injection techniques, assessments, and evaluations. BTA is an important part of

multimodal management, to support motor development and improve function when the targeted management of spasticity in specific muscle groups is clinically indicated. Individualized assessment and treatment are essential, and should be part of an integrated approach chosen to support the achievement of motor milestones. To this end, goals should be set for both the long term and for each injection cycle. The correct choice of target muscles is also important; not all spastic muscles need to be injected. A more focused approach needs to be established to improve function and motor development, and to prevent adverse compensations and contractures. The conclusion is that there is no uniform BTA treatment strategy in SCP (11). The main reason is primarily the diversity of clinical manifestations in children with SCP.

Extensive usage and long-term application of BTA in treating spasticity in children with CP have caused a lot of dilemmas, revised certain attitudes and opened a lot of questions. Some very important current dilemmas are those regarding the importance of rehabilitation after BTA application to the lower extremity spastic muscle in ambulatory children, as well as measurements and comparison of individual contribution of these two treatments. Functional tests have an important role in monitoring and evaluating the success of treatment outcomes. Most commonly used ones include Gross Motor Function Classification System (GMFCS) for the estimation of achieved functional motoric level of evaluated patients–the classification system for the estimation of adopted rough motoric functions in children with CP (13), and Gross Motor Function Measure (GMFM) score to measure the change in gross motor function during the follow-up of evaluated patients with CP we used (14).

BTA is licensed for use in the management of spasticity in children aged 2 years or over, so the majority of studies lack outcome effects in younger children (15). In ambulant children with SFCP, gait disturbances are evident at the age of two. During the period of abrupt growth period fixed contractures develop rapidly, as well as the resulting deformities. Besides the aforementioned, the recommendation of the Surveillance of Cerebral Palsy in Europe (SCPE) is that definitive diagnosis of CP should not be established until the age of 3 years, when motor impairment is evident (2).

Currently, application of BTA treatment in children under the age of two is being debated. By analyzing the results from 3 randomized trials, with the youngest participant aged 11 months, it has been demonstrated that BTA application resulted in reducing spasticity, preventing contractures, and postponing surgery interventions. But, there is no evidence regarding the improvement in general motor development. Further studies are needed to further the knowledge, as well as the development of reliable assessment tools for such young infants. It is important to point out that two studies included a rehabilitation modality as a part of compulsory therapeutic treatment after application of BTA, such as stretching program and occupational therapy (16).

The safety profile of the recommended doses of BTA is the same for children under two years as for older children (17).

According to the last international consensus from 2010, it is recommended to apply individualized rehabilitation approach encompassing physiotherapy (especially stretching and strengthening) and occupational therapy, after BTA treatment of lower limbs in children with SCP. The recommendations are based on the result of papers published up to 2010, stating that the aforementioned therapy combined with BTA therapy is more beneficial than occupational and physiotherapy alone (18), and is recommended in patients receiving BTA therapy (19). The authors recommend precise measurements of qualitative and quantitative data in order to obtain high quality assessment of the outcomes. Considering the fact that there is no measurement unit encompassing both values, it is recommended that outcome measures should include at least one objective measurement on limb mobility related to local response to BTA application, as well as at least one measurement of functional outcome and treatment satisfaction. There are numerous tests prepared for children, but the first recommendation states that in ambulatory children with CP, PRS (Physician Rating Scale for Gait Analysis) is used to describe the quality of gate. In clinical practice, description and function of gait are relevant not only in monitoring therapy effects, but also in selecting target muscles and possible orthotic applications. The gold standard for the comprehensive assessment of gait function in ambulant children with CP is 3-dimensional gait analysis (3DGA), but it is not available at all levels of the health-care system. Besides the aforementioned, it is also not applicable in many children with best results achieved by BTA treatment, aged from one to four years, children with restricted walking ability (GMFMC levels I-III), due to difficulties in cooperation with children and inadequate physical measurements for obtaining a complete 3DGA analysis. 3DGA is complex, expensive, not always available, thus being impractical for routine practice. Because of that, simplified methods of gait analysis have been developed for spastic cerebral palsy, using standardized scoring system from video recordings. In the absence of 3DGA, utilization of modified PRS is recommended for gait analysis by the International Committee for BTA treatment (19).

Kinesiotherapy and occupational therapy are not the only modalities of rehabilitation protocol after BTA application; they may include hydro- and thermotherapy along with orthotic applications, based on physician's assessment. The authors of this paper investigated the effects of BTA injection and the effects of rehabilitation on spastic equinus correction. The youngest participant was 2 years and 9 months old, and the oldest was 6 years old. Rehabilitation protocol included: physical therapy individually designed, including thermotherapy; kinesiotherapy (exercises included movement range increase, ankle dorsiflexion facilitation, muscle stretching, antagonists strengthening, gait training, coordination, and correction of acquired improper

motor functions); occupational and functional therapy, and prescription of adequate orthoses for deformity correction. The program was initiated 5 days after BTA-ABO application, with the plan of the standardized physical therapy to be performed 3 times weekly in duration of 1 h per child. In the literature, such a plan implies intensive physical therapy (IPT). For the purpose of this study the physical therapy program lasted for 16 weeks. The values of passive foot dorsiflexion after 6 months, even though they were close to the initial values, were still highly significantly different ($p < 0.05$). After 6 months there was non-significant change in the proportion of patients regarding spasticity levels, even though a distribution trend of lower spasticity levels was noticed. Mean values of GMFM-D score (motor functions related to abilities of standing up and standing) after 3, 8, 16 weeks, as well as after 6 months, statistically significantly differ (higher values) versus the initial value before treatment (20).

In children with SFCP gait disorders, BTA application in lower limbs was followed by iPT (3 times weekly, 45-60 min duration per child, during a 16-week period). By the protocol, after BTA application (5-7 days), the rehabilitation programme included paraffin therapy, kinesiotherapy (exercises to increase movement range, elongation of muscles with reduced length, strengthening of the antagonists, balance and coordination exercises, exercises for the correction of improperly developed motoric functions: sitting, crawling, walking and for the stimulation of non-developed motor functions), occupational and functional therapy and application of adequate orthoses for the correction of foot equinus deformity. The effects of the therapy were assessed by monitoring functional motor status, using the motor function standardization system in children with CP (GMFCS), by quantification of adopted motor functions (GMFM-88), and by spasticity measurements as well. It has been noticed that children with CP achieve a higher level of motor development through BTA treatment and IPT, they benefit from this development for longer than they would solely from the pharmacological effect of BTA (21).

The analysis of the protocol stating that it is necessary to conduct rehabilitation treatment after BTA application into the spastic musculature of lower limbs gives rise to the question on the contribution of each separate treatment. Although it is well known from scientific literature that such combinations are more effective than sporadic physical therapy, the contribution of expensive BTA injections in overall treatment effect is unknown. Schasfoort et al. (2018) (22) published a study in 2018 to determine the effectiveness of BTA treatment prior to intensive physiotherapy (iPT) in comparison to iPT alone in a group of 65 children with SCP, aged between 4 and 12 years. The effectiveness of the treatment was monitored by the measurements of the following parameters: leg muscle strength, muscle length and spasticity of several leg muscles, CP-related pain, walking speed, several gait parameters, the degree of achieved individually tailored therapeutic goals and general functioning reported by parents. The results showed no differences between the groups. This suggests that extensive prescribing and utilization of BTA in ambulatory children with SFCP in this age group requires critical reconsideration. The authors of the study do not question the working mechanism or efficacy of BTA, but they demonstrate the possibility of false indications for BTA treatment, although the medical specialists included in the study had enough experience with BTA. A possible answer is that clinical assessment of spasticity is a subjective method representing a response to passive muscle stretching. The assessment is complicated because it is difficult to make a reliable distinction between non-neural (tissue-related) and neural (central nervous system related) contributions to hyper-resistance (23). That is why future studies should employ instrumented/quantitative assessment of different components of hyper-resistance aiming at precisely defining indications and categories of patients who require BTA treatment. Unlike BTA treatment, the results revealed unquestionable effect of iPT in both groups. Of course, a revised international consensus is needed to determine time periods for intensive rehabilitation.

Conclusion

The treatment of lower limbs spasticity in ambulatory children with SFCP requires a multidisciplinary approach, primarily employing rehabilitation and BTA application.

However, despite widespread use of BTA, its role prior to period of iPT with orthoses utilization remains unclear. Critical evaluation of literature data on effectiveness of BTA shows that the conclusions have been made on implicated assumption that BTA is the most effective component of the combined modalities for spasticity treatment. For this reason, positive clinical experience in combining BTA and other therapies may unjustly be attributed to BTA injections. In current papers that compared contribution of BTA and iPT, iPT is undoubtedly the dominant component for effectiveness. Of course, the effectiveness of BTA in spasticity treatment is unquestionable; however, it is necessary to define the subgroup of children with gait impairment that is most likely to benefit from BTA treatment.

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UPOTREBA BOTULINUM TOKSINA A I NAREDNA REHABILITACIJA AMBULANTNA KOD DECE SA SPASTIČNOM CEREBRALNOM PARALIZOM – EFEKTI I DILEME

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Cerebralna paraliza (CP) po definiciji obuhvata grupu trajnih razvojnih poremećaja pokreta i držanja, koji uzrokuju ograničenje aktivnosti, a uslovljeni su neprogresivnom lezijom mozga u razvoju fetusa ili novorođenčeta. Najzastupljeniji tip je spastična cerebralna paraliza (SCP). Jedan od primarnih ciljeva lečenja dece sa SCP je omogućavanje aktivacija, promovisanje efikasnog kretanja, sprečavanje nastanka deformiteta koštano-zglobnog sistema i smanjenje bola. Deformiteti ekstremiteta predstavljaju najuočljiviju karakteristiku dece obolele od SCP, koja ih u velikoj meri ograničava u aktivnostima svakodnevnog života. Smanjenje spasticiteta i prevencija kontraktura, uz mogućnost pune aktivacije i odlaganje hiruške intervencije, uslovila je pravu ekspanziju primene botulinskog toksina tipa A (BTA) u terapiji dece sa CP poslednjih godina. Poznat kao najjači neurotoksin u prirodi, BTA nakon aplikacije smanjuje spazam ireverzibilnom denervacijom u nivou neuromišićne spojnice, dok je funkcionalni efekat vremenski ograničen. U tom vremenskom periodu neophodno je proceniti funkcijski motorički status deteta, jasno i realno definisati ciljeve u saradnji sa roditeljima i primeniti adekvatan rehabilitacioni protokol. Internacionalni konsenzus 2010 god. definisao je prokolol, doze, mesto aplikacije, rehabilitacioni protokol. Nakon dvoipodecenijske primene, pored ostalih, javile su se i dileme u pogledu efekta rehabilitacije nakon primene BTA.

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Ključne reči: *cerebralna paraliza, deca, botulinum toksin tip A, spasticitet, rehabilitacija*

Review article

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RADIOLOGICAL CHARACTERISTICS OF PERIOSTEAL REACTIONS

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The periosteum is a thin fibrous tissue in a form of fibro-vascular membrane which covers the outer surface of bones. On conventional radiography normal periosteum is completely invisible. The periosteum can be visualized when it is elevated by underlying pathology. Periosteal reaction can be unilateral or bilateral, or localized and generalized. Depends on how fast it takes periosteum to react, it can be divided into aggressive and non-aggressive. The role of computed tomography as superior method vs. nuclear magnetic resonance, ultrasonography or scintigraphy, has been noticed a long time ago.

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Key words: *periosteal reaction, radiology, differential diagnose, causes*

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Introduction

The periosteum is a thin specialized fibrous tissue in a form of fibro-vascular membrane which covers the outer surface of all bones, except at the joints of bones (which are protected by articular cartilage) (1, 2). Histologically, it contains the inner layer which is made of both osteoblasts and fibroblasts (so-called "cambium layer") and the outer layer which contains only fibroblasts (so-called "fibrous layer"). It is a membrane that has the ability for bone metaplasia. On conventional radiography normal periosteum is completely invisible. Today it is well known that the outer layer contains fibroblast, collagen, elastin, nervous and microvascular structures which all together provide stability to the periosteum. The inner layer is highly cellular (inner cellular layer- cambium), and these cells, such as mesenchymal progenitor cells, osteoblasts, fibroblast and symphatic nervous system, are responsible for bone growth and reparation. All of these characteristics allow the periosteum high bone regenerative potential. With aging there is a decrease in the number of the osteoblasts which leads to senile atrophy and thinning of the inner layer. The outer layer is vascularized by many branches of muscle blood vessels from attaching muscles. This way blood system nourishes the exterior of the cortex and anastomoses with bone blood vessels through Haversian system and Volkmann's canals. The periosteum (cambium) is responsible for normal lamellar bone apposition on cortical bone that grows in width (periosteal ossification) (3).

Inside of the periosteum there is a large number of endothelial pericytes (4). Perycites are cells that are in close contact with endothelial cells. In the certain circumstances, these cells may differentiate into osteoblasts; these are why these cells are considered to be important source of osteoprogenitor cells (5).

Periosteum can be visualized when it is elevated by underlying pathology (1). It takes up to ten days to three weeks for it to be visualized after the specific pathological process has begun. Through whole lifetime, periosteum can produce new bone layers and that is being used in orthopedic and maxillofacial surgery (6-8).

Inner layer of the periosteum and bones are bound together by collagen fibres called Sharpey's fibres that penetrate into bone. This location is subject to the traumatic separation followed with subperiosteal hemorrhage and hematoma (9).

Periosteal reaction is caused by anything that can irritate the bone. How it looks depends on the age of the patient, the cause, the intensity of the lesion, etc. Periosteal reaction is much more intense in childhood, because in this age periosteum is attached more loosely than in the adult age and there is less number of Sharpey's fibres which are

also shorter. The more intense pathology processes give more intense periosteal reaction (9).

The aim of our work was to show radiological characteristics of periosteal reactions with images of our patients with periosteal reactions on conventional radiography, computed tomography and nuclear magnetic resonance.

Types of periosteal reaction

Periosteal reaction can be unilateral or bilateral, or it can be localized and generalised. Bilateral can occur in systemic and unilateral in localized diseases (9). The most important localized processes that can cause periosteum to react are malignancies, infections and traumas.

Depends on how fast it takes periosteum to react, it can be divided into aggressive and non-aggressive. Non-aggressive periosteal reaction can be solid single layer or multilayered, forming a solid layer of new bone adjacent to the cortex. Solid single layer type is seen in benign lesions like osteoid-osteoma, trauma, chronic osteomyelitis, etc., while multilayered type that looks like "onion skin", is seen in Ewing's sarcoma. Aggressive type occurs in processes that are acute and rapid and in malignity. It can be spiculated (hair on end), form of periosteum that is perpendicular on the outer surface of the bone (Ewing's sarcoma) or it can be divergent – sunburst (osteosarcoma). Codman's triangle is a type of the periosteal reaction that is seen in aggressive lesions such as malignity, hemorrhage and osteomyelitis. With aggressive lesions, the periosteum does not have time to ossify with shells of new bone, so only the edge of the raised periosteum will ossify. Codman's triangle is consisting of triangular elevation of the periosteum with one or more layers of new bone (9).

So, as we have seen, there are several types of periosteal reactions (Figure 1). Periosteum can be seen as:

• *Nonaggressive or benign types of periosteal reactions:*

- Solid smooth:
  - ◇ Thin or single layer
  - ◇ Thick, linear osseous deposit may be separate from or merged with the underlying bone
- Solid buttress:
  - ◇ Thick irregular
  - ◇ Smooth
- Septated

• *Aggressive types of periosteal reactions:*

- Laminated or multilayered ("Onion-skin")
- Thin, linear osseous deposits extend in a direction perpendicular to the underlying cortex
- Spiculated pattern:
  - ◇ An irregular osseous excrescence with a spiculated contour merges with the underlying cortex (hair on end-parallel spiculated)
  - ◇ A sunburst pattern, in which linear deposits fan out from a single focus (divergent spiculated)
  - ◇ Sloping (velvet)
- Codman's triangle consisting of triangular elevation of the periosteum with one or more layers of new bone and
- Disorganized or complex type (10).

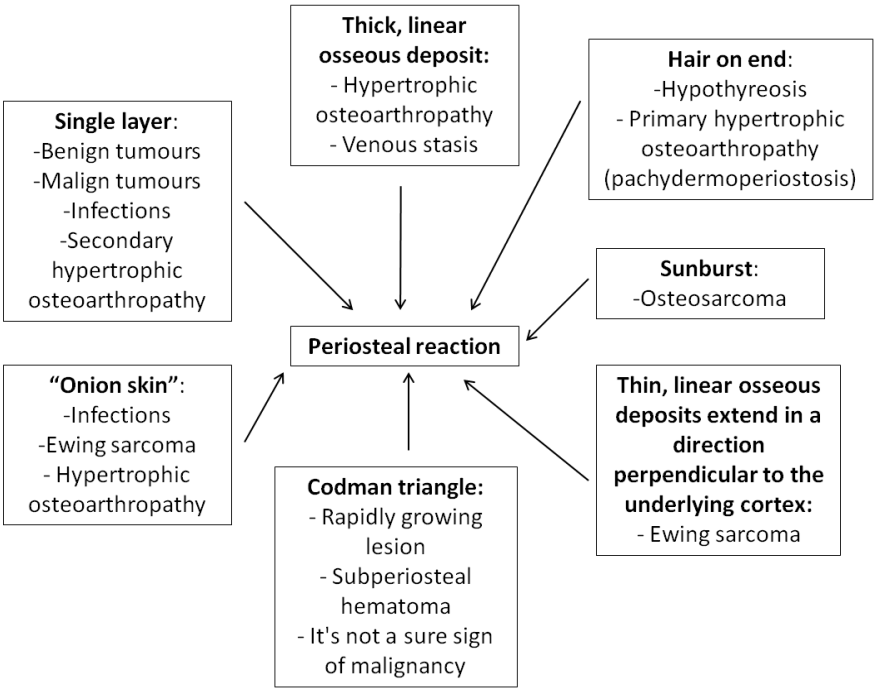

Figure 1. Differential diagnosis of periosteal reaction based on the radiographic (morphological) appearance

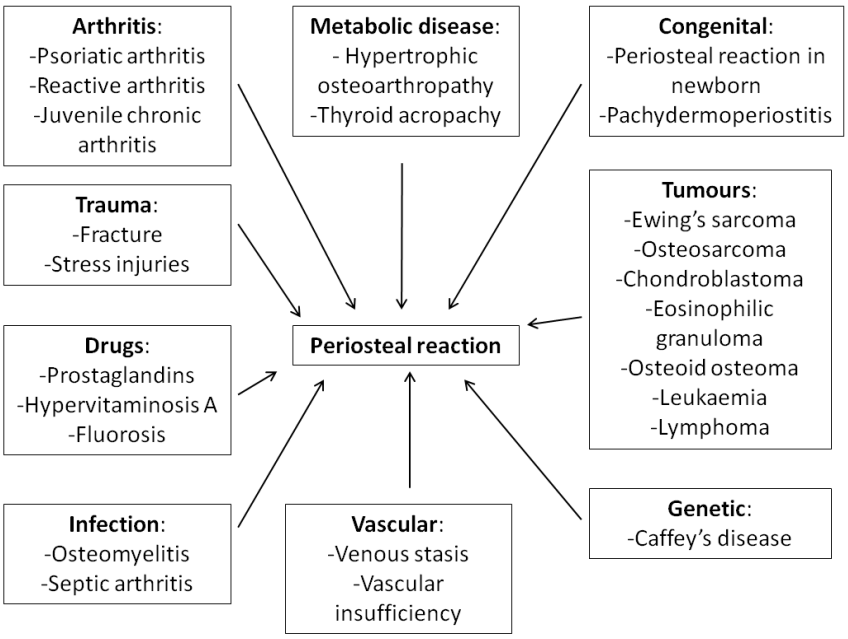

Figure 2. Differential diagnosis of periosteal reaction

Causes of periosteal reaction – differential diagnoses

There are many diseases that can cause periosteal reaction. They can be localized or systemic, or they can be hereditary and acquired (Figure 2).

Different causes irritate periosteum to react unilateral or bilateral. Unilateral reactions are seen in malignancies, infections and traumas. Bilateral are seen in adults, where they are symmetrical and are caused by hypertrophic ostheoartropathy, pachydermoperiostitis, vascular insufficiency, thyroid acropachy and abuse of fluoride. In children who are up to six months old, this type (bilateral symmetrical periosteal reaction) is seen in congenital syphilis, prostaglandin E1 therapy and Caffey's disease (infantile cortical hyperostosis). After that age (after six months old) they are symmetrical when caused by hypervitaminosis A, acute leukemia, metastatic neuroblastoma and juvenile chronic arthritis. Bilateral unsymmetrical periosteal reactions are seen in metastatic diseases, osteomyelitis, psoriatic and reactive arthritis, osteoporosis and osteomalacia, intention trauma, haemorrhagia and sickle cell dactylitis (9, 10).

Today as the population is getting older, many of the patients that take up to several drugs at the time in chronic therapy, there is also influence of the drugs on the periosteum (or bony tissue in general (11). Beside overdose with fluor (12) and prostaglandin E1, voriconazole can also cause periostitis (10, 13-17). Voriconazole lead to increase level of fluor in body, most often when there is kidney failure, and increased level of fluor leads to generalised periosteal reaction (14, 18). Risk factors for fluorosis are kidney damage, chronic abuse of voriconazole, slow metabolism of the drug and high levels of fluor in the body. All of these factors are responsible for high level of fluor - fluorosis and it causes periosteal reaction by increasing the osteoblastic activity (18).

Substance called 1,1-difluoroethane ("Dust-off") is refrigerant and aerosol propellant that can be inhaled and can, with same mechanism as voriconazole, cause generalised periosteal reaction. It also increases the level of fluor in the body. Dust-off is used for dusting the computers, key boards, photo equipment and other electronic devices, a lot of stuff for everyday use including windows, drapes, etc. Because of that, periosteal reaction in adolescents or people that have positive history of addiction can indirectly show the abuse of some substances (19).

Also, there is interleukin-11 that is thrombopoietic growth factor which stimulates production, proliferation and differentiation of megakaryoblasts into thrombocytes (11, 20).

Image methods for periosteal reaction visualization

Periosteum cannot be seen by conventional radiology in normal bone. Only when it is irritated by some pathological process and when there is periosteal reaction it can be observed (Figures 3-7). It takes 10 days for that to happen (21).

For diagnostic we can also use magnetic resonance imaging (MRI) and computed tomography (CT), which are more sensitive for the early visualization. The MRI is superior for detecting processes in soft tissue and periosteum (21, 22) (Figure 8). Even better is CT for detecting periosteal reaction and ossification of bones (23, 24). The role of CT as superior method has been noticed a long time ago (23) (Figure 9).

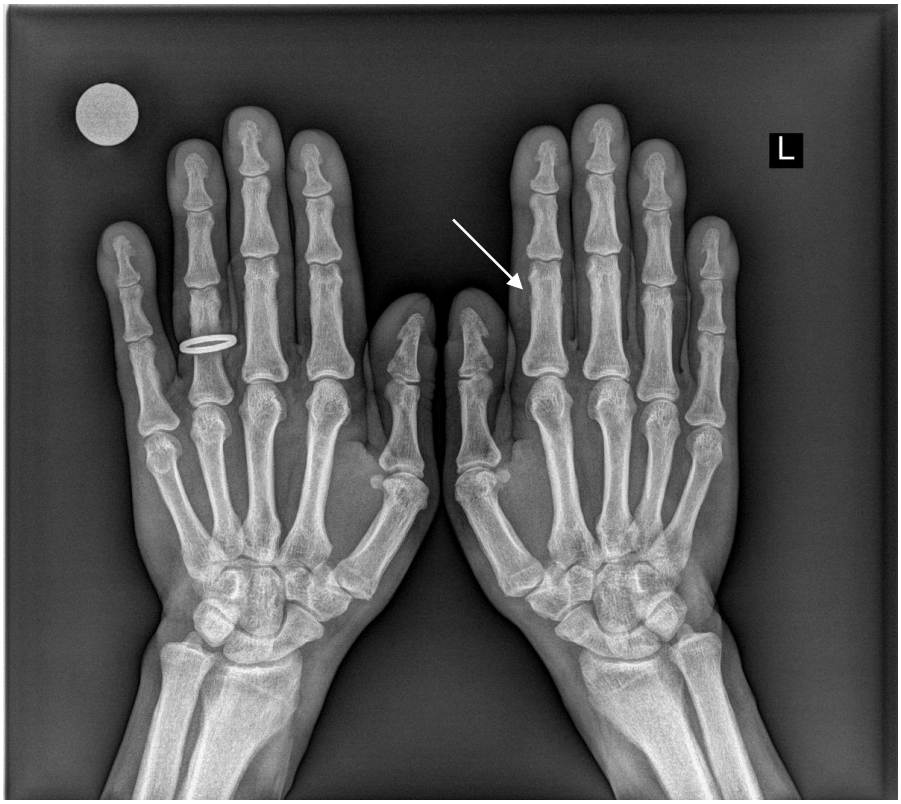

Figure 3. Left and right hand radiography at presentation show periosteal reaction involving middle and proximal phalanges of both hands in a fairly symmetric pattern - patient with hypertrophic osteoarthropathy

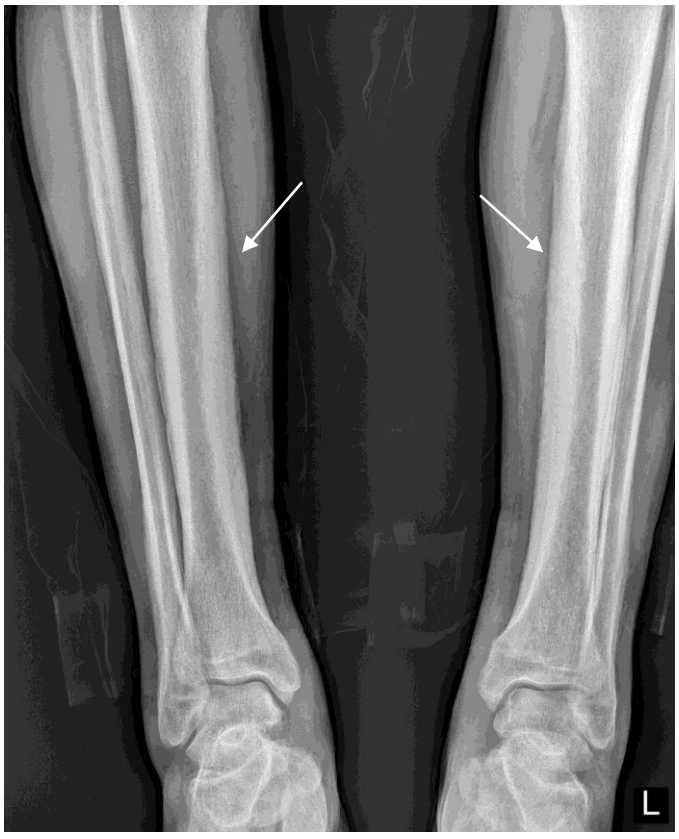

Figure 4. Left and right shin radiography at presentation show a periosteal reaction involving middle and distal diaphysis - patient with hypertrophic osteoarthropathy

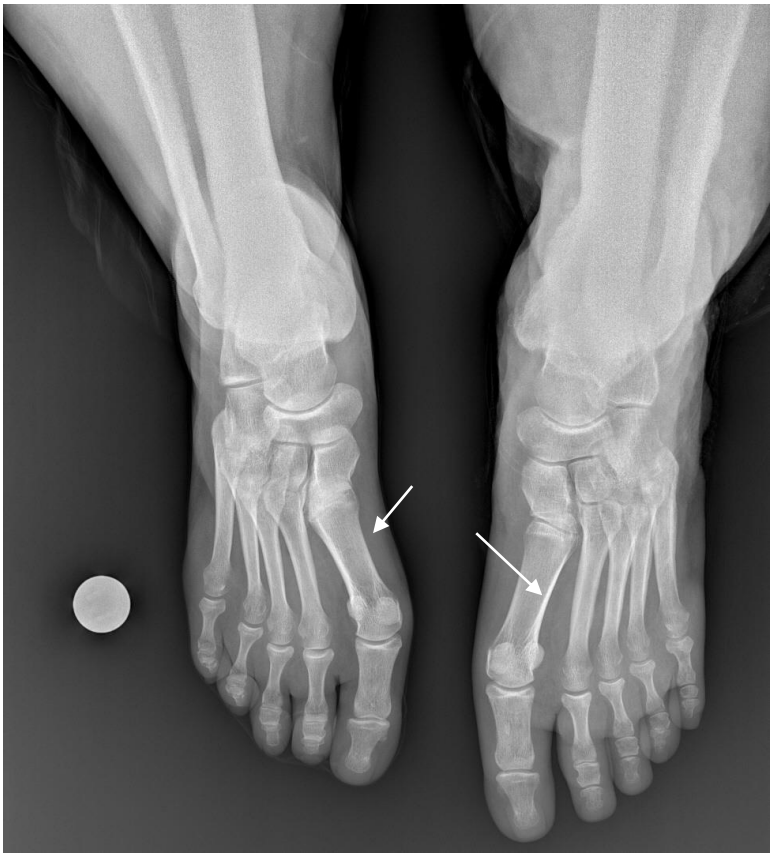

Figure 5. Left and right leg radiography at presentation show periosteal reaction involving proximal phalanges of both legs - patient with hypertrophic osteoarthropathy

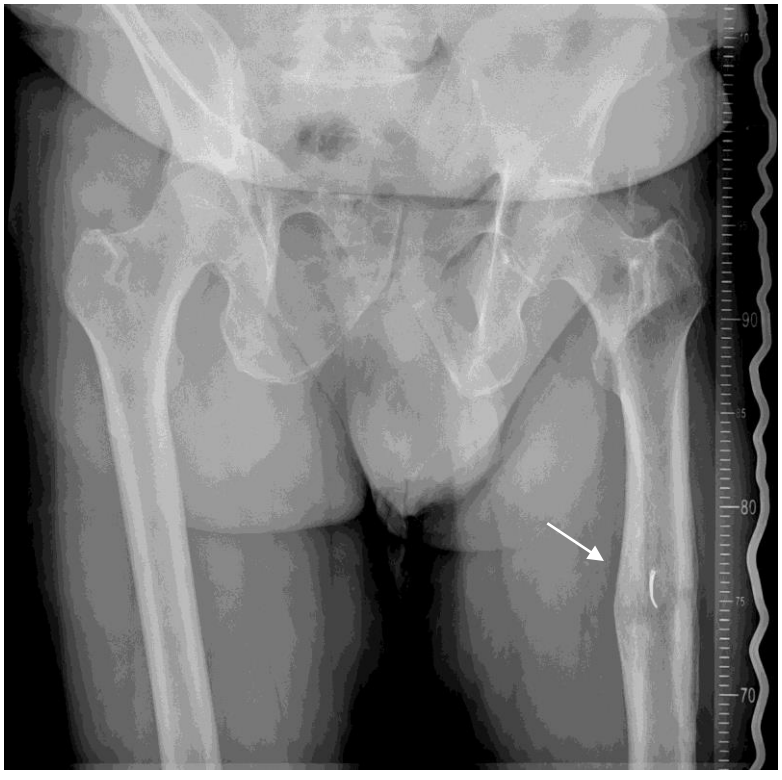

Figure 6. Left femur radiography at presentation show periosteal reaction involving middle and proximal diaphysis around the foreign body

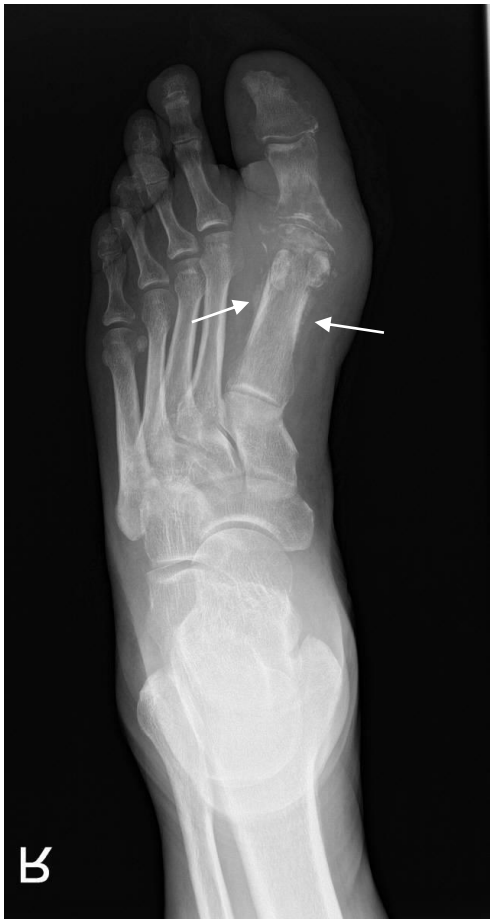

Figure 7. Right leg radiography at presentation show periosteal reaction involving first metatarsal bone after fracture and phlegmon

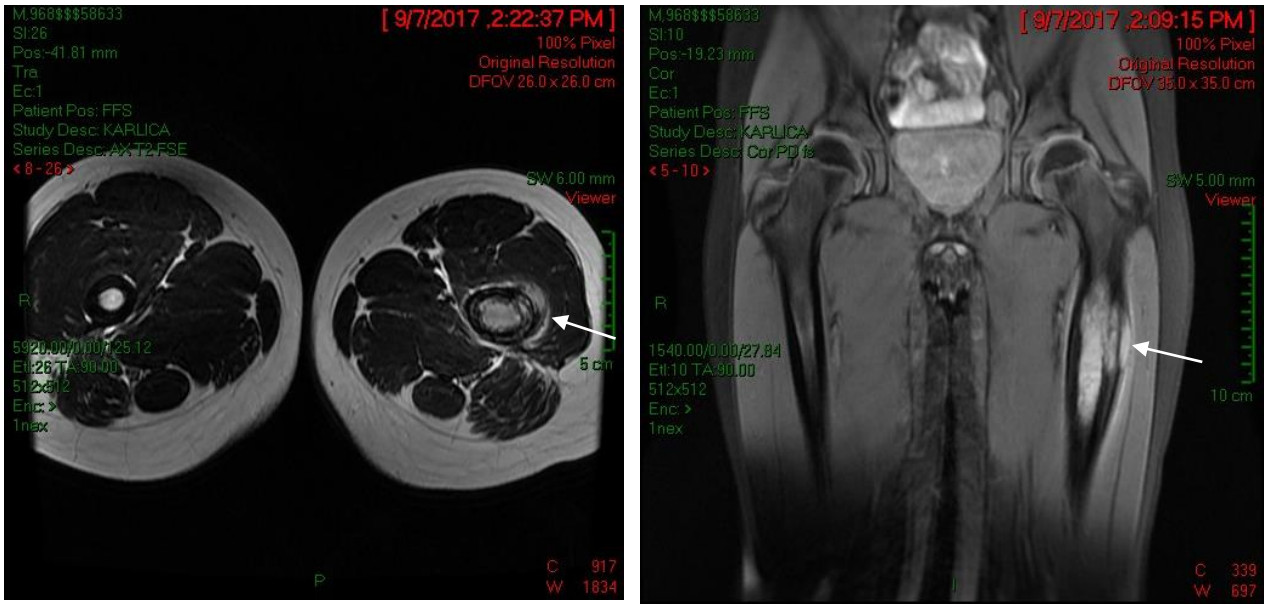

Figure 8. Left femur MRI images at presentation show periosteal reaction involving middle and proximal diaphysis around the foreign body- Ewing's sarcoma

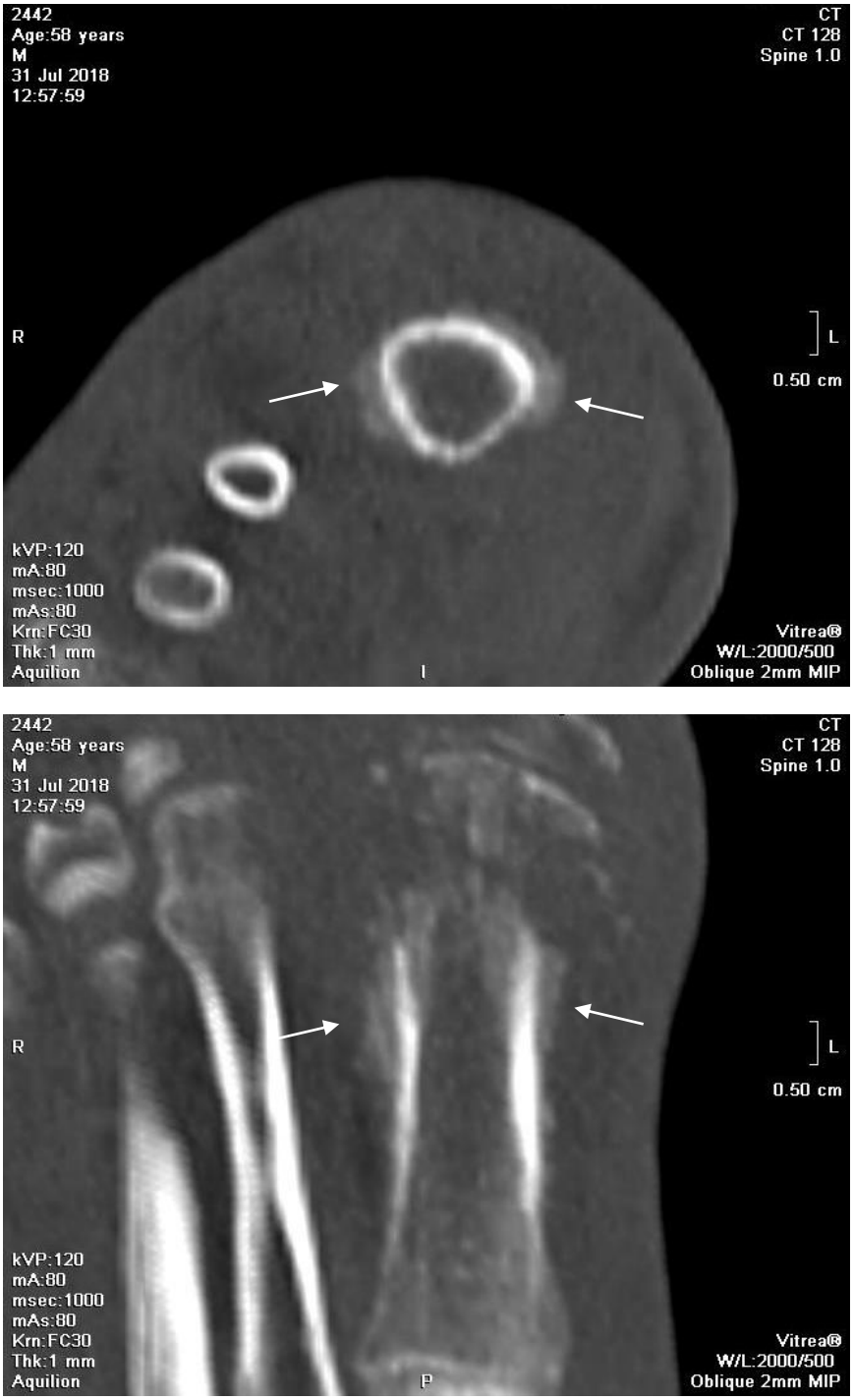

Figure 9. Right leg CT images at presentation show periosteal reaction involving first metatarsal bone (the same patient as in the Figure 7)

Ultrasonography has a minor role in visualization of the periosteum, but is used during biopsy of the changed tissue with fine needle and also is used for precise localization of these changes (21). Scintigraphy can also be used, but despite its high sensitivity it has low specificity (21).

Conclusion

Periosteal reactions are very important in radiology diagnosis. Many localized or systemic diseases can cause periosteum to react. Because of that, general knowledge of the periosteal reactions is necessary in radiology diagnostic of the skeletal system.

Conflict of Interest

The authors declare that they have no conflict of interest.

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RADIOGRAFSKE KARAKTERISTIKE PERIOSTNIH REAKCIJA

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Periost je tanko fibrozno tkivo (fibro-vaskularna membrana) koje prekriva spoljašnju površinu kosti. Na klasičnoj radiografiji normalan periost potpuno je nevidljiv. Može se vizualizovati kada je nadražen nekim patološkim procesom. Periostna reakcija može biti unilateralna ili bilateralna, odnosno lokalizovana ili generalizovana. Periostne reakcije dele se, u odnosu na brzinu kojom se javljaju, na agresivne i neagresivne. Uloga kompjuterizovane tomografije, kao superiorne metode, u odnosu na nuklearnu magnetnu rezonancu, ultrasonografiju ili scintigrafiju, pokazana je ranije.

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Review article

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THE ASSOCIATION OF GENETIC POLYMORPHISMS WITH DIABETES MELLITUS TYPE 1

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One of the most common endocrine disorders in children and adolescents, Diabetes mellitus (DM), is a chronic, polygenic and complex illness. Based on etiology, DM can be divided into two basic groups: Diabetes mellitus type 1 (DMT1) and Diabetes mellitus type 2 (DMT2). DMT1 is a disease mediated by immune mechanisms based on the autoimmune destruction of insulocyte by immunoreactive T cells and antibodies which leads to insulin resistance and hyperglycemia syndrome.

Several different molecular mechanisms contribute to the destruction of β-cells of Langerhans islets, such as: the production of autoantibodies directed against antigens on insulocytes, lysis of Langerhans islets mediated by cytotoxic CD8+ T lymphocytes, local production of cytokines (TNF and IL-1) by macrophages that damage insulocytes and delayed hypersensitivity reactions mediated by CD4+ Th1 lymphocytes.

The most important genes that show a high degree of association with DMT1 are found in the region which encodes MHC (major histocompatibility complex) of molecules class I and II. However, there is very strong evidence of association of other genes (outside of the MHC gene domain) with the expression of DMT1. These are: insulin gene (INS), Cytotoxic T-Lymphocyte Associated Protein 4 (CTLA4), Protein Tyrosine Phosphatase Non-Receptor Type 22 (PTPN22), Protein Tyrosine Phosphatase Non-Receptor Type 2 (PTPN2), C-type lecithin gene (CLEC16A, KIAA0350), interleukin 2 receptor α gene (IL2RA/CD25), interferon-induced helicase gen domain (IFIH1), Potassium Voltage-Gated Channel Subfamily J Member 11 (KCNJ11), Platelet and T Cell Activation Antigen 1 (CD226), vitamin D receptor gene (VDR), tumor necrosis factor gene (TNF) and lymphotoxin-α (LTA). In addition to these genes, researchers are still searching for other genes that are associated with the appearance and expression of DMT1.

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Key words: *Diabetes mellitus type 1, autoimmune endocrinopathy, non-HLA gene polymorphisms*

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Diabetes mellitus type 1

Diabetes mellitus type 1 (DMT1) is an immune-mediated disease based on the autoimmune destruction of Langernahs pancreatic insulocytes by immunoreactive T cells and antibodies, resulting in a progressive decrease in the number of insulocytes and their ability of insulin secretion leading to insulin resistance and hyperglycemia syndrome. This chronically progressive autoimmune disease occurs in a person with a genetic predisposition to its development. The first symptoms of this disorder most commonly occur early in childhood while the disease appears in puberty and progresses with age. According to the classifications given by the World Health Organization (WHO) and ADA, there are two types of DMT1. The more common type (1A) belongs to autoimmune diseases of complex etiology, while the rare type (1B) is characterized by idiopathic etiology. Both types are characterized by the destruction of β-cells Langerhans's isles in the pancreas in genetically predisposed individuals. This occurs under the influence of infectious or toxic environmental factors which consequently leads to a lack of insulin. Based on the appearance of typical symptoms of the disease (polydipsia, polyuria, polyphagia), and the presence of elevated blood glucose levels, the diagnosis of DMT1 is established.

Complications of DMT1 have been common including macroangiopathies, such as progressive atherosclerosis of the arteries leading to ischemic sclerosis of the extremities and internal organs and microangiopathy, i.e., microvascular obstructions leading to damage of the retina, renal glomeruli, and peripheral nerves. All mentioned complications of DMT1 can lead to renal and cardiovascular disease, blindness, and early death of adults, the insulin therapy is necessary both for disease control and for prolonging the life age. Most people with DMT1 depend on external insulin compensation and without insulin, they develop severe metabolic complications such as acute ketoacidosis and coma (1, 2).

Etiopathogenesis of DMT1

The mutual action of certain genetic, immunological and environmental factors leads to the initiation of the process that will affect the destruction of type B insulocytes. Environmental factors include viral infections (cytomegalovirus, mumps, rubeola, coxsackievirus), early infant feeding with cow's milk, additives (nitrosamines) and toxins in food. External factors include climate change, stress and vaccination. These factors can represent a trigger for the occurrence of DMT1. Viruses, for example, promote the development of DMT1 mainly by molecular mimicry (GAD – the glutamic acid decarboxylase antigen on type B insulocytes and *Coxsackie B4* virus have similar molecular properties) or non-specific cytokine-mediated reactions. The onset of diabetes is also affected by the secretion of certain hormones within physiological limits. The highest incidence of this type of diabetes is from 11 to 13 years of age when the growth hormone is most secreted as well as sex hormones that have the effect opposite to insulin (3–5).

There are several molecular mechanisms that contribute to the destruction of the β-cells of the Langerhans islets. The most important of these are:

a) production of autoantibodies directed against antigens on insulocytes,

b) lysis of Langerhans's islets mediated by cytotoxic CD8+ T lymphocytes,

c) local production of cytokines (TNF or IL-1) by macrophages that damage the insulocytes,

d) delayed-hypersensitivity mediated by CD4+ Th1 lymphocytes that are reactive to insulocyte antigen (including insulin) (6, 7).

Genetic factors that affect the appearance of DMT1

DMT1 is a complex, genetically determined disease whose development and manifestation also depends on environmental factors. Genes that can lead to this disease are found in 60 chromosomal regions. The most important genes that show a high degree of association with DMT1 are found in the region that encodes the MHC (*major histocompatibility complex*) class II molecule located on the 6p21 (HLA-D) chromosome, as well as the genes for MHC class I molecule. MHC genes are designated in humans as HLA (human leukocyte antigen) genes. Besides these, there are other genes that are associated with the manifestation of DMT1, but the evidence of their association is still investigated. These are: 5' end of insulin gene (INS), gene for protein 4 linked to cytotoxic T lymphocyte (CTLA4), gene for protein tyrosine phosphatase - 22 (PTPN22), gene for protein tyrosine phosphatase - 2 (PTPN2), gene for protein lecithin type C (*CLEC16A*, known as *KIAA0350*), gene for receptor of α interleukin 2 (IL2RA/CD25), gene region of interferon-induced helixase (IFIH1), KCNJ11, CD226, gene for vitamin D receptor (VDR), genes for tumor necrosis factor (TNF) and lymphotoxin-α (LTA) and gene regions on chromosomes 12q24, 12q13, 16p13 and 18p11. In addition to these genes, researchers are still searching for other genes that are associated with the appearance and expression DMT1 (2, 7, 8).

Human leukocyte antigen

The HLA system plays an important role in human immune responses. There are three classes of HLA systems with their corresponding functions: HLA I (HLA-A, HLA-B and HLA-C), HLA II (HLA-DR, HLA-DQ and HLA-DP) and HLA III. The genes HLA class I and II encode molecules that have a major role in the treatment and presentation of antigen by T lymphocytes (9, 10).

The HLA region of chromosome 6 is responsible for nearly 50% of the genetic predisposition to the development of DMT1. The subregions of DR4 and DR3 HLA class II are strongly related to the occurrence of DMT1 and the combination of DR3 / DR4 allele multiplies the risk of illness. It is believed that haplotype combinations of class II of the HLA gene DR4-DQB1-0302 and DR3-DQB1*0201 are the main markers of the tendency towards this disease. About 90% of children with DMT1 are carriers of one or both of these haplotypes of the gene. It is known that about 95% of Caucasians with DMT1 are wearing HLA-DR3, DR4 or both antigens. In carriers of DR3 and DR4 antigens, the risk of developing this disease is increased tenfold (2, 3, 9).

Gene for insulin (INS)

Insulin has a major role in the clinical, genetic and immunological aspects of DMT1. Gene for insulin is localized to 11p15 chromosome. This gene encodes a protein, pre-proinsulin, consisting of 110 amino acids, which is synthesized by beta cells of the Langerhans's islets. By reaction of proteases that eliminate the signal peptide pre-proinsulin is modified to proinsulin which is transformed into biologically active insulin after splitting of the C-peptide (11, 12).

The insulin gene locus is the second most important locus after the HLA locus which indicates a predisposition to the development of DMT1. The insulin gene comprises 3 exons and 2 introns, separated from each other by several polymorphisms in linkage disequilibrium (LD). The insulin locus represents a variable number of tandem repeats (VNTR)

in the INS gene promoter. The alleles of this region are divided into three classes, I, II and III. Alleles class I contain about 570 base pairs (bp) (26-63 repeats), allele class II have 1200 base pairs (around 80 repeats) and allele class III have 2200 base pairs (140-210 repeats). Studies have shown that allele class I are associated with a higher risk of developing DMT1 than allele class III that are considered to have a protective role (13–15).

The genetic susceptibility mechanism is explained in such a way that the own antigens for allele class I are less expressed in thymus compared to own antigens for allele class III that are two to three times more expressed in the thymus. This will affect the positive and negative selection of T lymphocytes in the thymus. There is the inverse pattern of expression in the pancreas (16, 17).

Gene for protein 4 associated with cytotoxic T-lymphocyte antigen-4 (CTLA4)

CTLA-4 gene is located on chromosome 2q33. It encodes the homonymous molecule localized on the surface of activated T-lymphocytes which is responsible for suppressing the immune response. Inactive T-lymphocytes do not express CTLA-4 on their surface while when activating T-lymphocytes this molecule binds to the receptor on antigen presenting cells (APC), which stops activation of T-lymphocyte thereby interrupting the immune response. The CTLA-4 binding to the APC receptor creates an inhibitory signal for the production of IL-2 and IL-2 receptors, as well as the continuation of the cell cycle. In this way, CTLA-4 plays an important role in the regulation of lymphocytic expansion which leads to a gradual reduction of the immune response. Changes in the expression of the gene for CTLA4 can lead to an increase in the autoreactivity or reactivity of T-lymphocytes, which may affect the onset of autoimmune diseases such as DMT1 (18, 19).

The onset of many other autoimmune diseases is associated with the mutation of the CTLA-4 gene. Mutation of this gene can cause asthma, Addison's disease, myasthenia gravis, Sjogren's syndrome, systemic lupus erythematosus, systemic sclerosis, ulcerative colitis and thyroiditis (20–23).

The following polymorphisms of the CTLA-4 gene have shown the association with DMT1: 49A/G in exon 1, CT60 in 3' UTR (untranslated region), -318C/T, -1661A/G i -1722C/T in the promoter region. Several studies in which the polymorphism of CTLA-4 gene was showed that the greatest association with DMT1 shows allele G polymorphism 49A/G of CTLA-4 gene. The SNP CTLA-4 gene is associated with the symptoms of DMT1 in several human populations (19, 24, 25).

Gene for protein tyrosine phosphatase-22 (*PTPN22*)

Gene for PTPN22 is localized to chromosome 1p13.3-13.1. This gene encodes lymphoid tyrosine phosphatase (LYP) which inhibits the proliferation and activation of T-lymphocytes (26). LYP in interaction with Csk, C-terminal tyrosine kinase, reduces signal transduction via TCR (T-cell receptor), thus regulating various cellular activities including cell growth, differentiation, division and apoptosis. The polymorphism at position 1858 within this gene, when cysteine is replaced by thymine leads to a mutation at the codon 620 for LYP. This mutation leads to the loss of interaction between LYP and Csk which creates conditions for inadequate prolonged activation of T-lymphocytes leading to autoimmunity and the possibility of DMT1 (8, 19).

The variant of PTPN22 R620W is associated with multiple autoimmune diseases such as lupus erythematosus, rheumatoid arthritis, juvenile idiopathic arthritis, multiple sclerosis, autoimmune thyroiditis, psoriasis and Addison's disease, as confirmed by more studies in Europe and the United States (27, 28).

Gene for the α interleukin 2 receptor (*IL2RA/CD25*)

The CD25 gene is located on chromosome 10p15. This gene encodes the α-chain IL-2 receptor which is expressed for several hours after antigen activation on CD4+ and CD8+, as well as on regulatory T-lymphocytes. Only after the formation of the third subunit of the IL-2 receptor (α-chain) this receptor can bind IL-2. IL-2 generates the same cells that this interleukin acts through its receptor. IL-2 is also referred to as T-lymphocyte growth factor, because its basic activity is stimulation of T-lymphocyte proliferation, that is, the stimulation of the entry of T-lymphocytes into the cell cycle and their consequent division. This leads to an increase in the number of antigen-specific T-lymphocytes. Expression of CD25 on regulatory T-lymphocytes is an important factor in suppressing the T-cell immune response and consequently also on the protection against the onset of autoimmune diseases. However, mutations on this gene in humans lead to severe forms of autoimmune diseases, including DMT1 (8, 29).

The genetic region for interferon-induced helicase (*IFIH1*)

The genetic region for interferon-induced helicase (IFIH1) is located on chromosome 2q24. IFIH1 encodes cytoplasmic viral sensor melanoma differentiation associated protein 5 (MDA5). After viral infection, MDA5 binds to viral RNA chains activating transcriptional factors (IRF7, IRF3, IRF1 and NFκB) which induce the formation and secretion of several proinflammatory factors, interferons and interleukins: INF-α, INF- β, INF- γ IL-1, IL-6, IL-8. Proinflammatory cytokines activate NK and T-lymphocytes, then attract dendritic cells, macrophages, and granulocytes to the site of infection leading to the destruction of beta cells of the pancreas. In this way, the presentation of the own antigens of the pancreatic beta cells to the immune system is increased resulting in an autoimmune reaction, which

is a trigger for the development of DTM1. This confirms the fact that viruses can contribute to the initiation of DMT1 (27, 30).

Several polymorphisms of IFIH1 gene locus, rs1990760 or Thr946Ala, rs2111485, rs3747517, and rs13422767, are associated with the emergence of DMT1 (31).

Gene for vitamin D receptor (*VDR*)

DMT1 is a common autoimmune endocrinopathy in which the deficiency of Vitamin D can be one of the causes of its initiation. It has been shown that the application of 1,25-dihydroxy vitamin D3, in the experimental model of animals suffering from T-cell-dependent autoimmune diabetes reduces the incidence of this disease. The way in which disease progression is stopped is not yet fully clarified but there is evidence of cytokine modulation of T helper (Th1/Th2) lymphocyte balance in local pancreatic lesions. Recent studies show that a significant pathophysiological role in the onset of diabetes mellitus has a specific vitamin D (VDR) nuclear receptor, which is particularly expressed on monocytes and activated T-lymphocytes. By binding the ligand to VDR receptor there is a decrease in secretion of cytokines (IL-1, IL-2, IL-6, IL-12, TNF, and interferon-γ) (32, 33) involved in the pathogenesis of autoimmune diseases. In this regard, it has been shown that VDR receptor polymorphism has an effect on the occurrence of DMT1 (34, 35).

The VDR gene is localized to a long arm of chromosomes 12 and 14. Although there are many polymorphisms of this gene only four SNPs have been tested so far:

- Bsm1 (rs1544410),
- Fok1 (rs10735810),
- Apa1 (rs7975232) and
- Taq1 (rs731236).

All of the aforementioned polymorphisms are associated with some of the autoimmune disorders in humans, such as celiac disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis and Hashimoto thyroiditis (36, 37).

Genes for tumor necrosis factor (*TNF*) and lymphotoxin-α (*LTA*)

The tumor necrosis factor (TNF) is a proinflammatory cytokine. It belongs to TNF superfamily of cytokines. It is also marked as TNF-α, to differ from TNF-β, which is also called lymphotoxin (LTA). Cells which secrete TNF are activated phagocytes, antigen-stimulated T-lymphocytes, NK cells and mastocytes (35).

There are two types of TNF molecules, membrane, and secretion. Membrane TNF (mTNF) is a non-glycose type II membrane protein with the intracellular amino-terminal end and a large extracellular carboxy-terminal end. Secretory TNF (sTNF) has a triangular pyramidal shape where each side of the pyramid forms on a subunit of this molecule. Biologically active forms are trimer forms of protein, homotrimer (23, 38).

Biological functions of TNF are carried out over two types of external membrane receptors TNFR1 and TNFR2. By binding the ligand to the receptors, signal pathways for apoptosis are activated, nuclear kappa factor B (NF-kappa B), ERK-kinase, p38 protein kinase, and c-Jun-N-terminal kinase. The biological effects are stimulation of migration and activation of leukocytes at the site of infection, induction of expression of adhesion molecules (selectins and integrins) on endothelial cells, inhibition or induction of apoptosis. TNF also works on macrophages, stimulating them to produce IL-1, which has a similar role to TNF (29, 35, 38, 39)

The TNF encoding genes are located on a short arm of chromosome 6, in class III of the MHC locus, between the HLA-B and the HLA-DR gene. It has been proven that there is a strong bond between THF allele with DR haplotypes, such as HLA-A1-B8-DR3 and Bw62-DR4. Both alleles are expressed in patients with DMT1. The most researched TNF polymorphisms associated with DMT1 are rs1800629 (at position 308) and rs361525 (at position 238) (40).

Gene CLEC16A and gene for protein tyrosine phosphatase-2 (PTPN2)

Gene CLEC16A (*C-type lectin domain family 16*, also known as KIAA0350) is localized on chromosome 16p13 and encodes a protein with Ca+ dependent or type C lectin binding structure. This protein plays important roles in binding carbohydrates, cell adhesion, and pathogen recognition. KIAA0350 is almost exclusively expressed in cells of the immune system (dendritic cells, NK cells, B lymphocytes). These cells are crucial in the pathogenesis of DMT1 which indicates that modulation of immunity contributes to the onset of disease. Several polymorphisms of the KIAA0350 gene (rs2903692, rs725613, rs17673553 and 12708716) are associated with the occurrence of multiple sclerosis and DMT1 (29, 41).

PTPN2 (also known as TC-PTP or PTP-S2) gene, which is localized on chromosome 18, encodes tyrosine phosphatase 2 which is a negative regulator of inflammatory processes and is particularly present in immune system cells (CD4+ T-lymphocytes and B-lymphocytes), but also in pancreatic β-cells and intestinal enterocytes. This molecule inhibits the STAT1 transcription factor in the IFN signaling pathway, whereby the downregulation type I (IFNα and IFNβ) and type II (IFNγ) IFN receptors are activated. The inhibition of PTPN2 expression in pancreatic insulocytes and intestinal enterocytes leads to the activation of STAT and the subsequent formation of apoptosis through Bim proteins and the mitochondrial pathway of activation. This suggests that polymorphism of the PTPN2 gene can cause β-cell apoptosis and loss of the intestinal epithelial barrier followed by the appearance of symptoms of DMT1. Too early start of intake of cow's milk or gliadin from wheat in children, as well as the viral infections, are the most common triggers that can cause the onset of DMT1 and β-cell autoimmunity,

caused by polymorphism of the PTPN2 gene (42, 43). Polymorphisms of this gene (rs478582, rs1893217, and rs2542151) were also identified in persons suffering from inflammatory intestinal disease, celiac disease and rheumatoid arthritis (28).

Potassium voltage-gated channel subfamily J member 11 (KCNJ11)

The KCNJ11 gene (gene for the potassium voltage channel, subfamily J, member 11) is localized on a short arm of chromosome 11 at position 15.1. This gene encodes the formation of four subunits of the hetero-octamer complex of the ATP-sensitive potassium channel (KATP), while the other four subunits are encoded by the ABCC8 gene. Namely, the KCNJ11 gene encodes Kir6.2 subunit (inward-rectifier potassium channel subunit) which participate in the formation of KATP pores, while the ABCC8 gene encodes the SUR1 (receptor sulphonylurea) regulatory subunits (44, 45).

KATP channels are transmembrane channels that are most commonly found in the plasma membrane of the β-cells of the Langer-Hans pancreatic islet. In addition to these cells, KATP channels can also be found on muscle and nerve cells. They open or close depending on the amount of blood glucose. Increasing intracellular metabolic activity leads to a change in the relationship between ATP and ADP in the cell and conditions the closure of the KATP channel. By closing these channels, depolarization of the pancreatic β-cells occurs, which is also a trigger for the secretion of insulin (44, 46).

Mutations of KCNJ11 and ABCC8 genes are present in about 50% of patients with DMT1. These mutations prevent the closure of the KATP channel and hence the insulin secretion in response to hyperglycemia. Also, in children with this disorder, neurological disorders are observed as well. One of the most serious disorders that can occur is DEND syndrome, which is characterized by neonatal diabetes, a developmental disorder, epilepsy and later onset of speech. To date, several polymorphisms of the KCNJ11 genes which are associated with the occurrence of DMT1 are found. The most prevalent in the population are E23K, A190A, L267L, L270V, I337V, K381K and S385C (45, 47).

Platelet and T cell activation antigen 1 (CD226)

CD226 or DNAM-1 (DNAX auxiliary molecule-1) is an immunoglobulin-like transmembrane glycoprotein found on plasma membranes of monocytes, macrophages, megakaryocytes, thrombocytes, NK and T-lymphocytes, as well as in some subpopulations of B-lymphocytes. This receptor participates in numerous immunological functions, such as cell signaling and adhesion, cytokine secretion, differentiation of naive T-cells, as well as proliferation and activation of cytotoxic T-lymphocytes, then migration of leukocytes to the site of infection, mastocyte degranulation, and thrombocyte aggregation. Ligands for the CD226 receptor are CD112 and CD155 (poliovirus receptor). Their binding to the receptor leads to cytotoxicity reaction (NK and T-lymphocytes) and cytokine secretion (48, 49).

In recent years, the CD226 gene polymorphism found at 18q22.3 is increasingly associated with the development of multiple autoimmune diseases including DMT1, rheumatoid arthritis, systemic lupus erythematosus, Grave's disease, and psoriasis. The polymorphism of this gene, with DMT1, occurs in exon 7 when cysteine is replaced by thymine (rs763361 C>T), which leads to the replacement of a glycine with serine at the site 307 (Gly307Ser). Lazano et al. (2013) have shown that by activating the CD226 receptor on T-lymphocytes, the balance between Th1, Th2 and Th17 immune responses changes. Activation of this receptor affects the increased production of proinflammatory cytokine and T-cell proliferation leading to a proinflammatory response, while its blockage affects the reduction of IL-17 secretion, indicating the potential for the use of a CD226 receptor blocker for the treatment of Th1 and Th17 autoimmune diseases (50, 51).

Conclusion

Although DMT1 is a disease known for more than a century, scientists still intensively research determination of its more precise etiology and genetic basis of the disease. For this purpose, the monitoring of the polymorphisms of genes for which there are already evidence of their association to DMT1, as well as the initiation of more research for testing of the new polymorphisms of the genes would provide a great contribution. Such discoveries which we could name "preventive genetics" would greatly contribute to the early detection of patients and the individualization of the therapy, which can also encourage the development of vaccine usage in patients with DMT1.

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UDRUŽENOST GENSKIH POLIMORFIZAMA SA POJAVOM DIABETES MELITUSA TIP 1

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Jedan od najčešćih endokrinih poremećaja kod dece i adolescenata, *Diabetes mellitus* (DM), predstavlja hroničnu, poligensku i kompleksnu bolest. Na osnovu etiologije, DM se može podeliti na dve osnovne grupe: *Diabetes mellitus tip 1* (DMT1) i *Diabetes mellitus tip 2* (DMT2). DMT1 predstavlja oboljenje posredovano imunološkim mehanizmima, u čijoj osnovi je autoimunska destrukcija insulocita od strane imunoreaktivnih T-ćelija i antitela, što dovodi do insulinske rezistencije i sindroma hiperglikemije.

Nekoliko različitih molekularnih mehanizama doprinose uništavaju β-ćelija Langerhansovih ostrvaca, kao što su: produkcija autoantitela usmerenih protiv antigena na insulocitima, liza Langerhansovih ostrvaca posredovana citotoksičnim CD8+ T limfocotitima, lokalna produkcija citokina (TNF i IL-1) od strane makrofaga, koji oštećuju insulocite, i reakcije kasne preosetljivosti posredovane CD4+ Th1 limfocitima.

Najvažniji geni, koji pokazuju visok stepen povezanosti sa DMT1 nalaze se u regionu koji kodira MHC (major histocompatibility complex) molekule I i II klase. Međutim, postoje veoma čvrsti dokazi o povezanosti i drugih gena (van MHC genskog područja) sa ispoljavanjem DMT1. To su: gen za insulin (INS), gen za protein 4 vezan na citotoksični T limfocit (CTLA4), gen za protein tirozin fosfatazu — 22 (PTPN22), gen za protein tirozin fosfatazu — 2 (PTPN2), gen za protein lecitin tipa C (CLEC16A, KIAA0350), gen za receptor α interleukina 2 (IL2RA/CD25), genska regija za interferon indukovanu helikazu (IFIH1), KCNJ11, CD226, gen za receptor vitamina D (VDR), geni za faktor tumorske nekroze (TNF) i limfotoksin-α (LTA). Pored ovih gena, istraživači i dalje tragaju za drugim genima koji su povezani sa pojavom i ekspresijom DMT1.

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Ključne reči: *Diabetes mellitus tip 1, autoimunska endokrinopatija, polimorfizmi non-HLA gena*

Case report

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RETINAL PIGMENT EPITHELIUM TEARS IN YOUNGER PATIENTS: CAUSES AND CONSEQUENCES

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We present three different cases of patients with retinal pigment epithelium (RPE) tear: one case with choroidal osteoma, one with central serous chorioretinopathy (CSHR) and one with idiopathic polypoidal vasculopathy (IPCV). Fotodocumentation, fluorescein angiography, optical coherence tomography (OCT) and OCT angiography showed typical choroidal neovascularization in patient with osteoma and IPCV. In patient with CSHR, large PED without choroidal neovascularization was present. PED tear was detected in all patients.

Various eye disorders, such as tear of retinal pigment layer, can complicate clinical picture and be associated with PED. Large height and diameter of PED, association with CNV, small ratio of CNV size to PED size, lines in infrared images, and duration of PED may predict the course of RPE tear. Therefore, it should be monitored for this complication. Treatment and outcome depends on cause of tear.

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Key words: *retinal pigment epithelium, tears, causes*

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Introduction

Retinal pigment epithelium (RPE) is a monolayer of pigmented cells in outer part of retina. The apical membrane of retinal pigment cells faces the light sensitive outer segments of the photoreceptors. The basolateral membranes of pigment epithelium cells are in contact with the choroid and the bloodstream. The cells of RPE have an important role in the function and survival of photoreceptors (1). Tear of RPE can be caused by different disorders and is complication involved with devastating visual loss (2, 3, 4, 5, 6). The mechanism and potential risk factors for RPE tear in patients with age related macular degeneration are defined while they still have been unclear in rare retinal disorders. (7, 8, 9, 10, 11).

The aim of our study was to present three different cases with the tear of RPE. The first patient was with central serous chorioretinopathy (CSHR), the second was with choroidal osteoma and the third with idiopathic polypoidal vasculopathy (IPCV). In such way, we want to emphasize that different etiology, then pathophysiological bases can underpin tear of RPE, and to estimate possible risk factors for its development.

First case

A 33 year old male presented with metamorphosia, blurred central vision and small decrease of visual acuity, a disturbance of color vision in the left eye presented as a dark spot in the central vision. Visual acuity tested by Snellen chart was 1.0 in the right eye and 0.5 in the left eye. Intraocular pressure was 14 mmHg in the right eye and 16 mm Hg in the left eye. A slit lamp biomicroscopy showed a normal appearance. Family and life history were negative. The patient suffered from sleep disorders.

A posterior segment examination showed edema of posterior pole between vascular arcade of superior and inferior temporal retinal arterial branches in the left eye (Figure 1a). In the left eye, small area of grey color was noted in the edematous zone close to the superior temporal artery (Figure 1a). The appearance of posterior pole of the right eye was normal. During fluorescein angiography, in the left eye, leaking as point in early phase and in form of umbrella in late phase was present (Figure 1b). OCT of macula on the left eye showed detachment

of RPE with present tear (Figure 1c). Central macular thickness on retinal maps was 586μm.

After two weeks, treatment with topical nonsteroid drops, drops with carbonanhydrase inhibitors and systemic administration of eplerenone 25 mg daily resulted in a smaller edema, which was resorbed after four weeks. Visual acuity restored to 0.9, by Snellen and small subjective color vision defects persisted for some time.

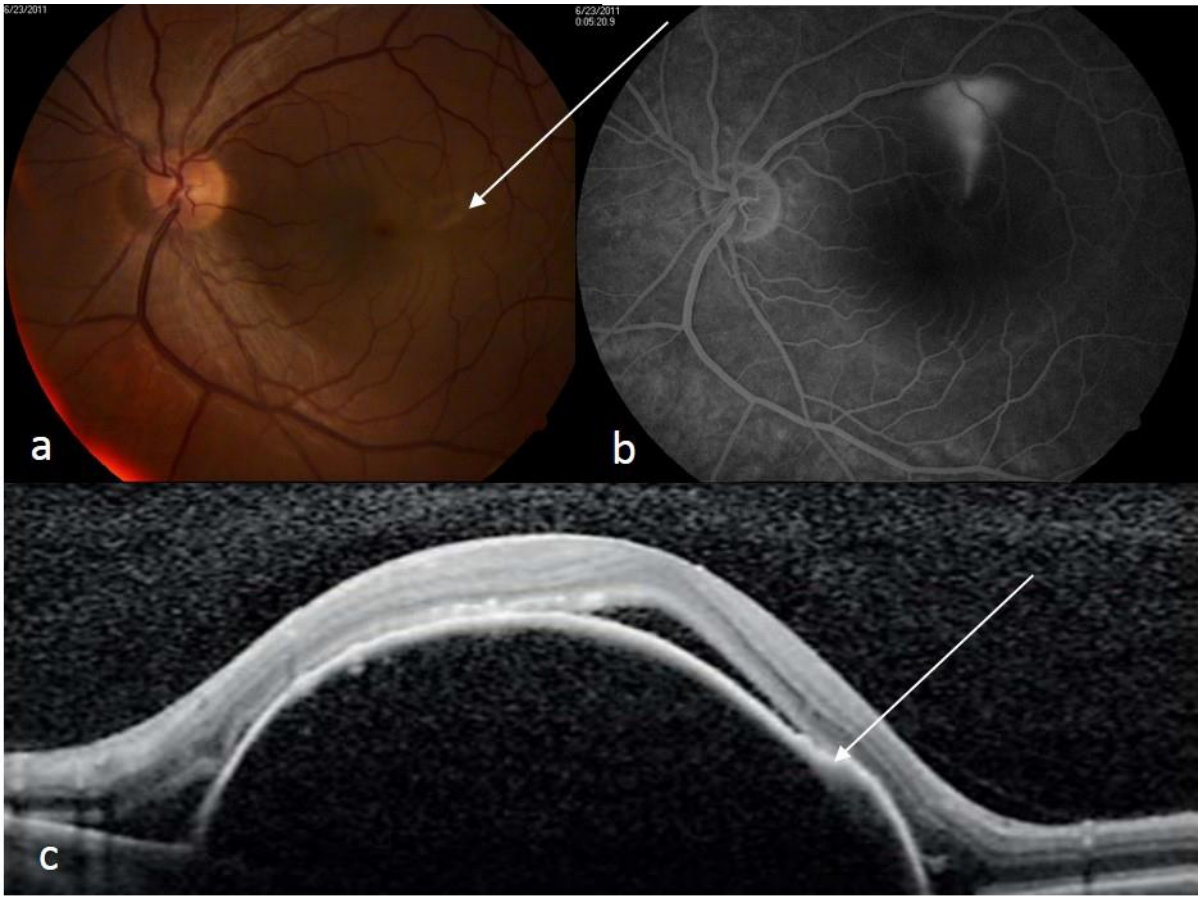

Figure 1. Patient with CSHR

a) Color fundus photography-arrow is pointing to a possible area of RPE tear
b) FA
c) OCT- arrow is pointing to RPE tear

Second case

A 14 year old girl with abrupt and painless vision loss in the right eye was presented. Family and previous life history were negative. Best corrected visual acuity tested in the right eye was counting fingers on 2 meters, and in the left eye 1.0 by Snellen. Intraocular pressure in both eyes was 14 mmHg. A slit lamp biomicroscopy showed normal appearance.

Posterior segment examination showed slightly and irregularly elevated yellow area with small vascular networks and hemorrhages on its surface (Figure 2a). Dark lines were notable on the surface of the lesion (Figures 2a, b), too. Line defect in the area of osteoma presented RPE tear (Figure 2a). Fluorescein angiography showed blockade of choroidal fluorescence in the zones of hemorrhage in early phase, and hiperfluorescence with leaking and pooling in later phase (Figures 2c and d). Leaking phenomena was caused by the presence of newly formed choroidal vessels (Figures 2b and c). Ultrasonography B scan presented highly reflective choroidal mass with acoustic shadowing (Figure 2f). OCT finding proved choroidal neovascularization with RPE tear (Figure 2e).

The patient started with anti VEGF therapy, without significant visual recovery.

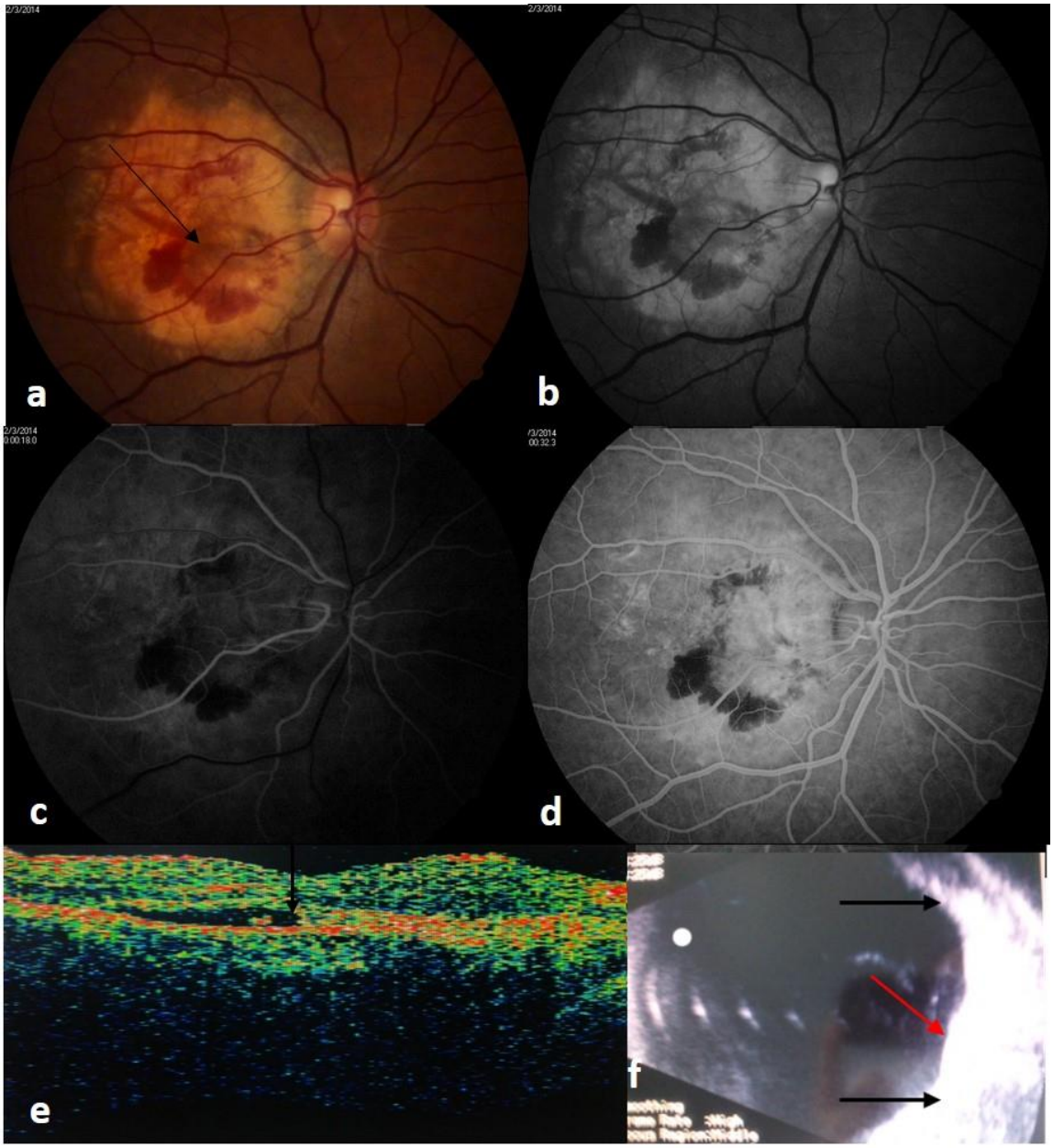

Figure 2. Patient with choroidal osteoma

a) Color fundus photography
b) Fundus photography green mode
c) FA-arrows are pointing to an area of possible RPE tear
d) FA-arrows are pointing to an area of possible RPE tear
e) OCT-arrows are pointing to RPE tear
f) B scan ultrasonography–red arrow is pointing to osteoma and black to secondary effect

Third case

A 46 year old male, with sudden visual loss in the left eye was presented at the Clinic for Eye Diseases. Family and previous life history were negative. Vision loss was painless and without any other manifestation after a hard workday. Best corrected visual acuity in the left eye was 0.1 by Snellen. Visual acuity in the right eye was preserved. Intraocular pressure in both eyes was 16 mmHg. A slit lamp biomicroscopy showed a normal appearance.

Preretinal hemorrhage and retinal hemorrhage were present in macular and paramacular region, as well as in the area of grey round atrophy of choroid (Figure 3a). Peripapillar atrophy was also noted on fundus photography (Figure 3a). Fluorescein angiography showed blockade of choroidal

fluorescence in the areas of hemorrhage and point hyperfluorescence during angiography in the area of noted choroidal atrophy (Figure 3b). On OCT PED and PED small RPE tear was noted (Figure 3c).

Spontaneous resorption of retinal and pre-retinal hemorrhages was present and discrete visual improvement with anti-VEGF therapy was noted.

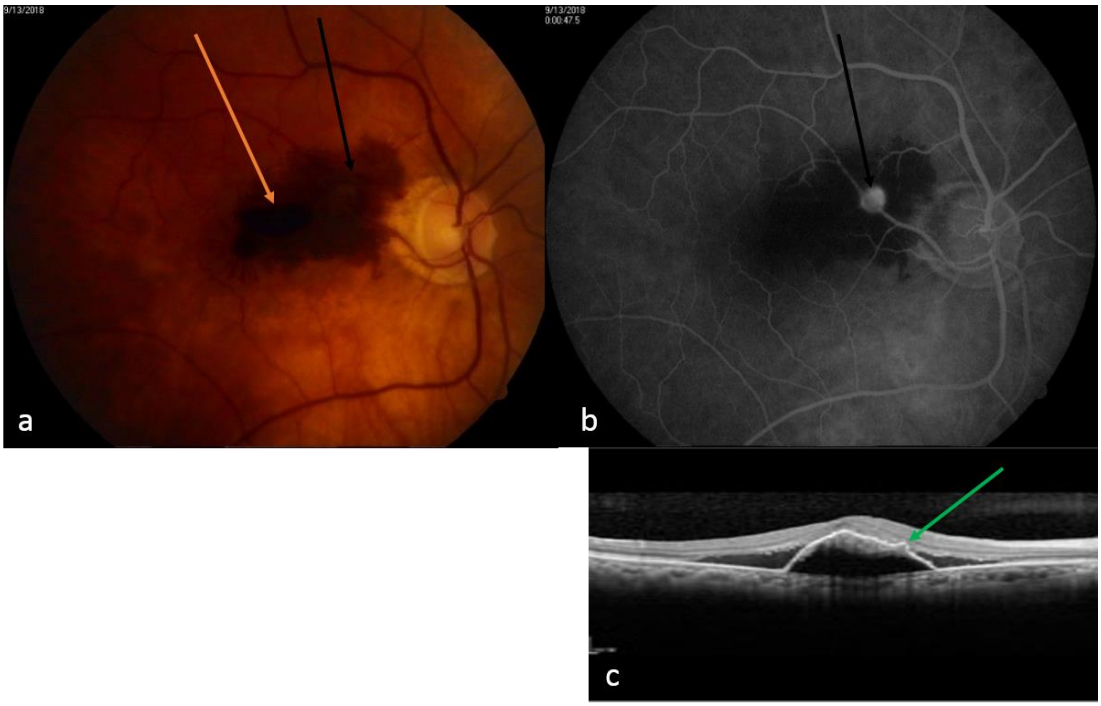

Figure 3. Patient with IPCV

a) Color fundus photography black arrow is pointing to a possible area of tear and red to preretinal hemorrhage
b) FA-arrow is pointing to a possible area of RPE tear
c) OCT-arrow is pointing to a possible area of RPE tear

Discussion

Hoskin et al. first reported RPE tears as a complication in patients with PED due to AMD (13). Today, it is well known that RPE tear can be part of the natural course of PED due to occult CNV or a complication of different procedures such as: anti-VEGF therapy, photodynamic therapy, laser photo-coagulation, or transpupillary thermotherapy (5, 6, 7, 12, 13).

Retinal pigment tear in younger patients is rare. It is described in cases such as: traumatic chorioretinopathy, high myopia, angioid streaks, choroidal tumors, light chain deposition disease, central serous chorioretinopathy, and polypoidal choroidal vasculopathy (PCV), retinal dystrophies and chorioretinitis (posterior uveitis) (2, 3, 4, 10).

We presented young patients with different retinal pathology and associated PED and RPE tear. Possible pathogenetic mechanism of RPE tear in case of patient with CSHR maybe large PED (Figure 1a, c) and duration of process, which was supported by umbrella leaking during fluorescein angiography (Figure 1). The tear is present in this case at the base of PED, and the possible factor for tear may be pressure of fluid in PED itself. Saffar et al. also proposed that large PED is highly susceptible for developing RPE tear due to forces of increased hydrostatic pressure that is present inside PED (11). These authors also proposed that tear caused by large PED may be the result of rupture of RPE at the weakest point near the base of the PED. Two types of repair process after RPE tear are observed (14). It seems that persistent subretinal fluid after tear leads to subsequent repair with thickening of proliferative tissues at the area of RPE defect. Other possible mechanism of repair is complete resolution of the fluid and direct attachment of outer retina and Bruch membrane (14).

In the case of the patient with osteoma, PED was also present. However, the risk factor for tear of pigment epithelium may rather be neovascular membrane. Present dark lines on fundus color photography, and infrared images can be factors for prediction of RPE tear. Spaide et al. assumed that

contracture of CNV adherent to the undersurface of the RPE applies traction at the junction of the attached and detached RPE which represents the weakest point (15). The force of CNV is acting perpendicular on RPE (Figure 2).

In the case of the patient with IPCV, PED is not large, but RPE tear was located in foveal and parafoveal zone, which caused low central visual acuity (Figure 3). Sudden visual loss may be explained by newly developed CNV. It has been reported that inverse relationship between the duration of the PED and a risk for RPE tear is caused by vascularized PED (9, 11, 16). A short duration of PED means that the neovascular process is new with immature vessels. Immature vessels are more vulnerable and susceptible to anti-VEGF therapy (16).

Conclusion

So, to conclude, different eye disorders can be complicated with tear of retinal pigment epithelium. Treatment and outcome depend on the cause of tear. In the large, PED, specially associated with CNV, may predict development of RPE tear as well as an atrophy of RPE. Duration of PED is important for preservation of visual acuity. Therefore, it should be counseled and monitored for this complication, which may limit visual prognosis.

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Conflict of Interests

No conflict of interests

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Prikaz bolesnika

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RASCEP PIGMENTNOG EPITELA RETINE KOD MLADIH PACIJENTA – UZROCI I POSLEDICE

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Cilj rada je prikaz tri različita slučaja bolesnika sa rascepom pigmentnog epitela: bolesnika sa dijagnozom osteoma, bolesnika sa centralnom seroznom retinopatijom (CSHR) i bolesnika sa idiopatskom polipoidnom vaskulopatijom (IPCV). Urađena fotodokumentacija, fluoresceinska angiografija optička koherentna tomografija (OCT) i optička koherentna angiografija (OCTA) dokazala su horoidalnu neovaskularizaciju (CNV) kod bolesnika sa osteomom i bolesnika sa dijagnozom IPCV. Kod bolesnika sa CSHR istim dijagnostičkim metodama dokazana je i potvrđena velika ablacija RPE (PED) bez CNV. Rascep retinalnog pigmentnog epitela bio je prisutan kod svih prikazanih bolesnika.

Rascep RPE može se sresti kao komplikacija kod različitih oboljenja oka, nakon ablacije RPE. Velika ablacija RPE, po visini i dijametru, zatim udruženost sa CNV, mali odnos CNV i PED, kao i dužina trajanja PED, mogu ukazivati na razvoj rascepa RPE. Zato je neophodno stalno i obazrivo praćenje nastanka ove komplikacije. Lečenje kao i ishod zavise od uzroka.

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Ključne reči: *retinalni pigmentni epitel, rascep, uzroci*

Case report

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CYSTIC DUPLICATION OF STOMACH: A CASE REPORT

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Cystic gastric duplications represent an extremely rare surgical entity. Persistent vomiting is the most common clinical presentation due to its compressive effect on pylorus. Additional confusion is created by their localization mimicking pyloric stenosis.

A female infant at 3 months of age was admitted to the clinic for persistent postprandial vomiting and weight loss. Abdominal X ray examination confirmed the presence of aeroliquid levels in the upper abdominal portions. Echosonographic and NMR examination indicated the presence of bilocular cystic formation in the region of the pylorus, near visceral contour of the spleen, with a total diameter of 31 mm.

Two partially interconnected cystic formations forming hourglass mass were found during surgery. The complete enucleation was done. Postoperatively, a rapid recovery occurred and the child was discharged home on the 5th day. Pathohistological finding: the lesion corresponds to cystic duplication, with pylorus type mucosa.

Peripyloric cysts are an extremely rare clinical entity. Symptomatology is conditioned primarily by a mechanical, compressive effect on the pylorus, which, differentially diagnostically, often mimics pyloric stenosis. Therapy is surgical.

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Key words: *cystic duplication, stomach, children*

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Introduction

Cystic duplications are defined as spherical lesions surrounded by a muscular wall that is in intimate contact with the associated part of the digestive tube. They can be located along the entire length of the digestive tract from the mouth to the anus. They represent the result of a defect in the gut. These are extremely rare developmental disorders (1/4000-5000). They are most commonly localized in the small intestine (40%), and extremely rare (<5%) in gastric projection (1). The most common localization of cystic duplications is an area of great curvature, and at the level of the pylorus are exclusive. Clinical manifestations depend on location, size, and mucosal pattern. Rarely seen in adults because of their compressive effect, vomiting, and symptoms of gastric outlet obstruction, or even palpable mass, they are diagnosed at an early age, in 2/3 of cases appear before one year of age (2). Very often, they contain ectopic pancreatic tissue, lymphatic tissue and respiratory epithelium.

Case report

A female infant at 3 months of age was admitted to the Clinic for persistent vomiting after each meal and weight loos. Physical examination, routine blood tests and biochemistry were unremarkable. Abdominal X ray examination confirms the presence of aeroliquid levels in the upper abdominal portions. Echosonographic (Figure 1) and NMR examination indicate the presence of bilocular cystic formation in the region of the pylorus, near visceral contour of the spleen, with a total diameter of 31 mm.

The patient underwent an exploratory laparotomy. Two partially interconnected cystic formations forming hourglass mass were found during surgery. The smaller lesion was of solid and thick walls in intimate contact with the pylorus. The narrow canal was connected to a larger cyst (30 mm) lying in the meso between the colon and the stomach, thin walls filled with liquid content (Figure 2). The complete enucleation was done (Figure 3). The cyst was separated by a sharp and blunt preparation from most of the pylorus canal. The part of the pylorus duct in

intimate contact with the cyst was open longitudinally and the part of the wall was resected together with the cyst and then closed transversely, preserving the patency of the pyloric lumen (Figure 4).

Histopathologic examination of the lesion revealed a 3 cm bilocular cyst, with pylorus type mucosa and well-developed ectopic pancreatic tissue.

In the postoperative period, a rapid recovery occurred and the child was discharged home on the 5th day after the intervention.

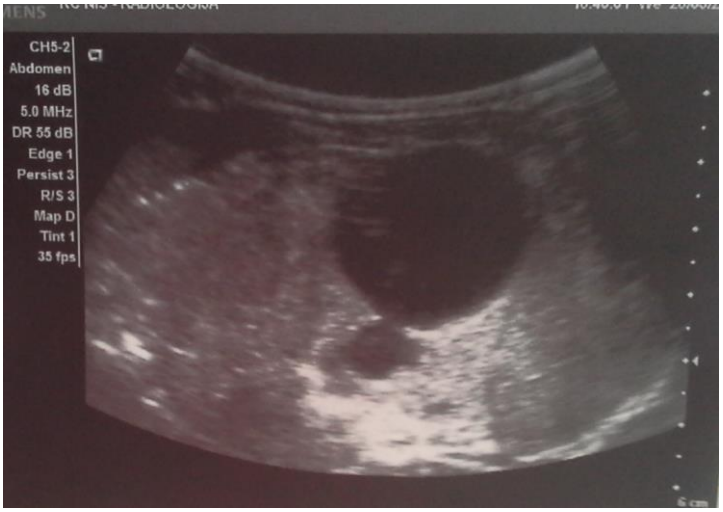

Figure 1. Echosonographic examination revealed the presence of bilocular cystic formation, in the region of the pylorus

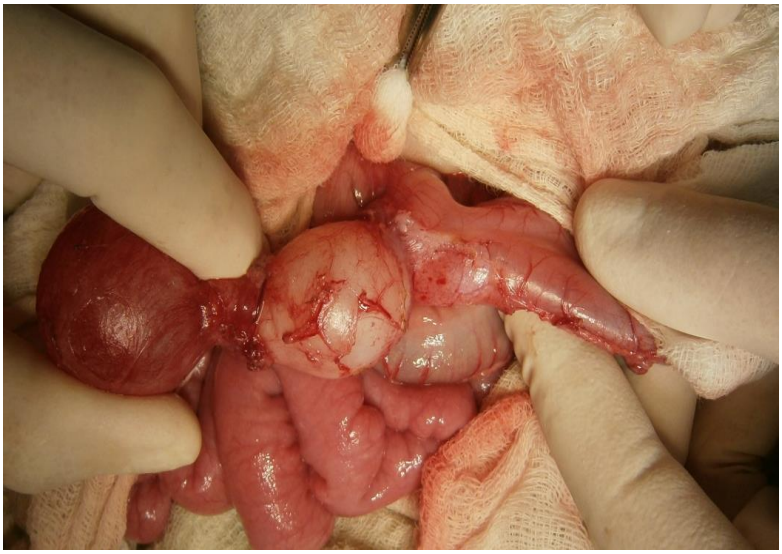

Figure 2. Two partially interconnected cystic formations forming hourglass mass. Cysts are connected with a narrow canal

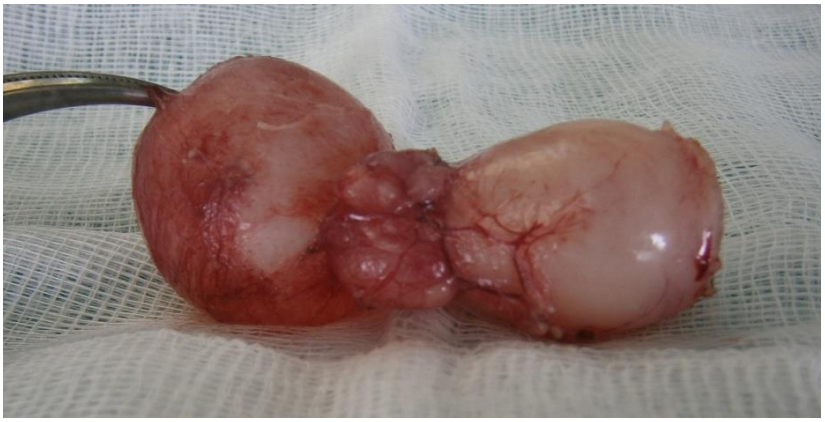

Figure 3. Complete enucleated hourglass cyst

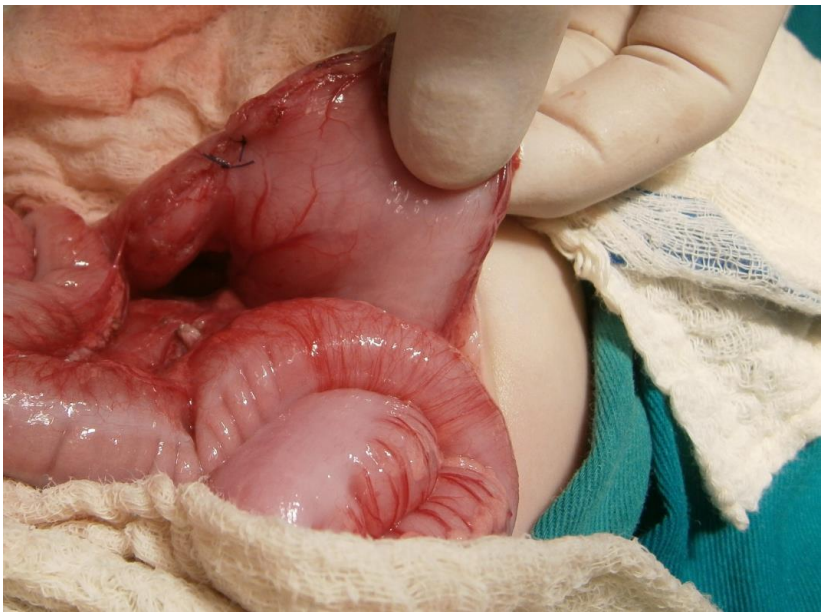

Figure 4. Appearance of pylorus after enucleation of the cyst

Discussion

Duplications of gastrointestinal tract are very rare congenital anomalies. They occur along the entire digestive tube and are most rarely in the projection of the pylorus (< 5%), with the majority of patients diagnosed within the first 3 months of life and rarely in adults (2).

The basic criteria for the diagnosis of cystic duplication of the stomach are: a. common wall between the cyst and adjacent portion of the stomach; b. the cyst is in spherical shape surrounded by a muscular tissue, that extends to the muscular layer of the wall of the stomach; c the cyst is covered by the stomach type mucosa or mucosa of other parts of the digestive tube (3, 4).

There is no definite explanation in the literature regarding the occurrence of cystic gastric duplications. Several theories have been proposed but none has been proven. Bremer proposed the theory of errors of recanalization and fusion of longitudinal folds (5), while McLetchie suggested that adhesion of notochord and embryonic endoderm might not elongate as quickly as its surrounding structures, causing traction diverticulum leading to duplication cyst formation (5). Hypoxia, persistent embryonic diverticulum are just some of the theories.

The clinical presentation can be very different from asymptomatic cases to patients with abdominal pain to nausea, vomiting, weight loss, dysphagia, and epigastric mass on physical examination (3). As in our case of pyloric localization of the cyst nonbilious vomiting and weight loss may be the dominant symptom. A very small subset of patients can remain asymptomatic.

The most common location of cyst is the distal greater curvature, communicating or noncommunicating with the gastric lumen (6). Presence of gastric and ectopic pancreatic tissues, what is most common entity is associated with complications such as bleeding, perforation, peptic ulcer, pancreatitis or even malignancy (7).

Ultrasound is a noninvasive imaging modality used as first tool in infants for examining the upper gastrointestinal tract. In the majority of the cases it shows hypoechogenic cystic lesion in the upper abdomen seen usually adjacent to the stomach, pancreas, the liver and biliary tracts.

Imaging studies including the CT and MRI are important to determine the cystic nature of the lesion as well as its extent and relation with the adjacent structures.

The purpose of treatment is complete surgical excision of the cyst (8). It is recommended not only for symptomatic relief as seen with a gastric outlet obstruction, but also because of the risk of malignant degeneration. There are published 14 cases of adenocarcinoma diagnosed in gastric cystic duplications (9). If malignancy is suspected, surgical resection is the golden standard.

Conculsion

Peripyloric cysts are an extremely rare clinical entity. Symptomatology is conditioned primarily by a mechanical, compressive effect on the pylorus, which, differentially diagnostically, often mimics pyloric stenosis. Therapy is surgical in all patients, and especially if malignancy is suspected.

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Prikaz bolesnika

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CISTIČNA DUPLIKACIJA ŽELUCA – PRIKAZ SLUČAJA

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Cistične duplikacije želuca predstavljaju izuzetno redak hirurški entitet. Zbog kompresivnog delovanja na pilorus, uporno povraćanje predstavlja najčešću kliničku prezentaciju. Dodatnu konfuziju stvara njihova lokalizacija, te često diferencijalno-dijagnostički upućuju na stenozu pilorusa.

Žensko novorođenče u 3. mesecu života primljeno je u kliniku zbog upornog povraćanja, posle svakog obroka, i nenapredovanja. Nativna grafija trbuha potvrđuje prisustvo hidrogasnih nivoa u gornjim partijama trbuha. Ehosonografski i NMR pregledi ukazuju na prisustvo bilokularne cistične formacije, u regiji pilorusa i uz viscelarnu konturu slezine, ukupnog promera 31 mm.

Intraoperativno je evidentirana promena izgleda peščanog sata, koju čine dve delom međusobno povezane cistične formacije. Učinjena je kompletna enukleacija cisti. Postoperativno, dolazi do brzog oporavka i dete je 5. dana otpušteno kući. Patohistološki nalaz: lezija odgovara cističnoj duplikaciji sa mukozom pilorusnog tipa.

Peripiloričnе ciste predstavljaju izuzetno redak klinički entitet. Simptomatologija je uslovljena, pre svega, mehaničkim, kompresivnim efektom na pilorus, što diferencijalno-dijagnostički često imitira stenozu pilorusa. Terapija je hirurška.

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Ključne reči: *cistična duplikacija, želudac, deca*

Case report

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Nd: YAG LASER ANTERIOR CAPSULOTOMY IN CAPSULAR PHIMOSIS IN THE EYE WITH PEX AND ZONULAR LESION

Aleksandar Veselinović, Marija Cvetanović, Dragan Veselinović

Secondary cataract formation is significantly present after cataract surgery and develops at different time intervals after the intervention. Blepharophimosis or anterior capsule contraction may result in numerous problems in the operated eye. In patients with cataract associated with pseudoexfoliation syndrome the risk of anterior capsule contraction is greater, especially if small capsulorhexis had previously been performed.

The paper presents blepharophimosis of the anterior capsule with phacodonesis and secondary cataract eight months after phacoemulsification with intraocular lens implantation. Due to anterior capsule opacification and formation of capsular phimosis, visual acuity decreased and monocular diplopia developed in the operated eye. After sectoral YAG laser capsulotomy of adjustable intensity was performed, visual acuity improved and the patient's subjective complaints disappeared.

YAG laser capsulotomy is a significant method in managing secondary cataract. Management of secondary cataract due to anterior lens capsule opacification and the presence of anterior capsule phimosis associated with expressed pseuoexfoliations and zonular lesion poses a particular challenge to ophthalmologists and requires a certain amount of experience in the management of these conditions.

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Key words: *YAG laser capsulotomy, capsular phimosis, pseudoexfoliation*

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Introduction

Capsular phimosis or anterior capsule contraction syndrome has been described as one of the potential complications in patients with cataract. Capsular phimosis occurs as a postoperative complication resulting from a small diameter capsulorhexis and postoperative inflammatory reactions in the eye (1, 2, 3, 4). After cataract surgery residual lens epithelial cells may proliferate over the posterior lens capsule and on the intraocular lens surface as well, causing myofibroblast transdifferentiation (4, 5). A small capsulorhexis diameter most commonly presents a risk for capsular phimosis either as a consequence of inexperience of the surgeon or due to a small pupil size in cataract patients. Most commonly registered associated condition is pseudoexfoliation syndrome, but it has also been described in patients with retinitis pigmentosa, diabetes, uveitis and Behcet's syndrome, high myopia, uveitis, pars planitis, and myotonic dystrophy (5, 6, 7, 8, 9). A small anterior capsule opening makes surgery more difficult and may cause numerous intraoperative complications. Capsular contraction syndrome may also occur after combined cataract and glaucoma surgery and due to intraoperative complications that may result in prolonged postoperative inflammation. According to some authors' experience, intraocular lens material, especially haptic lenses, may be contributing factors for capsular phimosis (10, 11, 12, 13).

Postoperatively, subsequent constriction of the anterior capsular opening may occur, capsular phimosis, as a consequence of proliferative and inflammatory processes. Severe anterior lens constriction, especially in patients with pseudoexfoliation syndrome, may result in subluxation of artificial lenses due to zonular lesions. As a consequence of severe anterior lens constriction, tractional ciliary body detachment may also occur accompanied with choroidal effusion (14, 15).

Case report

Patient Z.M. aged 72, presented to her doctor with the complaints of monocular diplopia and de-

creased vision in the last three months in the left pseudophakic eye. She had had her left eye operated for cataract eight months before she came to see her doctor, with performed phacoemulsification of mature cataract in the eye with pseudoexfoliation glaucoma. A single-piece, hydrophilic acrylic lens with two haptics was implanted during the surgery. The postoperative course was normal. The aforementioned problem with a small size pupil was associated with smaller capsulorhexis during the surgery, but it did not affect the course of the operation. Postoperative visual acuity in the left, operated eye after seven days was 1.0. Three months after the operation the patient reported decreased visual acuity in the left eye with occasional signs of diplopia. The symptoms intensified in the last few weeks. On examination, visual acuity was 0.5 with the presence of phimosis and fibrosis on the inferior portion of the anterior lens capsule. The deposition of pseudoexfoliation material was noted on the anterior capsule and on the zonulae. Zonular defect was evident at the 5-6 o'clock position. Incipient cataract with pseudoexfoliation changes of the pupillary rim was present in the other eye. Intraocular pressure in both eyes was 18 mmHg with topical antiglaucomatous therapy sol. Cosopt 2x1.

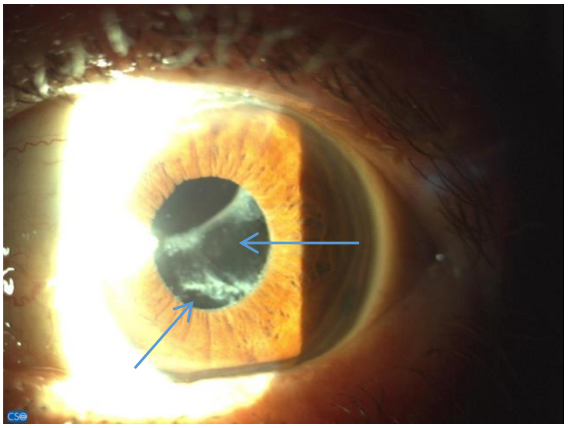

Figure 1. Pseudophakic eye with PEX with secondary cataract and capsular phimosis

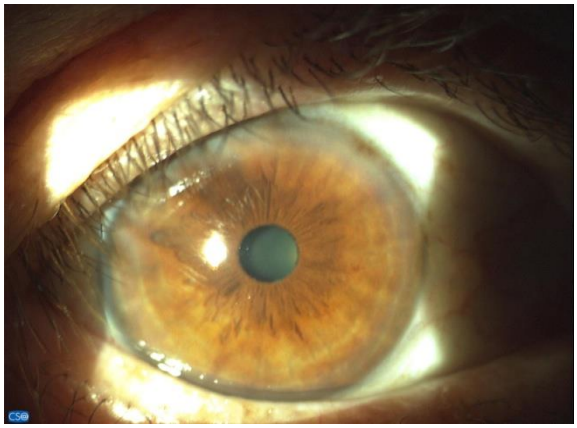

Figure 2. The other, 'healthy eye'

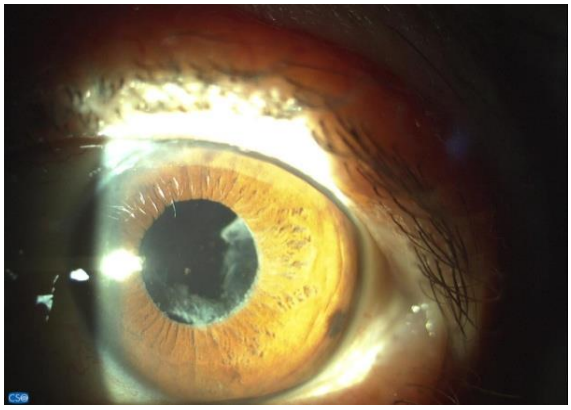

Figure 3. Triangle Yag capsulotomy

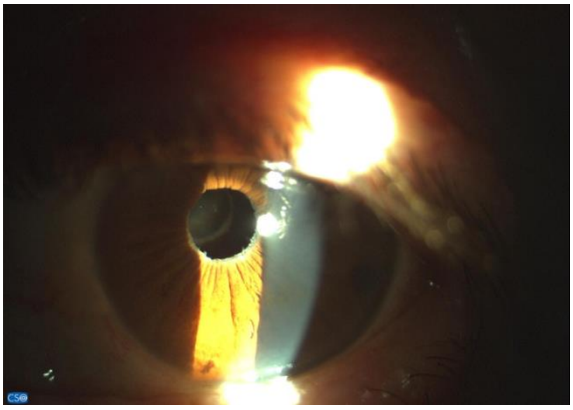

Figure 4. Patient's eye 7 days after the intervention

YAG laser capsulotomy was performed using 7 shots, power level of 5 mJ, with extreme caution regarding zonular weakness in the sector of the performance. The first shot by YAG laser was performed at the very edge of capsulorrhexis where the greatest thickening of the anterior capsule was present, causing higher power level of 5 mJ to be used. A triangle opening was created later and the visual acuity was improved, VOS: 0.8. Prescribed therapy for the patient included sol. Neodexacin 3 x 1 and tbl. Huma-Zolamide 1 x 1 for their anti-inflam-

matory properties and because of possible transient increase of intraocular pressure.

Follow-up examination revealed that visual acuity in the left eye was 1.0, IOP: 18 mmHg with regular antiglaucomatous treatment. The patient had no more complaints of diplopia and was satisfied with the effects of the intervention.

Discussion

Anterior capsular contraction syndrome has multiple causes and its occurrence can greatly diminish primary effect of cataract surgery. The most common causes of capsular phimosis include a small diameter capsulorhexis in patients with chronic inflammatory processes, uveitis, diabetes and pseudoexfoliation syndrome (2, 3, 4). Good postoperative acuity in these patients may greatly be decreased and result in patient's condition similar to preoperative one. If a tendency for capsular phimosis is noted in early postoperative period, relaxing radial incisions along the rim may be performed using the YAG laser to prevent further constriction (16). Due to strong contractions and intraocular lens decentration, monocular diplopia, glare and binocular vision problems are very common.

Application of the YAG laser is of extreme importance in secondary cataract treatment and in patients with postoperative anterior capsular contraction. A special problem in performing the YAG laser anterior capsulotomy in blepharophimosis is a condition accompanied with zonular lesion, as well as phacodonesis and intraocular lens decentration. In patients with blepharophimosis it is necessary to perform anterior capsulotomy requiring adjustment of the laser shock waves intensity in order not to damage anterior surface of the artificial lens. In some cases, it is necessary to use YAG laser with higher intensity due to anterior capsular thickening and fibrosis, which makes correct dosage and the surgeon's experience very important. Additional YAG laser treatment, due to exerted stress on the zonulae, may in some cases produce more zonular lesions and lens decentration, thus increasing monocular diplopia intensity and the patient's subjective visual disturbances. In severe contraction syndrome dislocation of artificial lens towards the vitreous body is often present, resulting in refractive changes and hypermetropia. The YAG laser anterior capsulotomy results in capsular bag relaxation and a possible refractive change regarding the reduction of hypermetropia (17). There were no refractive anomaly changes in our patient.

Conclusion

Before anterior laser capsulotomy is performed, the evaluation of anterior capsular blurriness and fibrosis degree should be evaluated to determine the intensity of the laser beam. Anterior capsular extension must not be performed in the direction of zonular defects and the first wave shot should be directed to capsulorhexis edge. A triangular anterior capsule opening in the eye with dilated pupil should be big enough to avoid glare that may be caused by peripheral portion of anterior capsular remnants after normal pupil size is achieved.

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Prikaz bolesnika

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Nd: YAG LASER KAPSULOTOMIJA PREDNJE KAPSULE KOD KAPSULOFIMOZE U OKU SA PEX-OM I LEZIJOM ZONULA

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Pojava sekundarne katarakte nakon operacije katarakte prisutna je u značajnom broju i nastaje u različitom vremenskom periodu od intervencije. Blefarofimoza ili konstrikcija prednje kapsule može prouzrokovati brojne probleme na operisanom oku. Kod bolesnika kod kojih je katarakta udružena sa pseudoeksfolijacionim sindromom postoji veći rizik za nastanak kon-strikcije prednje kapsule, posebno ukoliko je predhodno urađena mala kapsuloreksa.

U radu je dat prikaz pojave blefarofimoze prednje kapsule, sa fakodonezom i sekundarnom kataraktom, osam meseci nakon urađene fakoemulzifikacije sa ugradnjom intraokularnog sočiva. Usled zamućenja prednje kapsule sočiva i pojave kapsulofimoze, došlo je do smanjenja oštrine vida i pojave monokularnih diplopija na operisanom oku. Nakon izvođenja sektoraste YAG laser kapsulotomije dozirane snage, došlo je do poboljšanja oštrine vida i gubitka subjektivnih tegoba bolesnice.

Yag laser kapsulotomija prestavlja značajnu metodu u rešavanju pojave sekundarne katarakte. Rešavanje problema sekundarne katarakte usled prisustva zamućenja prednje kapsule sočiva i prisustva fimoze prednje kapsule, udružene sa izraženim pseudoeksfolijacijama i lezijom zonula, predstavlja poseban izazov za oftalmologa i zahteva iskustvo u rešavanju ovakvih stanja.

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Ključne reči: *Yag laser kapsulotomija, kapsulofimoza, pseudoexfoliatio*

Case report

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ACUTE DACRYOADENITIS ASSOCIATED WITH INFECTIOUS MONONUCLEOSIS

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The paper presents a case report of a patient with unilateral acute dacryoadenitis caused by infectious mononucleosis. The aim of the paper was to point out therapeutic and diagnostic possibilities in patients with dacryoadenitis.

Hyposecretion of tears was determined by Schirmer's test. MRI revealed lacrimal gland enlargement with signs of inflammation in the surrounding orbital tissue. Laboratory tests for Epstein Barr virus infection were positive.

Clinical manifestation of infectious mononucleosis was proved by a pediatrician.

The application of local and general therapy resulted in withdrawal of the lacrimal gland enlargement, stabilization of inflammation symptoms and normalization of a grey eye in our patient.

Acute dacryoadenitis is a relatively rare pathological condition requiring appropriate diagnostics for proper treatment, as it was the case with our patient in whom infectious mononucleosis caused the disease.

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Key words: *dacryoadenitis, infectious mononucleosis*

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Introduction

The frequency of dacryoadenitis is low; it occurs in only one case per 10,000 ophthalmic patients. There is no predilection for race, gender or age for the onset of the disease. Dacryoadenitis can be acute or chronic (1, 2, 3).

Acute dacryoadenitis is usually unilateral and characterized by painful swelling of the eyelid with the signs of chemosis, conjunctival hyperemia, erythema of the lids and lymphadenopathy (submandibular). Its characteristic features include swelling of the outer third of the lid, proptosis, occasionally ocular motility restriction and painful globe movement (1, 2).

It is commonly an independent disease (viral, bacterial infections) with good prognosis. Factors that may cause acute dacryoadenitis are mostly due to infections with viral etiologies (mumps, Epstein-Barr virus, herpes zoster virus), or bacterial etiologies caused by staphylococcus, streptococcus, haemophilus influenza, gonorrhea or chlamydia. Infection may spread to the lacrimal gland hematogenously, transneuronally, from the inflamed conjunctiva or via traumatic injuries (4, 5, 6, 7).

Chronic dacryoadenitis is usually bilateral and painless with minimal ocular signs. Chronic condition is accompanied by enlargement of the lacrimal gland present for months (8, 9, 10).

It is usually associated with a systemic disease (sarcoidosis, tuberculosis, Mikulicz's syndrome, Sjogren's syndrome, syphilis, leukemia). Prognosis depends on the underlying cause of the systemic disease. Symptoms of mild to severe dry eyes are also present (11, 12, 13).

Case study

A girl aged 14 presented to her doctor with the complaints of pain and swelling of her right outer upper eyelid.

The local finding revealed the absence of tarsal and bulbar conjunctival inflammation, enlarged right lacrimal gland with the signs of inflammation and edema in that region. Mild proptosis and upper lid swelling were present in the outer part with characteristic "comma-like" appearance (Figure 1). The outer upper portion of the eyelid was painful on palpation, and the patient reported the feeling of

scratching and dryness in the right eye, as well as pain on the right eye movement. Schirmer's test registered hyposecretion of tears in the right eye, while the finding of the left eye was within the reference range (Figure 2).

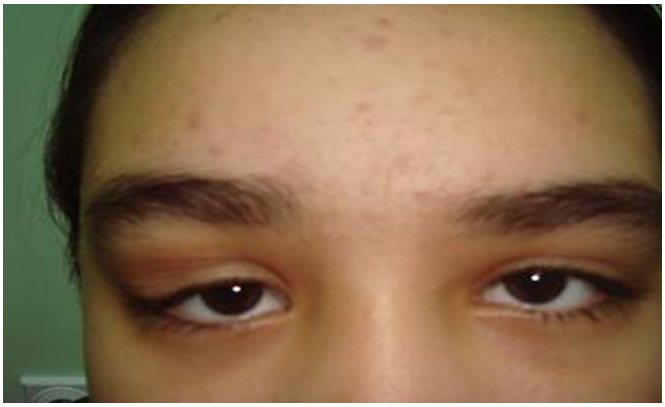

Figure 1. Acute dacryoadenitis of the right eye

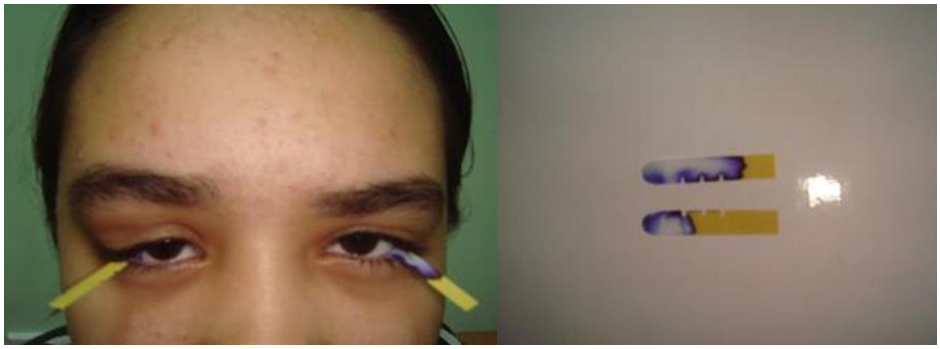

Figure 2. Shirmer's test

Magnetic resonance imaging showed enlarged lacrimal glands with the signs of inflammation in the surrounding orbital tissue. Laboratory findings were positive for Epstein Barr virus.

The presence of infectious mononucleosis was confirmed by a pediatrician based on clinical examination and laboratory finding. After the consultation with a pediatrician, systemic corticosteroids (40 mg Lemod Solu) for five days and local corticosteroid therapy in the form of drops and ointments were administered, resulting in the reduction of inflammation signs in the area of the lacrimal gland and in disappearance of the general symptoms of the disease. The girl did not show up for further controls, so the control check-up to prove the normalization of tear secretion in the eye with dacryoadenitis was not possible.

Discussion

Acute dacryoadenitis has a characteristic clinical manifestation and can easily be identified (1, 2). It is most commonly unilateral, but in rare cases it can be a bilateral disease (10). This relatively rare lacrimal gland disorder requires determination of the causative agents as early as possible for timely and adequate therapy. One of the common causes of dacryoadenitis can be an infection caused by Epstein Barr virus within infectious mononucleosis. Infectious mononucleosis occurs sporadically and primarily affects young adults, but it can also affect people of any age. Infectious mononucleosis is an acute disease caused by a virus that most commonly infects the liver and lymphoid tissue (13, 14). Clinical manifestations of the disease include fatigue, muscle pain, increase in body temperature and headache. Within the disease, the liver and spleen are enlarged with accompanying symptoms. The disease commonly has benign course and the symptoms withdraw after two weeks.

The signs of dacryoadenitis are common presentation in patients with infectious mononucleosis. In a study of 16 patients with dacryoadenitis there were 6 patients with the signs of infectious mononucleosis and serological testing positive for Epstein-

Barr virus infection (3). Acute dacryoadenitis caused by Epstein-Barr virus most commonly occurs in younger population, rarely in elderly people (1, 2). In our patient characteristic symptoms were manifested unilaterally. Hyposecretion of tears in the affected eye was associated with other symptoms of the disease.

Conclusion

Acute dacryoadenitis can often be associated with infectious mononucleosis and it can be manifested in children and young adults. Upon the confirmation of the presence of infectious mononucleosis a pediatrician and an infectologist should be consulted for appropriate therapy. Despite the fact that some authors support conservative treatment, administration of general corticosteroid therapy speeds up the recovery of general symptoms in these patients and local symptoms of dacryoadenitis as well. Hyposecretion of tears in dacryoadenitis may be a characteristic symptom of the disease. It disappears with the treatment and the loss of inflammatory signs.

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AKUTNI DAKRIOADENITIS UDRUŽEN SA INFEKTIVNOM MONONUKLEOZOM

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U radu je dat prikaz bolesnika sa jednostranim akutnim dakrioadenitisom prouzrokovanim infektivnom mononukleozom. Cilj rada je da se ukaže na terapijske i dijagnostičke mogućnosti kod bolesnika sa dakrioadenitisom.

Hiposekrecija suza dokazana je Schirmerovim testom. MR pokazala je uvećanje suzne žlezde sa znacima inflamacije okolnog orbitalnog tkiva. Registrovani su pozitivni laboratorijski nalazi na Epstein-Barr virus.

Od strane pedijatra, potvrđena je klinička slika infektivne momonukleoze.

Na primenu lokalne i opšte terapije došlo je do povlačenja otoka suzne žlezde, smirivanja simptoma inflamacije i normalizovanja simptoma sivog oka kod bolesnika.

Akutni dakrioadenitis je relativno retko patološko stanje i zahteva odgovarajuću dijagnostiku, nakon koje je olakšana terapija, što se pokazalo i u slučaju našeg bolesnika, kod kojeg je bolest bila prouzrokovana infektivnom mononukleozom.

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Ključne reči: *dakrioadenitis, infektivna mononukleoza*

Case report

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SURGICAL TREATMENT OF GIANT PERICARDIAL CYST THROUGH THE LATERAL THORACOTOMY

Stefan Timčić[1], Igor Živković[1], Staša Krasić[2], Marko Andjelković[1], Ivan Nešić[1], Milan Ćirković[1], Miodrag Perić[1,3]

Pericardial cysts are uncommon benign tumors with the prevalence about 7% of all mediastinal tumors. Patients are mostly asymptomatic unless when cysts compress major anatomic structures in the chest cavity. We represented a patient with a pericardial cyst near to the apex of the heart. Magnetic resonance examination revealed 9 x 4 cm cystic formation. Surgical treatment was performed through left side lateral thoracotomy without cardio-pulmonary bypass support. Tumorous formation was completely resected and sent for the pathohistological examination. Surgical or percutaneous treatment for pericardial cysts might be occasionally necessary, depending on the location of the cyst and its relationship with the adjacent structures. Morbidity and mortality are low. Surgery has been demonstrated as the only definitive curative treatment.

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Key words: *pericardial cyst, lateral thoracotomy*

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Introduction-Background

Pericardial cysts are uncommon benign tumors with prevalence about 7% of all mediastinal tumors (1, 2). They usually arise from failure of fusion of one of the mesenchymal lacunae that form the pericardial sac (3). Etiology of pericardial cyst could be congenital, inflammatory (rheumatic pericarditis, bacterial infection particularly tuberculosis, echinococcosis), traumatic and after cardiac surgery (4). The size of the cysts varies from 2 to 28 cm (5). They are usually found in the third or fourth decade of life, male and the females are affected equally.

Patients are mostly asymptomatic unless major anatomic structures compressed with tumor or rupture of the cyst occurs. The most common symptoms are dyspnea, chest pain, or persistent cough. Hemoptysis, fever, and pneumothorax are unusual presentations (6).

In 70% of the cases, these tumors are located in right cardiophrenic angle, 22% in the left cardiophrenic angle and in 8% cases are located in the posterior or the anterior-superior part of the mediastinum.

However, rarely, this pathology is associated with serious complications such as cardiac tamponade, cyst rupture or even sudden death (7).

Case presentation

A 69 year old male with a history of cardiac weakness and arrhythmia was admitted to the hospital. The chest roentgenography presented supradiaphragmatic oval homogenous shading on the right side and enlarged cardiac vessel silhouette. The transesophageal examination showed large non-homogeneous mass (dimensions 4.5 x 9 cm), near to the lateral wall of the left ventricle without compressive effect on the left heart. Magnetic resonance scan of the heart revealed tumorous formation dimension 90 x 50 mm (Figure 1). The pericardial cyst was near to the left ventricle without communication with chamber.

Surgical treatment was performed through the lateral thoracotomy, without cardio – pulmonary bypass and pericardial sac dissection. On the right side and outside the pericardium single, soft and filled with liquidity pericardial cyst was found (Figure 2).

Intraoperative dimension was approximately 9 x 4.5 cm in diameter. Total surgical excision was performed (Figure 3).

The further postoperative period was uneventful.

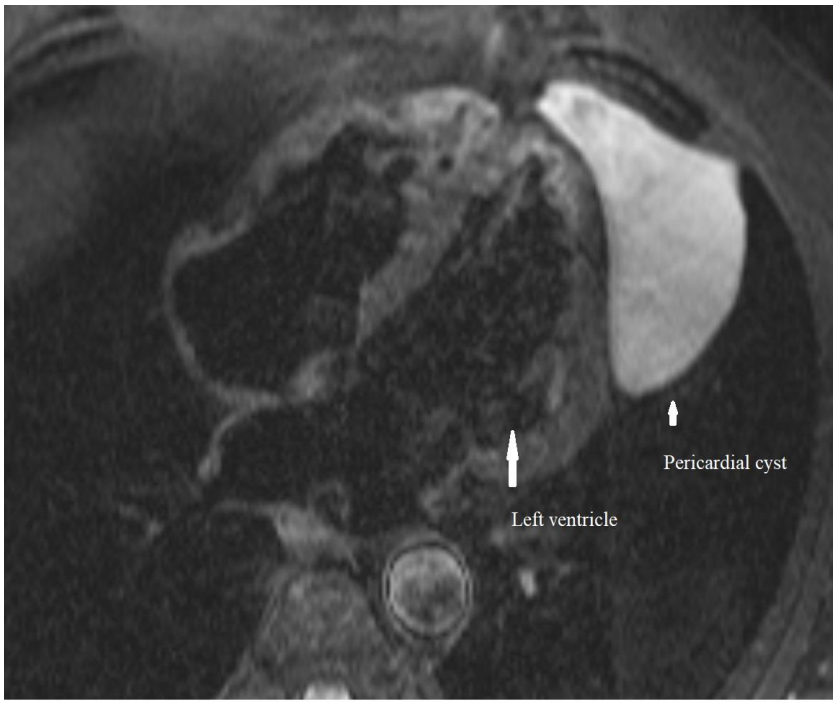

Figure 1. Magnetic resonance scan revealed pericardial cyst close to the left ventricle

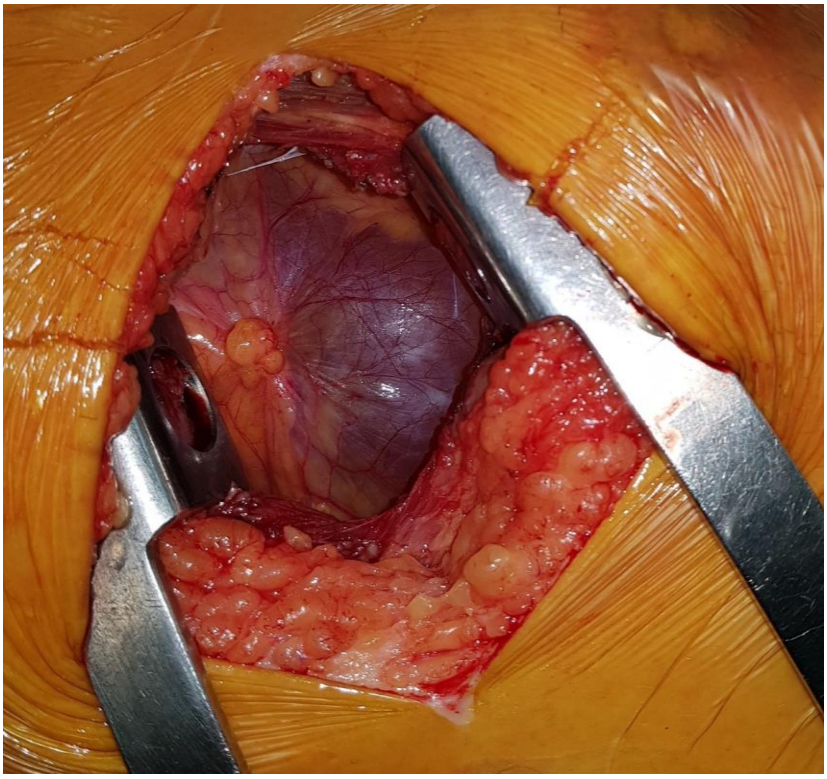

Figure 2. Surgical view on the pericardial cyst through the left thoracotomy

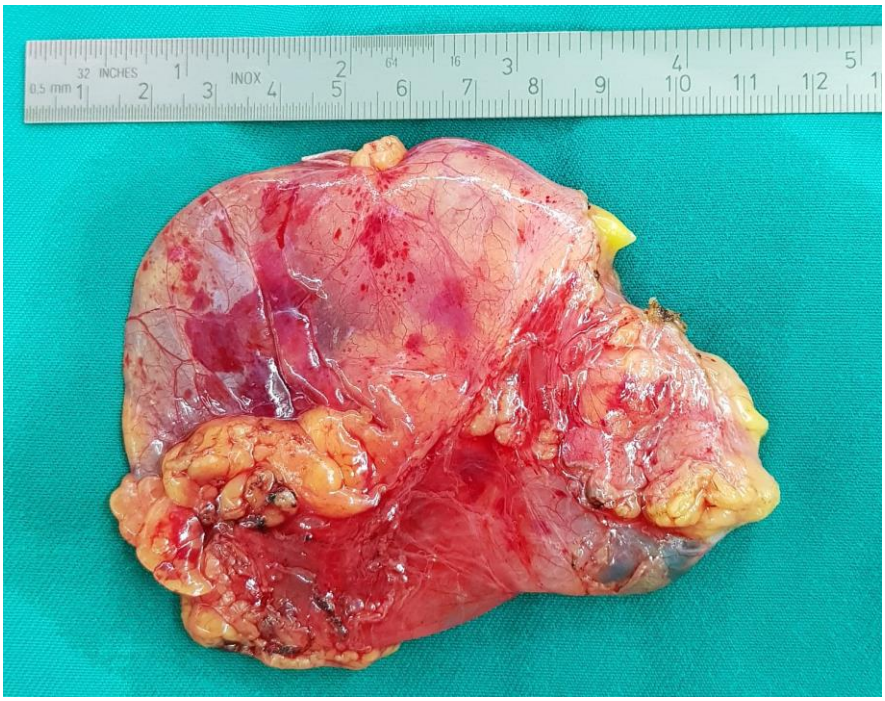

Figure 3. Dimensions of extirpated pericardial cyst

Discussion

Pericardial cyst is an uncommon benign congenital anomaly. The cyst walls are composed of connective tissue and a single layer of mesothelial cells, and they usually contain clear fluid (7). Most of them are asymptomatic (50%-75%) and found incidentally during routine chest roentgenography or echocardiography. Only 25% to 30% of patients complain of chest discomfort, cough, dyspnea, or paroxysmal tachycardia. Serious complications such as infection, vascular erosion, ventricular outlet obstructions or sudden cardiac death are extremely rare (8). The differential diagnosis should take into consideration solid tumors, hydatid cysts and mesotheliomas (9). Additional diagnostic modalities that may find pericardial cysts include transthoracic echocardiography, CT, and MRI of the chest (7). Computerized tomography scan (CT scan) is considered as best modality for diagnosis and follow up as it provide excellent delineation of the pericardial anatomy and can aid in the precise localization and characterization of various pericardial lesions, including effusion, pericardial thickening, pericardial masses, and congenital anomalies (10, 11). Magnetic resonance imaging is another useful imaging modality and the fluid in the pericardial cyst produce hyperintense signal on T2-weighted MRI images and hypointense signals on T1-weighted images (11). Management of pericardial cysts depends on their symptom. If the patient is asymptomatic, serial echocardiography is enough, but if the patient is symptomatic or reveals an increase in the size of the cyst or has solid component in the cyst cavity in the serial follow-up, a cyst resection has been the most favored approach with thoracotomy, sternotomy or video-assisted thoracic surgery (VATS) (7). Since operative risks of minimally invasive techniques are extremely low, it would seem reasonable to offer resection for all pericardial cysts in otherwise healthy patients for whom the risk of surgery is low. Aspiration is another method, but one of third have shown recurrence. Morbidity and mortality are low. Surgery has been demonstrated as the only definitive curative treatment (10).

Conclusion

Mortality and morbidity of this pathology are very low. Surgical treatment could be a good option for treatment of giant pericardial cyst. With the aim to exclude complications after median sternotomy, lateral thoracotomy might be a perfect alternative approach for surgical treatment. Surgery has been demonstrated as the only definitive curative treatment.

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HIRURŠKO LEČENJE GIGANTSKE PERIKARDNE CISTE KROZ LATERALNU TORAKOTOMIJU

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Perikardne ciste predstavljaju retke benigne tumore sa ukupnom prevalencijom oko 7% svih medijastinalnih tumora. Obično su bolesnici bez simptomatologije, izuzev kada cista komprimuje anatomske strukture unutar grudnog koša. Mi smo predstavili veliku perikardnu cistu lokalizovanu bluzu srčanog vrha. Magnetna rezonanca je kod bolesnika detektovala cističnu formaciju dimenzija 9 cm x 4 cm. Hirurška procedura izvedena je kroz levu lateralnu torakotomiju bez primene kardiopulmonalnog bajpasa kao podrške. Čitava tumorska formacija izvađena je i poslata na dalja patohistološka ispitivanja. Hirurška ili perkutana procedura neophodna je za lečenje perikardnih cisti, zavisno od lokalizacije i odnosa sa ostalim strukturama. Stope morbiditeta i mortalita su niske. Hirurška intervencija predstavlja jedini definitivni način lečenja.

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Ključne reči: *perikarda cista, lateralna torakotomija*

Review article

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GESTALT PSYCHOTHERAPY: SCIENCE OR QUASI-SCIENCE?

Jana Milić

The term Gestalt appears for the first time in Gestalt psychology, created by Wertheimer, Keller, Koffka and Rubin, to mark an entity, good form. Elements do not determine an entity, and an entity is not a mere set of elements. It should be specified that a particular part of an entity is not the same when alone or included in some other entity because that part absorbs the characteristics coming from its place and function in various entities (for example, a scream in an empty street is not the same as a scream made by children playing).

The proof for gestalt therapy's success is an introspective report on subjective perception of a person's improvement, or more precisely "maintenance and development of balance and good condition of the whole organism."

To scientifically approach gestalt psychotherapy, it is necessary to operationalize positive effects or changes that this therapy brings. All terms used to validate the effects of psychotherapy are subjective constructs. An introspective perception of improvement is an indicator of a positive effect of psychotherapy. Intersubjective consent, that clients give in psychotherapy, after some time of applying gestalt therapeutic techniques results in improvement, is a guarantee that the achieved therapeutic effect is objective. Gestalt psychotherapy is a branch of psychotherapy that bases its theory on a so called "empty chair". This psychotherapeutic school's basic goals are solving the problem of the now and here, and as the other psychotherapeutic schools, it bases usually its research methods on a case study. This is clearly seen in journals and articles which discuss this psychotherapeutic school. As a therapy, it is suitable for treating mild mental disabilities and anxiety. Its therapists are constantly improving themselves and students, or psychotherapists to be, consider it to be challenging.

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Key words: *psychotherapy, individual growth and development, work on oneself, time and man*

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Introduction

Gestalt psychotherapy is a branch of psychotherapy which bases its theory on gestalt theory, especially on Kurt Lewin's theory, and it bases its therapeutic techniques on a so called "empty chair". This psychotherapeutic school's basic goals are solving the problems of the here and now, and as other schools of psychotherapy, it usually bases its fundamental methods on the analysis of a case. That is clearly seen in journals and articles which discuss this psychotherapy school.

Historically speaking, gestalt therapy was created by Fritz Perls in 1942. The practitioners of this branch of psychotherapy are Laura Perls, Yontef, Zinker, as well as the Parisian's gestalt school practitioners, such as Gonzague Masquelier, Brigitte Martel, Giles Delisle from Montreal and Edoardo Giusti from Rome. Considering that this branch of psychotherapy has its main support in the gestalt theory, we will mention only the key concepts and principles of this theory.

The Basic Scientific Principles in Gestalt Theory and Psychotherapy

Beginning with Descartes, it was thought if we would want to objectively comprehend and analyze a phenomenon or behavior, then we would need to find their causes which are usually found in the past. Causal determination is based on connecting the past events and their current consequences. Once we see the cause of a phenomenon, then it becomes predictable for us and we can control it.

However, contemporary physicians want us to understand there is no such thing as an "objective" analysis because a mere observation of a phenomenon changes it, and predict the most of the

causes is found in the future! (For instance: I went to bed earlier last night so that I would be well rested today as I could teach an important class, so, the cause is in the future), says Ginger (1). This only proves that theological determination and explanation by comprehension have always been present in psychology, since the ancient division of psychology to a natural science and a spiritual science. Where do gestalt theory and psychotherapy belong to? What kind of determination it deals with?

In the beginning of the development of this psychological movement, the object of analysis was the observation or the laws under which observation was constructed. The laws of organizing stimuli into observation have been experimentally proven and verified. Hereby the disciples of this school in psychology have chosen an original approach to access perception, as a phenomenon which is dynamic, not static, organized, not chaotic, predictable, but not unexpected. The term Gestalt is a term which appears for the first time in Gestalt psychology founded by Wertheimer, Keller, Koffka and Rubin, to signify a whole, good form. Elements do not determine a whole, and a whole is not a mere set of elements. It should be said that a particular part of a whole is not the same when alone or included in some other whole, because that part gets the characteristics coming from its placement and its role in various wholes (for example, a scream in an empty street is not the same scream of children who play together).

Disciples of gestalt school in psychology started off with a radically new approach to phenomenology of observation, which are then experimentally tested and confirmed, thus making a significant step in comparison to theories which had dealt with perception until then. These principles (the significance of restructuration of the observational field-figures and backgrounds, significance of nearness, similarity, good form and common faith) found while explaining and understanding the processes of observation were generalized as other cognitive processes, whereas their followers to psychotherapy.

When we speak about "Gestalt psychotherapy", we emphasize that gestalt psychotherapists consider that a man's behavior cannot be understood outside of the context it is developed in, the context of the ***field***.

a) **Field** is a scientific construct which the disciples of the gestalt principle in psychology and psychotherapy rely on. It refers to the notion that life is an open system in which there is interaction between organisms and environment. An organism is an active participant in the creation of its own reality and can be the basic force in shaping our experience. Perls thought that a man and his behavior cannot be understood out of context in which the behavior is being developed (2, 3). We are the people in context!

b) **Dialogue**-Me: You conversation, the now and here.

c) **Phenomenology**-stretches towards the understanding based on what is obvious or what is discovered by the situation itself, but not on interpretation of an observer (3).

Apart from these terms, according to Ginger's systematization there are 20 more constructs of gestalt therapy and they are:

1) The now and how,
2) Process,
3) Awareness,
4) The limits of contact,
5) The field and system,
6) Creative adaptation,
7) The experience cycle,
8) Unfinished businesses/ Unfinished gestalt,
9) Resistance,
10) Homeostasis,
11) The Potentiation of responsibility,
12) Experimentation,
13) The right on diversity-unrepeatability of every man,
14) The Attitude for sympathy,
15) The Holistic approach to a man,
16) The Polarities which complete each other,
17) The Involvement of emotions and body,
18) Aggression,
19) Creativity and imagination,
20) Individual inside a group.

The effects of gestalt psychotherapy and diagnostics

Proving the success of gestalt therapy is an introspective statement on impressions of the *subjective better* in a person, or more precisely "maintaining and developing balance and good condition of the whole body. Health is not just the absence of a disease or defect, but the condition of a complete physical, mental and social wellness" (1).

This principle, as Ginger (1) puts it in his book, states that Gestalt therapy's task is not treatment but maintaining balance and wellness of the whole organism, leads to the "rejection" of discussing normality and disorder which opposes Van Baalen's statement, the author of the article "Gestalt diagnosis" from 1999, which is about finding the appropriate diagnostic criteria for evaluation of the severity and gravity of a patient's psychopathology (4).

In his article, Van Baalen (4) gave detailed instructions on the criteria to use before diagnosing. He accurately explained the theory of Gestalt psychotherapy, trying to be very specific so that the reader truly understands the interaction among parts within the field, in the way that the sense of the figure and background is that every figure has its own background, and that sometimes it can happen for the background to be primary and stand out, or that these two (the figure and background) change in accordance with the focus. He also described the role of a therapist as the part which engages into interaction within a field with a client. The accent is on the change (behavioral or emotional) in a client, which would be closely related to Fajgelj (5), a methodologist, whose theory is about wrong patterns in behavior that change through relations, which would be closely related to psychology's task classified as a behavioral science. The scientific value of the article is high, given the fact it gives directions to Gestalt therapists in understand-

ing the process of diagnosing while being closely connected to the clinical practice of psychology.

Baalen's theory is very similar to Mann Dave's (6) theory, which states that a good Gestalt is clear, or the relation between the figure and background is clear, that is, between a primary need and the background, hence the energy flow which every person should have in a certain moment depends on the dominant need.

The founding source of pathology, according to Gestalt, is the incomplete emotional Gestalt or the "unfinished business" (2).

To scientifically approach gestalt psychotherapy, it is needed to operationalize positive effects or changes which this therapy provides. All the terms which validate the effect of psychotherapy are subjective constructs. The introspective perception of improvement is the indicator of the positive effect in psychotherapy. Intersubjective consent, that clients give in psychotherapy, after some time of applying gestalt therapeutic techniques results in improvement, is a guarantee that the achieved therapeutic effect is objective. The generality of these findings, their systematic record and evaluation, as well as the development of application and methods, confirm Ginger's and Van Baalam's scientific approach.

Gestalt psychotherapy in scientific journals and articles

According to the data in KoBSON database, there is a journal that publishes articles about Gestalt psychotherapy. It is the journal called *South African Journal of Psychology*. The journal is not strictly related to the field of Gestalt psychotherapy, as British Gestalt journal, but it publishes numerous articles about Gestalt psychotherapy. It is found by using the key words gestalt psychotherapy.

Susanne Jacobs often publishes her articles in this journal. In her articles she always refers to the founders of Gestalt psychotherapy, Yontef and Zinker, which would imply she doesn't digress from the primary theory and the methods of Gestalt psychotherapy, and is consistent in maintaining the tradition (7). Her article which deals with humor in Gestalt therapy consistently follows the theory of Gestalt therapy. The method of the research is the case study, which belongs to the clinical method but the author has precisely explained the science behind this method, referring to various authors who use it in their research. Susanne states that the facts will be collected before and after a treatment, gestalt theory will be consistently applied in practice and that the method of observation will be used. The author defined a theory which will be applied, illustrated to the details the method which will be used and systematically showed the results of the observation, that is, the case study. The article is consistent in following scientific methodological norms by applying the Gestalt theoretical approach, describes detailed observations so that the process could be checked. Her work is quite close to a scientific approach, which separates her greatly from many other researchers in the field hence many authors refer to and quote her. This is an extraordinary example how a serious scientific methodology could be applied to a field of psychotherapy, which by its nature belongs on the border between science and pseudoscience.

The reference Susanne uses is the book *Skills in Gestalt Counselling and Psychotherapy* by authors Joyce i Sills (8). The book describes how to lead the Gestalt session and operate with the terms related to religion. One part of the book is about the difference between a normal condition and a transcendental experience and esoteric, describes how a man regresses to the level of an infant, has psychotic reactions, and even can be autistic. Religion does not have an empiric proof. Various religions are founded on a religious experience of an individual, and are characterized by the feeling of admiration for God. The main controversy between various representatives of materialism and religion lies beyond the borders of experimental, verifiable, and refers to the issue of the genesis of the universe. Materialists claim that the universe is endless in space and time, and numerous religions consider the universe has its own Maker, whom not even the greatest minds, such as Einstein, renounced. He explains that his religion is based on the belief of a Higher reason's existence, who reveals itself in the world available for cognition (9). However, if the meanings of numerous mentioned terms (esotery, transcendental experience, religion, deity) are understood from the point of view we have nowadays, Gestalt therapy in this case, approached by Joyce and Sills, is not a science.

Valerie Aiach Dominitz following the theory of Gestalt psychotherapy applied in the practice of the case study (10). The terms are defined and explained as well as the work with a client. Therefore, this journal publishes articles about Gestalt psychotherapy. Among its authors, we can encounter the founders of Gestalt therapy and Zinker, Yontef, Perls.

In his article published in Gestalt Journal, Edwin S. Hariss, describes the attitude towards God and disease (11). The article is not consistent in following the norms of Gestalt psychotherapy. The method is based on an experiment in which one speaks to God and gives the answer to oneself from God's perspective. The therapy is conducted with abused clients and those angry with God. A man's world is spiritual, immaterial; spiritual being here defined as recognizing the reality greater than the one which is a convulsion of all visible things. Edwin S. Hariss says that Gestalt psychotherapy is more and more in collaboration with psychoanalysis, which Karl Poper does not consider to be a science because it does not provide the refutation, that is, from the beginning it was untrusting and in a mythical way approaches to behaviour (12).

Yontef in *British Gestalt Journal* points out that Gestalt therapy has founded many useful and creative innovations related to theory and practice in psychotherapy (13). Kohler (1959 cited by Yontef, 1996) confirms that. He states that enthusiasm and ventures of the early Gestalt's psychologists were virtues because they created new observations and discoveries. Moreover, Yontef says that many of

these discoveries and techniques are integrated into the general practice, many times with no credit. However, Gestalt techniques and methods represent a good model of psychotherapy and should be applied and developed forwardly.

Some articles are about the connection between Gestalt psychotherapy, existentialism and Zen Buddhism. Existentialist narrowness results in noogenic neuroses (Frankl, 1994) which imply overaccented existential frustration, which disrupts free action and development. It can lead to alienation ("My mind contradicts my feelings", a quote from the novel "The Stranger" by Albert Camus). This narrowness is called "the existential vacuum" which refers to the lack of sense or the state of boredom. Since Frankl's logotherapy represents therapy involving sense, then the constant frustration disrupts sense. The greatest sense of a man's existence is love.

Cannon Bard studied philosophy while studying Gestalt therapy (14). She thought that the Sartre's existential philosophy could be connected to Gestalt therapy. Her opinion is that Gestalt therapy can learn a great deal from existentialists about anxiety. Existentialist psychotherapy still did not find its Freud. She relied on and supported the work of Frederick Fritz Perls and having compared existentialist and gestalt psychotherapy, said that both deny the Freudian unconscious, whereas unconscious in their sense is a phenomenological relation between consciousness and its objects. The fundamental thread which connects them is the sense of emptiness (there is nothing there). That would be the idea of Zen Buddhism, and Perls was a student of Zen. "There is nothing but happenings, that is, things happen". Neurotic people cannot see, because their eyes are always given to others so to reflect themselves as objects (Cannon, 2009). Freedom is nothing, emptiness, detachment from things, the sense of emptiness, but Kant says there where the sense of emptiness is, is God. The scientific value of the article is funded on pointing out the clear similarities between Sartre's existential philosophy and Gestalt therapy. The similarity is the sense of emptiness- unreality.

In her article, *Gestalt Therapy in Psychological Practice*, Palmer A. Kendra discusses "phenomenological" or "experienced" approach, which relates to a patients' experience-the way he or she experiences things (15). Phenomenology is a discipline that helps patients digress from their usual way of thinking so they could make a difference between what they are really experiencing at the moment and what is a consequence of the past events (Yontef, 1993, cited by Palmer, 2011). Therapy, phenomenological and existential, focuses on people's existence-relationships with others, joys and sufferings, and for example, immediate experience. Patients have freedom, but they are given the responsibility for their actions and thoughts. The challenge is to take responsibility for themselves in the present (Corey, 2009: cited by Palmer, 2011). Patients' specific reality is under research, while the diagnosis and prognosis are mainly ignored (Corey, 2009, cited by Palmer, 2011).

Gestalt therapy is efficient (16). This is a positive, humanistic therapy. It helps practitioners (both clients and therapists) become responsible for their own experiences and experiment with new ways of behaviour and thinking. It helps them practice and completely experience key moments and emotions in their lives (17). Awareness about important topics or patterns urges practitioners and clients to become more authentic and open for transition or change.

Many articles talk about the significance of the connection between Gestalt psychotherapy and meditation. Meditation (of the eastern religion) is equal to praying in a Christian religion. One who meditates, thinks about a particular content, goes deep in thoughts or strives to achieve relaxation using a breathing method. To hear and accept a prayer cannot be considered scientific.

Conclusion

Since its inception, Gestalt therapy, until today, has evolved into psychotherapy which field of interest is open to religion, meditation, play therapy, psychoanalysis, but primarily its concepts were based on understanding an individual's existence and it used the phenomenological method. So, the description of experience of the here and now. It could be said it is not rigid in relation to other psychoterapies' points of view and allows combining and eclecticism. What I have noticed is that the authors of the articles are always relying on the founders of Gestalt psychotherapy, Perls, Yontef, Zinker, which would imply that theoretical basis of psychotherapy should not be changed. The methodology of Gestalt psychotherapy is based on constructs of the gestalt theory; the principles of causability are manifested in therapeutic situation or field, whereas change in one part of the field causes changes in other parts of the field. The truth it strives for is the patient's truth. Gestalt psychotherapy confirms itself through the subjective better in a patient, that is, in a way a kind of a measurable subjective experience. Nowadays it establishes connection to religion for what there are no facts for proof. That would be the step backwards in science.

Gestalt psychotherapy, in general, does not belong to a scientific field exactly because of the narrower connection to religion and meditation, but of course, it is possible to advance the methodology of verification and measure the effects of psychotherapy. In that sense, the gap, scientifically and pseudonically does not have to be that deep, at least when it is about gestalt psychotherapy, given that the theory which is based on experimental research of perception and possibility to explicitly define at least some, if not all, parameters of improvement which therapy can give to a person, that is, to people.

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GEŠTALT PSIHOTERAPIJA: NAUKA ILI KVAZINAUKA?

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Pojam geštalt je pojam koji se prvi put pojavljuje u geštalt psihologiji, čiji su stvaraoci Verthajmenr, Keler, Kofka i Rubin, kako bi označio celinu, dobru formu. Elementi ne determinišu celinu, a celina nije puki zbir elemenata. Treba naglasiti i to da određeni deo unutar neke celine nije isti deo kada je izdvojen ili kada je uključen u neku drugu celinu, jer taj deo poprima osobine koje proizilaze iz njegovog mesta i njegove uloge u različitim celinama (npr. vriska u pustoj ulici nije isto što i vriska dece koja se igraju).

Dokaz o uspešnosti geštalt terapije je introspektivni izveštaj o doživljaju subjektivog boljitka kod osobe ili preciznije rečeno "održavanje i razvijanje ravnoteže i dobrog stanja celog organizma". Za naučni pristup geštalt psihoterapiji potrebno je operacionalizovati pozitivne efekte ili promene koje ova terapija donosi. Svi termini kojima se validiraju efekti psihoterapije subjektivni su konstrukti. Introspektivni doživljaj boljitka pokazatelj je pozitivnog efekta psihoterapije. Intersubjektivna usaglašenost klijenata, koji su na psihoterapiji, da posle izvesnog vremena, uz primenu geštalt terapijskih tehnika osećaju poboljšanje, jeste garancija da je postignuti terapijski efekat objektivan. Geštalt psihoterapija je grana psihoterapije koja svoju teoriju bazira na geštalt teoriji i to pre svega na teoriji polja, a svoje terapijske tehnike zasniva na tzv. praznoj stolici. U okviru ovog psihoterapisjkog pravca, bazični ciljevi su usmereni na rešavanje problema *sada i ovde*, a kao i ostale psihoterapijske škole, svoje istraživačke metode bazira uglavnom na studiji slučaja. To se jasno vidi u časopisima i člancima u kojima se razmatra ova psihoterapijska škola. Kao terapija, pogodna je za lečenje blagih mentalnih poremećaja i anksioznosti. Njeni terapeuti konstantno napreduju, a učenici ili budući psihoterapueti smatraju je izazovnom.

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Ključne reči: *psihoterapija, individualni rast i razvoj, rad na sebi, vreme i čovek*

Review article

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THE APPLICATION OF INFORMATION TECHNOLOGIES IN THE PROCESS OF NURSING CARE

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Healthcare professionals are witnessing numerous innovations and technologies that have made it possible to perform more complex diagnostic procedures, treatments and numerous multidisciplinary researches.

This paper presents the importance of information technology from the aspect of its applicability in the process of nursing.

A systematic review of quantitative studies published in the Serbian Library Consortium for Coordinated Acquisition (KOBSON).

The use of information technologies significantly improves the work of healthcare institutions. In addition to the application in the nursing process, proper design and implementation of information technology should contribute to significant improvement in the education and research work of nurses.

The use of information technologies is essential in the daily work of nurses in the health care process.

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Key words: *health care, information technologies, education, scientific research*

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Introduction

Independently or in a team work, nurses are constantly working to promote and improve health, to treat and rehabilitate patients, provide health care, as well as organize and provide continuing education. Therefore, the success of a nurse's work depends not only on the application of nursing care, but also on the ability to apply modern technology, which helps the advancement of the nursing profession through continuous learning and improvement.

Nursing care process

In the professional literature, Nursing Health Care is cited as a basic discipline in nursing theory and practice within which a system of specific nurs-ing knowledge and skills is studied (1). It is also implemented in all forms of organization of health, social and child care, including private practice (2). Medical and nursing records can be defined as do-cuments created in healthcare institutions (3). A broader concept of documentation is found in the law of the Republic of Serbia, according to which health documentation is defined as:

- patient and population health monitoring document;
- fulfilling the obligations of all health care entities;
- environmental risk factor and assessment of their impact on population health;
- health care resource;
- continuous improvement of the quality of health care;
- financing health care;
- health care planning and programming;
- monitoring and evaluating the implementation of health care curriculum;
- conducting statistical and scientific research;
- informing the public;
- fulfillment of international obligations in the field of health care and for the development of health care and health insurance systems.

From this it follows that health institutions, private practices and other legal entities are obliged to keep health records, in the manner and according to the procedure and within the deadlines set by this law. Health records can be kept in written or electronic form (4).

Nursing care users are primarily patients with impaired medical conditions, but they may also be healthy patients who can be influenced by the nurse to preserve and prevent diseases in the area of their competence (5).

Nursing health care can be interpreted as a standard based on the rules of the profession (6). In order to implement care procedures as soon as possible, standards and criteria need to be set (7).

There are several defined stages through which the nursing care process takes place:

- identifying nursing care needs
- making a nursing diagnosis
- planning
- performing care and
- evaluation of the results achieved (1).

Collecting data in the health care process is the first stage in the patient's health (8). The implementation of the process is shown in Table 1.

Table 1. The nursing care processes supported by data collection

Better organization, systematization and implementation of healthcare
Provision of individualized and problem-oriented healthcare
Flexibility in care - fast adjustment of care to the changing needs of users and conditions
Active participation of users in the care, protection and promotion of health
Documenting all stages of work in health care
Continuity in healthcare delivery
Better coordination within the nursing team, and implementation of the process provides cost-effective care
Better communication and better coordination of nurses with physicians and health and other professionals

Objective

This paper presents the importance of information technology from the aspect of its applicability in the process of nursing.

Method

A systematic review of quantitative studies published in the Serbian Library Consortium for Coordinated Acquisition (KOBSON).

Results and discussion

Application of information technologies

Information technology is a term that describes computers and related technologies (9).

Medical or health informatics is a discipline that deals with the theory and practice of information processes in medical work and is first mentioned in the 1970s in Europe. Information processes include data transfer, i.e., data processing, development of methods that can be used in a more efficient, reliable and economical way to use medical data, knowledge, information sharing and can make it accessible to a wide range of medical users through computer networks. Because of all of the above, information technologies are a modern tool for medical informatics (10). Information technology is global, and as a result, the amount of information available is increasing (11). Today, in the 21st century, attention has been focused on improving the quality of overall health care (12). Proper use of the medical information system should contribute to the advancement of education and research (13).

Medical informatics is an interdisciplinary science that is also linked to medical records and other disciplines in and out of medicine. In order to avoid misuse of patient data and impairment of quality of life, all data must be protected (14).

Medical information is said to be an integral part of the information system, which is why computer scientists need to provide a secure system for implementation, and make it available to certain healthcare providers. The introduction of information technology into the healthcare system is an important segment that helps to rationalize healthcare resources. This enables healthcare institutions to plan well and provide adequate treatment at anytime, anywhere (15). Nurses use data in all workplaces, both in decision-making in nursing process and for management purposes.

It is of particular importance to emphasize the quality of the data used by nurses, because information that is inaccurate and incorrect can cause harm (16). In their professional work, they face the challenge of applying information technology in the nursing care delivery process (17).

An effective healthcare system must be seriously designed and must use modern information, electronic and telecommunication achievements. According to the World Health Organization definition, healthcare system is a part of a general information system and involves a mechanism for collecting, processing, analyzing and receiving information required for the organization and delivery of health care, as well as for research and organization in healthcare (15). Health information systems are one of the most important trends in health care development, as the possibility of reducing health services prices, conducting quality control and increasing efficiency of health care.

The importance of application of information technologies in the nursing process

The use of modern information technology such as electronic health, telehealth, telemedicine etc. is widespread (18).

Citation in the literature indicates that in medicine, 5,000 types of medical information-communication-technical devices are used to provide about 1,000,000 health care services. Technology is thought to improve health care efficiency and safety, but can also lead to errors and adverse events if not used in accordance with regulations (19).

The use of modern information technology in healthcare according to some authors enables: access to the healthcare system using mobile devices, functions designed for patient management, diagnostics and therapy via the Internet, automation of the health system and management system, delivery of health services based on the application of information technology, relocation of the healthcare system in the Internet environment, as well as the implementation of an electronic way of conducting business processes within healthcare institutions to increase efficiency and reduce costs (18). Nurses need the knowledge, skills and equipment to use informatics and data to achieve better quality of healthcare services and performances in workplace (16).

Healthcare computerization is implemented in several ways, and one of the basic forms is computer equipment, nursing organization and nursing education. The organization enables the available resources to be applied, and the training empowers the nurses to plan, implement and evaluate, thus facilitating communication and accessibility of information, regardless of physical distance (8).

In countries with advanced information technologies, numerous programs are being implemented for patient care and care processes, nursing education, nursing scientific research, and improving nursing service organization. The nursing care process has proven to be very suitable for the application of information technology and it is precisely in the countries in which it is applied that they achieve significant quality of healthcare care (20).

In the world, the nursing care information system is defined as a priority and has six goals:

1. Nursing Dictionary Development
2. Establishment of a clinical database
3. Develop an information system to support measures for patient care
4. Developing a decision support system based on patient care
5. Development of nurse work units of the information system associated with the integrated information system
6. Develop methodologies that evaluate the impact of the information system on the development of health care and patient/user care (7).

The electronic method of record keeping enables the evaluation of nurses' work and the quality of services provided (20). According to some authors, a classification was made shown in Table 2.

The proper functioning of the health information system requires the application of technical, technological infrastructures, education, ethics and legislation (17). According to the requirements of the World Health Organization, education of a nurse in the field of informatics should enable all of the following in Table 3.

Table 2. Classification of nursing care by technology application

1.	**Care with direct application of technology**
2.	**Care with the indirect application of technology**
3.	**Communication technologies**
4.	**Patient and Nurse Assistance Devices**
5.	**Diagnostic and monitoring devices**
6.	**Remote monitoring device**
7.	**Teaching Aids**
8.	**Identification systems**

Table 3. Presentation of the World Health Organization's IT requirements for nursing education

Selection of information for health care planning, implementation and evaluation
Application of research findings relevant to nursing practice
Independence in solving problems related to nursing practice
Participation in the organization and development of nursing care
Participating in training and monitoring of others
Valuing your own work achievements
Collaboration with all institutions that contribute to the well-being of individuals, family, community
Understanding Ethics in nursing care and its impact on nursing practice
Application of current legal regulations in the implementation of nursing care
Tracking changes that affect nursing care delivery

The application of information technologies in nursing contributes to better rationalization and organization (20). Nurses must embrace, understand and apply the concept of information technologies in the nursing healthcare process (21).

The importance of practical implications and research

Nurses need to participate in the selection of technologies and equipment, and should test and give their opinion on what is best for patients (19).

The application of monitoring in hospital conditions is very important, which implies continuous monitoring of vital functions of patients. The alarm systems within the monitoring record changes in the set parameters and alert healthcare professionals about various disturbances.

The use of pulse oximetry and electrocardiography (23) is significant in pre-hospital conditions, on the field, in the ambulance, and during transportation. Information technologies are becoming indispensable in large healthcare institutions (22).

Planning for the introduction of a central information system in the future will enable faster and better communication between all health profiles, regardless of physical distance, faster dissemination of data and better information exchange. All this will enable the integration of information systems into a single, centralized system (8). Nurses around the world use health portals to promote questions related to their field of work in health promotion. The health facilities on their websites contain a 24-hour nurse contact who is in charge of educating patients and families (24).

Through day-to-day work and contact with new technologies, nurses have a significant role in the early detection of errors during nursing care process, and are required to report adverse events and incidents that may contribute to patient illness, injury, or death.

One example of modern technology is electric lifts that assist them through transfer during various interventions. If the lift fails because the batteries are not sufficiently charged, the nurse is at risk of injury, as is the patient. At the suggestion of the nurse, the manufacturer installed an alarm and battery indicator, which contributed to safety (19). They must also have sufficient knowledge and skills regarding the technology they use (22).

Every healthcare institution is obligated to improve nursing care through the use of information technology (19).

Conclusion

Law and the profession require the nurse to document procedures in the implementation of the nursing process, so that work can be monitored and evaluated, in order to allow ongoing monitoring of the patient and to prevent unnecessary duplication of data. The application of information technologies increases the quality and efficiency of work, reduces the possibility of error, saves time and costs and thus provides high quality nursing care.

The application of medical information technologies is very important in the process of education of students, medical staff, patients and their families. They are also widely used for research and professional development purposes.

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PRIMENA INFORMACIONIH TEHNOLOGIJA U PROCESU ZDRAVSTVENE NEGE

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Zdravstveni radnici svedoci su brojnih inovacija i tehnologija koje su omogućile izvođenje složenijih dijagnostičkih postupaka, lečenja i brojnih multidisciplinarnih istraživanja.

Ovaj rad predstavlja značaj informacione tehnologije, sa aspekta njene primenljivosti u procesu zdravstvene nege.

Dat je sistematski pregled kvantitativnih studija, objavljenih u Konzorcijumu biblioteke za objedinjenu nabavku (KOBSON).

Upotreba informacionih tehnologija značajno poboljšava rad zdravstvenih ustanova. Pored primene u sestrinskom procesu, pravilno oblikovanje i primena informacionih tehnologija trebalo bi da doprinesu značajnom poboljšanju obrazovnog i istraživačkog rada medicinskih sestara.

Upotreba informacionih tehnologija od suštinskog je značaja u svakodnevnom radu medicinskih sestara u procesu zdravstvene zaštite.

Acta Medica Medianae 2020;59(1):164-169.

Ključne reči: *zdravstvena zaštita, informacione tehnologije, obrazovanje, naučna istraživanja*

JEDINSTVENI KRITERIJUMI ZA OBJAVLJIVANJE NAUČNIH RADOVA U BIOMEDICINSKIM ČASOPISIMA

Ideja o postavljanju jedinstvenih kriterijuma za objavljivanje radova u časopisima za biomedicinske nauke iskristalisana je 1978. godine u Vankuveru. Ovi kriterijumi za rukopise, uključujući pravila za pisanje bibliografije, prvi put su objavljeni 1979. godine. Vankuverska grupa je vremenom prerasla u Međunarodni komitet urednika medicinskih časopisa — International Committee of Medical Journal Editors (ICMJE). Trenutno je na snazi peta revizija kriterijuma za objavljivanje radova u biomedicinskim časopisima, doneta 1997. godine.

Kriterijumi za citiranje i navođenje referenci

Reference se obeležavaju arapskim brojevima u zagradama, pri čemu se reference obele-žavaju brojevima onim redosledom kojim se pojavljuju u tekstu. Reference citirane jedino u tabelama ili legen-di moraju se obeležiti brojem u skladu sa redosledom pojavljivanja u tekstu.

Naslove medicinskih časopisa treba pisati u skraćenom obliku onako kako su navedeni u poglavlju **List of Journals Indexed in Index Medicus**. Lista skraćenih naziva medicinskih časopisa objavljuje se svake godine u januarskom broju ***Index Medicusa***. Ova lista se takođe može naći na adresi **www.nlm.nih.gov**

Izbegavati upotrebu apstrakata kao referenci, već koristiti samo izvorne tekstove (*in extenso* članci). Reference koje se odnose na radove koji su prihvaćeni, ali još nisu odštampani, treba označiti sa "u štampi", pri čemu autor mora imati pismeno odobrenje da citira takve radove i da priloži pismeni dokaz da je citirani rad prihvaćen za štampu. Informacije iz rukopisa koji nisu prihvaćeni za štampanje mogu se citirati u tekstu kao "neobjavljeni rezultati", ali sa pismenom dozvolom autora.

Izbegavati citiranje prethodnih saopštenja (personal communication) ukoliko ona ne obezbeđuju esencijalne rezultate koji još nigde nisu objavljeni. U ovom slučaju, neophodno je u zagradi navesti ime osobe i datum usmenog saopštenja rezultata. Za objavljivanje ovih podataka neophodno je pismeno odobrenje autora.

Kriterijumi za pisanje referenci korišćenih u radu

U ovom pregledu biće obrađena pravila za pi-sanje literaturnih referenci samo za najčešće korišćene tipove publikacija.

Članci u časopisima

1. Standardni članak u časopisu

Navesti prvih šest autora, ukoliko ih je više iza šestog dodati **et al.** ukoliko je referenca na engleskom jeziku ili **i sar.** ukoliko je referenca na srpskom jeziku.

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Acta Medica Medianae (AMM) je tematski časopis iz oblasti medicinskih nauka. Časopis objavljuje originalne radove koji nisu prethodno publikovani.

U AMM se objavljuju: uvodnici, naučni i stručni članci, prethodna ili kratka saopštenja, revijski radovi tipa opšteg pregleda, aktuelne teme, meta-analize, prikazi slučajeva, prikazi knjiga i drugi prilozi. Radovi se štampaju na srpskom ili engleskom jeziku sa apstrak-tom na srpskom i engleskom jeziku. Radovi na engles-kom jeziku se prezentuju u elektronskom formatu na sajtu Medicinskog fakulteta u Nišu, kao i na među-narodnim sajtovima iz oblasti medicinskih nauka. Acta Medica Medianae izlazi četiri puta godišnje, od 1962 godine.

Svi radovi koji se objavljuju u AMM podležu anonimnoj recenziji, a Uređivački odbor određuje redosled njihovog štampanja. Primedbe i sugestije urednika i recenzenata dostavljaju se autoru radi konačnog oblikovanja. Radovi se predaju u pisanom ili elektronskom obliku na srpskom i engleskom jeziku. Rukopisi radova prihvaćenih za štampu ne vraćaju se autoru.

Rukopis treba predati sa jednostrukim proredom, formata A4, sa levom marginom od 3 cm.

Prva strana rada treba da sadrži: a) naslov rada b) puna imena i prezimena autora c) puni nazivi ustanova i organizacijskih jedinica u kojima je rad realizovan i mesta u kojima se ustanove nalaze, d) znacima *, **, ***, #, ##, ###,…označavaju se redom autori i njihove institucije e) puna adresa, broj telefona i e-mail osobe zadužene za korespondenciju u vezi predatog rukopisa.

Druga strana treba da sadrži samo naslov rada, rezime i ključne reči, bez imena autora i institucija. Veličina rezimea za naučne i stručne članke, revijske radove tipa opšteg pregleda i meta–analize može da bude do 250 reči. Ispod rezimea sa podnaslovom "Klučne reči" navesti 3-5 ključnih reči ili izraza. Poželjno je da autori za ključne reči koriste odgovarajuće deskriptore, tj. definisane termine iz *Medical Subject Heading* (MeSH) liste *Index Medicus-a*. Prva i druga strana se predaju na srpskom i engleskom jeziku i ne obeležavaju se brojevima.

Tekst članka: Naučni i stručni članci, kao i opšti pregledi i meta-analize ne smeju prelaziti 11 stranica sa prilozima; aktuelne teme– 6 stranica; kazuistika 6–stranica; prethodna saopštenja– 5 stranica, a izveštaji sa skupova i prikazi knjiga 2 stranice. Naučni i stručni članci obavezno treba da sadrže poglavlja: uvod, cilj, materijal i metode, rezultati, diskusija i zaključak. Zahvalnost ili komentar povodom sponzorstva rada dati na kraju teksta članka iza poglavlja "zaključak".

U tekstu naznačiti mesta priloga i obeležiti ih onako kako su obeleženi u prilogu.

Literatura se daje u posebnom poglavlju, pri čemu se navodi onim redosledom kojim se citati pojavljuju u tekstu. Broj literaturne reference se u tekstu označava arapskim brojem u zagradi. Za navođenje literature koristiti pravila Vankuverske konvencije. Strane se numerišu arapskim brojevima u donjem desnom uglu

Priloge u vidu teksta, tabela i ilustracija (grafikoni, crteži i dr.) ne unositi u tekst članka, već na kraju teksta, na posebnim stranicama obeleženim u gor-njem levom uglu sa "Tabela, Grafikon, Ilustracija" i arapskim brojem redosledom pojavljivanja u tekstu (npr. Tabela 1, Grafikon 1 i dr.) i svakoj se daje kratak naslov. Kratka objašnjenja i skraćenice daju se u fusnoti. Za fusnotu koristiti sledeće simbole: *, **, ***, #, ##, ###, …itd. Tabele, grafikone i ilustracije treba praviti korišćenjem nekog od programa iz Microsoft Office paketa. Izbegavati upotrebu boja kod izrade grafika.

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Ukoliko je tabela ili ilustracija već negde objavljena, citirati izvor i priložiti pismeno odo-brenje, ukoliko se radi o zaštićenom materijalu. Ukoliko je na fotografiji prikazan bolesnik tako da se može prepoznati, potrebno je njegovo pismeno odobrenje, u suprotnom, delovi fotografije se moraju izbrisati da bolesnik ne može biti identifikovan.

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Rad treba otkucati u programu ms office Word verzija 2003. ili novija. Za verziju na engleskom jeziku koristiti font Verdana, veličine 9 pt, kodna stranica (English). Za verziju na srpskom jeziku koristiti font Verdana, veličine 9 pt, kodna stranica (Serbian lat ili Croatian).

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SECRETARIAT

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All research articles published in this journal undergo rigorous peer review, based on initial editor screening and anonymized refereeing by at least two anonymous referees. Remarks and suggestions made by the editors and reviewers are sent to the author for final revision. The papers in English are to be submitted by e-mail: acta@medfak.ni.ac.rs. Manuscripts accepted for publication are not returned to authors.

The first page of a research article must contain: a) article title b) full name of author(s) c) full name(s) of institutions and/or address(es) of department(s) where either reasearch was conducted or research article written d) following signs *, **, ***, #, ##, ### signifying author(s) and institutions e) full address, phone number and e-mail of a corresponding author.

The second page should contain only research article title, abstract and key words without names of author(s) and institution(s). Abstract for research and professional articles, review articles and meta-analyses should have up to 350 words while abstract for all other types of publications should consist of 250 words. Key Words section should have up to 5 key words or phrases related to a submitted article. It is desirable that authors use corresponding descriptors from Medical Subject Heading (MeSH) that can be found on Index Medicus list for key words. The first and the second page should not be numbered.

Body of a Research Article – Research and professional articles, as well as general surveys and meta-analyses should not exceed 11 pages altogether; current topics - 6 pages, casuistics - 6 pages and proceding statements - 5 pages, history of medicine articles - 3 pages while conference reports and book reviews - 2 pages. Research and professional articles should comprise the following mandatory chapters: introduction, aim(s), material and method(s), result(s), discussion and conclusion(s). Result(s) and discussion can be comprized into one chapter. Acknowledgments of any kind in a submitted article should be written at the end of the paper after "Conclusion(s)". It is necessary to clearly mark a place for additions in the text.

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