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Novi Centralni medicinski blok - Klinički centar Univerziteta u Sarajevu
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Novi Evropski vodič za prevenciju tromboembolizma kod A Fib

CHA₂DS₂-VASc skor za procjenu rizika od tromboembolizma kod A Fib!

Risk factor-based point-based scoring system - CHA ₂ DS ₂ -VASc	
Risk factor	Score
Congestive heart failure/LV dysfunction	1
Hypertension	1
Age ≥75	2
Diabetes mellitus	1
Stroke/TIA/thrombo-embolism	2
Vascular disease*	1
Age 65-74	1
Sex category (i.e. female sex)	1
Maximum score	9

*Prior myocardial infarction, peripheral artery disease, aortic plaque. Actual rates of stroke in contemporary cohorts may vary from these estimates.



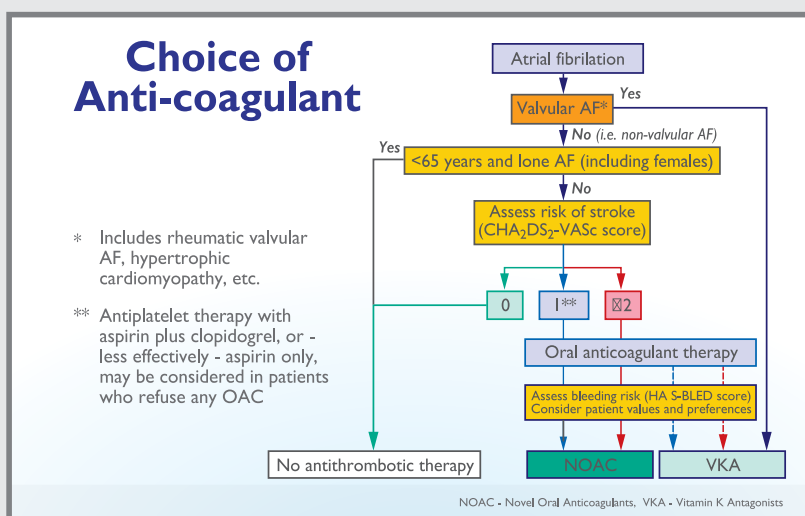
Major i non-major riziko faktori za procjenu tromboembolizma kod A Fib!

Risk factors for stroke and thrombo-embolism in non-valvular AF	
Major risk factors	Clinically relevant non-major risk factors
Previous stroke	CHF or moderate to severe LV systolic dysfunction [e.g. LV EF ≤ 40%]
TIA or systemic embolism	Hypertension
Age ≥75 years	Diabetes mellitus
	Age 65-74 years
	Female sex
	Vascular disease

AF = atrial fibrillation; EF = ejection fraction (as documented by echocardiography, radio nuclide ventriculography, cardiac catheterization, cardiac magnetic resonance imaging, etc.); LV = left ventricular; TIA = transient ischaemic attack.



Algoritam antikoagulantne terapije nakon procjene CHA₂DS₂VASc i major risk faktora!



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Immunohistochemistry-based molecular subtypes of breast cancer in Bosnian women: single-institution pilot study

Imunohistohemijski-određeni molekularni podtipovi karcinoma dojke kod bosanskih žena: pilot studija iz jednog centra

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ABSTRACT

Introduction: to evaluate the frequency and significance of immunohistochemistry-based molecular subtypes of breast cancer and investigate their association with traditional pathological features for breast cancer among Bosnian women. **Materials and methods:** this study included 100 female patients with primary invasive breast cancer. Immunohistochemical analyses for estrogen receptor (ER), progesterone receptor (PR), HER-2 and Ki-67 were performed to define four biological subtypes: luminal A, luminal B, HER-2-positive and triple-negative. **Results:** the frequency of luminal A, luminal B, HER2-positive and triple-negative subtypes of breast cancer was 44%, 39%, 11% and 6%, respectively. Molecular subtypes of breast cancer among Bosnian women showed to be independent of traditional pathological features ($p > 0.05$). Ki-67 showed significant difference regarding luminal B tumor type, where high ($\geq 14\%$) Ki-67 score was predominantly represented in 36 (92.3%) cases ($p < 0.001$). **Conclusion:** immunohistochemistry-based molecular subtypes of breast cancer in Bosnian women somehow vary in pathological features, i.e. luminal A subtype in this sample comprised mostly ductal histological type, moderate differentiation with the involvement of lymph nodes, known as worse prognostic factors, although with no statistical significance.

Key words: breast cancer; molecular subtypes; immunohistochemistry

SAŽETAK

Uvod: ispitati učestalost i značaj imunohistohemijski određenih molekularnih podtipova karcinoma dojke, kao i njihovu povezanost sa tradicionalnim patološkim faktorima za karcinom dojke kod bosanskih žena. **Materijali i metode:** studijom je obuhvaćeno 100 žena sa primarnim invazivnim karcinomom dojke. Urađena je imunohistohemijska evaluacija za estrogenske (ER) i progesteronske receptore (PR), humani epidermalni faktor rasta-2 (HER-2) i Ki-67 proliferativni indeks. Definirana su četiri molekularna podtipa karcinoma dojke: luminal A, luminal B, HER-2 pozitivni i tripl-negativni. **Rezultati:** učestalost molekularnih podtipova luminal A, luminal B, HER-2 pozitivni i tripl-negativni bila je sljedeća: 44%, 39%, 11% i 6%, respektivno. Nije nađena statistički signifikantna povezanost između molekularnih podtipova karcinoma dojke i tradicionalnih patoloških faktora ($p > 0.05$). Ki-67 proliferativni indeks je pokazao statistički značajnu razliku među molekularnim tipovima, pri čemu je visok Ki-67 proliferativni indeks ($\geq 14\%$) dominantno bio zastupljeni u grupi luminal B karcinoma dojke sa 36 (92.3%) slučajeva ($p < 0.001$). **Zaključak:** može se reći da imunohistohemijski-određeni molekularni podtipovi karcinoma dojke kod bosanskih žena dijelom variraju u kliničkopatološkim karakteristikama. Tako je luminal A podtip obuhvatao pretežno duktalne karcinome, umjerene diferencijacije sa metastazama u limfnim čvorovima, koji su poznati kao lošiji prognostički faktori, iako bez statističke značajnosti.

Ključne riječi: karcinom dojke, molekularni podtipovi, imunohistohemija

INTRODUCTION

Breast cancer, as one of the most common malignancies in women, represents heterogeneous disease with wide spectrum of clinical, pathological and molecular features (1). Traditional histopathological characteristics alone, i.e. tumor size, histological grade, lymph node involvement, estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) status, are not capable to meet its multifaceted criteria, although they are widely accepted factors for the assessment of the prognosis and adjuvant

treatment decision-making in breast cancer patients (1,2). In order to elucidate its complexity and provide more personalized therapy for breast cancer patients, in recent years various microarray-based gene expression studies were performed, leading to initial identification of four breast cancer intrinsic subtypes (3). Gene expression profiling is available, but due to its high cost and complexity, it has not been adopted in routine diagnostics, unlike immunohistochemical assessment of ER, PR, HER2 and Ki-67, which are widely used as routine analysis in most pathological laboratories. In order to improve therapeutic possibilities, on St' Gallen meeting held in 2011, surrogate classification,

based on immunohistochemical expression of ER, PR, HER2 and Ki-67, was recommended. This classification implies the following subtypes: Luminal A: ER and/or PR positive, HER2 negative, Ki-67 < 14%; Luminal B (HER2 negative): ER and/or PR positive, HER2 negative, Ki-67 ≥ 14%; Luminal B (HER2 positive): ER and/or PR positive, any Ki-67, HER2 over-expressed or amplified; HER2 positive (non luminal): HER2 over-expressed or amplified, ER and PR absent; Triple negative: ER and PR absent, HER2 negative (4). Recent studies have developed and proposed the usage of the 4-IHC score prognostic model (5,6) as another attempt to establish practical, simple and clinically useful classification for therapeutic and prognostic purposes. Despite the great effort of previous studies, molecular subtyping of breast cancer still has many limitations and unresolved issues, as lack of standardized definitions and unique methodology for determination of molecular subtypes, leaving them only as additional option in prognostic and therapeutic management of breast cancer compared to common clinicopathological parameters (5).

Considering all mentioned facts, as well as the fact that biological differences among breast cancer may reflect genetic influences, differences in lifestyle or nutritional and environmental exposures (7), we aimed to evaluate the prevalence of immunohistochemistry-based (IHC-based) different molecular subtypes of primary invasive breast cancer in Bosnian women, and investigate their possible association with common pathological features for breast cancer.

MATERIALS AND METHODS

Patients and pathological analysis

The study included 100 patients with invasive breast cancer, diagnosed in the period from 2014 to 2015 at Department of Pathology, Faculty of Medicine, Sarajevo, Bosnia and Herzegovina. All patients underwent mastectomy with axillary dissection. Patients on neoadjuvant chemotherapy and/or radiotherapy, patients with distant metastases at the time of diagnosis, bilateral breast cancer or malignancy other than breast cancer in their medical history, were excluded from the study.

Pathohistological data (tumor size, histological type, histological and nuclear grade, ER, PR and HER2 status, Ki-67 proliferative index, axillary lymph node status and lymphovascular invasion) were obtained for all patients. Tissue samples were fixated in 10% neutral formalin, embedded in paraffin, sectioned in 3-5 microns slices and stained with standard hematoxylin-eosin (HE) method. Histological classification of the tumor was performed according to World Health Organization (WHO) criteria (8) and staged according to AJCC TNM classification (9). Microscopic study of the tumor included determination of the following characteristics: size of the tumor (measuring in millimeters two biggest diameters), number of tubular formation (<10%, 10-75%, >75%), number of mitoses in 10 high magnification microscopic fields (<10/hpf, 11-20/hpf, >20/hpf), nuclear pleomorphism (slight, moderate and severe). Ductal infiltrative breast cancers were graded according to the Bloom-Richardson scheme modified by Elston and Ellis (well differentiated or grade I; moderately differentiated or grade II; poorly differentiated or grade III) (10); presence of lymphovascular invasion, and the analysis of the total number of axillary lymph nodes. Tumor's status was determined according to the criteria for determination of breast cancer's status given by the American Joint Committee (AJC) and International Union against Cancer (IUC) (11). In each axilla, minimum of 14 lymph nodes were examined.

Immunohistochemical analysis and molecular classification

Immunohistochemical analyses of the expressions of ER, PR, HER2, and Ki-67 were performed according to routine procedures. All tissue specimens were fixed in 10% neutral formalin and embedded in paraffin. Briefly, 5-μm sections of tumor tissues and non-neoplastic (peritumoral) breast tissue were mounted on poly-D-lysine coated slides. Thin sections were deparaffinized in xylene and rehydrated in a series of ethanol solutions (100%, 90%, and 80%) for 5 minutes each, washed in distilled water and three times in 0.05 mol/L PBS (pH 7.4), immersed in 10 mmol/L citrate buffer (pH 6.0) and put in a microwave for 5 minutes at 60°C for antigen retrieval. Afterwards, they were placed in methanol containing 3% H₂O₂ for 30 minutes at 4°C to block endogenous peroxidase activity and incubated with rabbit serum for 10 minutes to block non-specific antibody binding sites. After blocking the endogenous peroxidase and non-specific binding, the sections were incubated with primary antibodies: ER (clones ID5 and ER-2-123, Dako) and PR (clone PgR 1294, Dako) were recorded as Allred scores (12); HER2 (HercepTest, Dako) was scored as positive if >10% of tumor cells showed 3+ membrane staining (12,13). Ki-67 (clone MIB-1, Dako) was scored as % of any intensity nuclear stain (14). The primary antibodies were applied at a working concentration and incubated for 2 hours at 4°C. The secondary antibody and the avidin-biotin-peroxidase complex (ABC) were applied to slides. 3,3'-Diaminobenzidine (DAB) was used as a chromogen and sections were counterstained with Mayer's hematoxylin. Negative controls were obtained by replacing the primary antibody by non-immunized rabbit or mouse serum.

Breast cancer molecular subtypes according to immunohistochemical profile were classified as follows: luminal A (ER and/ or PR positive, HER2 negative, Ki-67 < 14%), luminal B (ER and/ or PR positive, HER2 negative, Ki-67 ≥ 14%; ER and/ or PR positive, HER2 over-expressed, any Ki-67), HER2 enriched (ER and PR negative, HER2 over-expressed), and triple-negative (ER, PR and HER2 negative) (4). Adjacent normal breast parenchyma was used as internal positive control.

Interpretation of immunohistochemistry

ER and PR positivity were defined as any positive nuclear staining (i.e., ≥ 1%) (15). HER2 immunolabeling was measured according to the HercepTest scoring system (Dako Cytomation), and weak to strong complete membrane staining of more than 10% of tumor cells was defined as overexpression of HER2 (13). Ki-67 proliferation index was expressed as percentage of positive cells from a count of 500 tumor cells. The cut off was 14% of positive nuclei on 500 counted tumor cells. Tumor cells displaying a nuclear positivity were considered positive.

Statistical Analysis

Results are expressed as median and interquartile range (IQR) in case of non-normal distributed continue variables. The inspection of histograms and quantile diagrams and the Kolmogorov-Smirnov test with a Lilliefors significance level were used for testing normality of distribution of continuous numerical variables. Statistical analysis comparing the two groups was performed using Mann-Whitney U-test for continuous non-normal distributed variables. In case of categorical variables, counts and percentages were reported. Categorical data were analyzed with Pearson's Chi-Square test or Fisher's exact test. A p-value

<0.05 was considered as significant. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS Release 19.0; SPSS Inc., Chicago, Illinois, United States of America) software.

RESULTS

Clinicopathological features

Among 100 women with invasive breast carcinoma included in the study, nineteen (n=19; 19.0%) were in their premenopausal period. The median age was 59.5 y (51.0 to 71.0). Most widely encountered type of breast cancer was invasive ductal carcinoma (n=79; 79%).

Other types included neuroendocrine, medullary, papillary and mixed carcinoma. Tumors were mostly pT2 size (n= 47; 47.0%), grade 2 (n=54, 54%), pN2 (n=45; 45%) with more than three metastatic lymph nodes, and mostly without detected lymphovascular invasion (n=61; 61%).

The prevalence and histopathological characteristics in different molecular subtypes

The most common subtype was luminal A (n=44; 44.0%). The second most common type was luminal B (n=39; 39.0%), comprising luminal B (HER2 positive) and luminal B (HER2 negative) tumors. Triple negative type was represented in eleven cases (n=11; 11.0%), and HER2 enriched, as the least frequent subtype, with 6.0% (n=6). All clinicopathological features according to molecular subtypes of breast cancer are presented in Table 1. Luminal A patients were older, had predominantly smaller (pT1 and pT2), moderately differentiated (G2) tumors, but with present metastases in axillary lymph nodes (pN2). Luminal B patients were also older, but mostly had larger tumors (pT2), with moderate differentiation (G2) and involvement of lymph nodes (pN2). Triple negative tumors were predominantly poorly differentiated (G3), lymph node positive tumors with higher incidence of lymphovascular invasion (45.0%) compared to luminal A (36.4%) and luminal B (38.5%) subtypes. HER2 positive tumors tended to occur in older patients (≥ 50), moderately differentiated, with positive regional lymph nodes (pN2) and equal percentage of tumors with present and absent lymphovascular invasion. None of commonly used pathological factors for breast cancer showed statistically significant association with IHC-based molecular subtypes of breast cancer ($p>0.05$) (Table 1).

Table 1 Molecular subtypes of invasive breast cancer.

Variables	Luminal A	Luminal B	Triple negative	HER2	p-value
Age at diagnosis (y)					
<50	9 (20.5%)	7 (17.9%)	2 (18.2%)	1 (16.7%)	0.973
≥ 50	35 (79.5%)	32 (82.1%)	9 (81.8%)	5 (83.3%)	
Tumor grade					
1	5 (11.4%)	8 (20.5%)	2 (18.2%)	0 (0.0%)	0.198
2	29 (65.9%)	18 (46.2%)	3 (27.3%)	4 (66.7%)	
3	10 (22.7%)	13 (33.3%)	6 (54.5%)	2 (33.3%)	
pT					
1	20 (45.5%)	14 (35.9%)	2 (18.2%)	3 (50.0%)	0.437
2	19 (43.2%)	17 (43.6%)	9 (81.8%)	2 (33.3%)	
3	2 (4.5%)	4 (10.3%)	0 (0.0%)	1 (16.7%)	
4	3 (6.8%)	4 (10.3%)	0 (0.0%)	0 (0.0%)	
pN					
0	17 (38.6%)	12 (30.8%)	4 (36.4%)	1 (16.7%)	0.633
1	8 (18.2%)	7 (17.9%)	1 (9.1%)	3 (50.0%)	
2	19 (43.2%)	18 (46.2%)	6 (54.5%)	2 (33.3%)	
3	0 (0.0%)	2 (5.1%)	0 (0.0%)	0 (0.0%)	
Hystological type					
Ductal	35 (79.5%)	30 (76.9%)	8 (72.7%)	6 (100.0%)	0.423
Lobular	1 (2.3%)	5 (12.8%)	1 (9.1%)	0 (0.0%)	
Others	8 (18.2%)	4 (10.3%)	2 (18.2%)	0 (0.0%)	
LVI					
Negative	28 (63.6%)	24 (61.5%)	6 (54.5%)	3 (50.0%)	0.686
Positive	16 (36.4%)	15 (38.5%)	5 (45.5%)	3 (50.0%)	

* LVI- Lymphovascular invasion

Characteristics of examined tumors according to Ki-67 proliferating index

Generally, the majority of tumors (54.0%) showed low Ki-67 proliferation index ($Ki-67 \leq 15\%$). The majority of patients had pT2 (47.0%) or pT1 (39%) tumors, respectively. Regarding the tumor size, low-pT stages were associated with low expression of Ki-67, but with no statistical significance ($p>0.05$) (Table 2). Concerning the nodal status, it was shown that node-negative tumors were associated with low expression of Ki-67, but were not statistically significant ($p>0.05$) (Table 2). Ki-67 expression with a cut-off of 14% was significantly differently distributed between the molecular subtypes, being the highest in luminal B type tumors ($p<0.001$) (Table 2). Tumors with lymphatic and vascular invasion (LVI) had higher expression of Ki-67 compared with tumors without LVI (48.7% vs. 44.3%, respectively), but this difference was not statistically significant ($p>0.05$) (Table 2).

Table 2 Characteristics of 100 patients with invasive breast cancer according to Ki67 cut-off (14%).

Variables	Ki67 <14%	Ki67 $\geq 14\%$	p-value
Age at diagnosis (Me) (IQR)	62.5 (52.0 to 72.5)	57.0 (50.8 to 66.8)	0.101
Tumor grade			
1	7 (46.7%)	8 (53.3%)	0.326
2	33 (61.1%)	21 (38.9%)	
3	14 (45.2%)	17 (54.8%)	
pT			
1	23 (59.0%)	16 (41.0%)	0.545
2	25 (53.2%)	22 (46.8%)	
3	2 (28.6%)	5 (71.4%)	
4	4 (57.1%)	3 (42.9%)	
pN			
0	21 (61.8%)	13 (38.2%)	0.768
1	10 (52.6%)	9 (47.4%)	
2	22 (48.9%)	23 (51.1%)	
3	1 (50.0%)	1 (50.0%)	
Hystological type			
Ductal	42 (53.2%)	37 (46.8%)	0.618
Lobular	3 (42.9%)	4 (57.1%)	
Others	9 (64.3%)	5 (35.7%)	
Molecular type			
Luminal A	44 (100.0%)	0 (0.0%)	<0.001
Luminal B	3 (7.7%)	36 (92.3%)	
HER2	5 (45.5%)	6 (54.5%)	
Triple-negative	2 (33.3%)	4 (66.7%)	
LVI			
Negative	34 (55.7%)	27 (44.3%)	0.686
Positive	20 (51.3%)	19 (48.7%)	

* LVI- Lymphovascular invasion

DISCUSSION

This study evaluated frequency of molecular subtypes of breast cancer among Bosnian women, and found luminal A as the most frequent subtype (44%) which corresponds to previous studies (16, 17, 18). Luminal A breast cancer is considered a subtype with the best prognosis (16). It is defined by high expression of hormone receptors, low expression of the cell-growth marker Ki-67 and the oncoprotein HER2, but the study identified some poor prognostic factors, i.e. the luminal A group of tumors in this series comprised mostly ductal histological type, moderate differentiation (grade 2) and involvement of lymph nodes (pN2), although without statistically significant association ($p>0.05$).

The study established luminal B, as the second most frequent subtype (39%), often detected in older women (≥ 50), frequently ductal histological type, mainly moderately or poorly differentiated, with tendency to be node-positive, expressing high Ki-67 proliferating index, similar to findings of other studies (16,17). Ki-67 expression detected by immunohistochemistry is one of the most reliable indicators of the proliferative status of cancer cells. Breast cancers with higher Ki-67 expression are associated with more active growth, invasion and more aggressive

behavior (19).

Since the cut-off value for Ki-67 has not been standardized yet (20,21), and given that we classified IHC-based molecular subtypes according to 2011 St' Gallen recommendations, we used cut-off value of 14% for Ki-67 to distinguish luminal A and luminal B molecular subtype. We evaluated Ki-67 proliferation index with respect to molecular types of breast cancer. It showed significant difference regarding luminal B tumor type, where high ($\geq 14\%$) Ki-67 score was predominantly represented in 36 (92.3%) cases ($p < 0.01$). Also HER2 type and triple-negative subtype of breast cancer showed higher percentage of high Ki-67 proliferation index, although statistical significance was not found ($p > 0.05$), which supports the opinion that Ki-67 index is one of the factors influencing molecular subtypes. Comparing Ki-67 proliferation index with demographical and clinicopathological data, significant differences were not found in our study ($p > 0.05$). Other studies found higher Ki-67 index to correlate significantly with young age, large tumors, positive lymph nodes, negative ER/PR, p53 over expression, and positive HER2 (19, 22).

Triple-negative tumors, which are typically ER-negative, PR-negative and HER2-negative, predominantly represented in breast cancer developing during the postmenopausal years (> 50) in Bosnian women, frequently tended to be of ductal histological type, poorly differentiated (G3), mainly high stage (pT3), with tendency to be node-positive (pN2) showing high expression of the cell-growth marker Ki-67, although statistically significant association was not observed ($p > 0.05$). Previous studies which compared survival in triple negative and other types of breast cancer found triple negative subtype as the one with poor overall and disease free survival (23,24,25). HER2-positive tumors were least frequent subtype in our sample with only 6%.

The present study has a limitation. The sample size ($n = 100$) was fairly small, and to draw clearer conclusions, another study with a larger sample size and prospective design will be necessary.

CONCLUSION

This study suggests that IHC-based molecular subtypes of invasive breast cancer in Bosnian women vary in pathological features, i.e. the luminal A subgroup cancers in this series comprised mostly ductal histological type, moderate differentiation with involvement of lymph nodes, which are known as worse prognostic factors, although statistical significance was not determined. The significance of this study is in the attempt to uncover the associations between traditional prognostic indicators (TNM staging and pathological grade) and molecular subtypes of breast cancer. In each case hormonal status and subtyping on the basis of immunohistochemical evaluation is important given that these different molecular subtypes may lead to different prognosis and different targeted therapies.

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Testing the efficiency of individual cognitive-behavior psychotherapy among patients with chronic inflammatory bowel disease

Ispitivanje efikasnosti individualne kognitivno-bihevioralne psihoterapije kod ispitanika sa hroničnom upalnom bolesti crijeva

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ABSTRACT

Introduction: inflammatory bowel disease is characterized by chronic inflammation of the gastrointestinal system, or some of its parts, which etiology has remained unexplained. Two entities, specifically Crohn's disease and ulcerative colitis are defined clinically and post-mortem. Changes in Crohn's disease may affect all parts of the gastrointestinal system, from the mouth to the anus. The disease is characterized by frequent exacerbations, with various clinical manifestations and chronic unpredictable course of the disease. Ulcerative colitis is an inflammatory bowel disease characterized by recurrent episodes of inflammation limited to the mucosa of the colon. It almost always includes the final part of the colon, rectum, and can be extended to other parts of the colon. Psychotherapy is still not applied in routine treatment protocols despite numerous researches and clinician's impression of personality disorders of patients with chronic inflammatory bowel disease. Cognitive behavioral psychotherapy (CBT) and psychoeducation has proved to be a useful complement to the primary therapy in the treatment of chronic inflammatory bowel disease. Goal: the research goals were to examine the effectiveness of individual CBT therapy in patients with chronic inflammatory bowel disease, to reduce anxiety, depression and use strategies to cope with stress. Materials and methods: the study included 30 patients with clinical diagnosis of chronic inflammatory bowel disease, of both sexes, age between 19 and 40 years, who met the inclusion criteria and signed consent for inclusion. The study included the following measuring instruments: Questionnaire for registration of respondent's socio-demographic characteristics, Beck questionnaire on depression and anxiety and Stress coping scale, during two measurements before and after the CBT. All patients had eight sessions during eight weeks of CBT individual psychotherapy once a week for an hour. The study was conducted at the Psychiatric Clinic of the Clinical Center University of Sarajevo (CCUS). The study is a prospective, longitudinal, comparative, analytical and descriptive. Results and conclusion: the results of our research showed statistically significant efficacy of individual

SAŽETAK

Uvod: upalne bolesti crijeva karakterizirane su hroničnom upalom gastrointestinalnog trakta, odnosno pojedinih njegovih dijelova, za sada nerazjašnjene etiologije. Klinički i patoanatomski definirana su dva entiteta i to Crohnova bolest i ulcerozni kolitis. Kod Crohnove bolesti promjene mogu zahvatiti sve dijelove gastrointestinalnog trakta, od usne šupljine do anusa. Bolest je karakterizirana čestim egzacerbacijama, s raznovrsnim kliničkim manifestacijama, te hroničnim nepredvidljivim tokom bolesti. Ulcerozni kolitis je upalna bolest crijeva obilježena ponavljanim epizodama upale ograničene na sluznicu debelog crijeva. Gotovo uvijek uključuje završni dio debelog crijeva, rektum, a može se proširiti i na ostale dijelove debelog crijeva. Psihoterapija se još uvijek ne primjenjuje u rutinskim terapijskim protokolima i pored brojnih istraživanja i utiska kliničara o poremećajima u strukturi ličnosti oboljelih od hroničnih upalnih bolesti crijeva. Kognitivno bihevioralna (KB) psihoterapija i psihoedukacija se pokazala kao korisna nadopuna osnovnoj terapiji u liječenju hroničnih upalnih bolesti crijeva. Cilj: ciljevi istraživanja su ispitati efikasnost individualne KB terapije kod ispitanika sa hroničnom upalnom bolešću crijeva, na redukciju anksioznosti, depresivnosti i korištenje strategija suočavanja sa stresom. Materijali i metode: u istraživanje je uključeno 30 ispitanika sa klinički postavljenom dijagnozom hronične upalne bolesti crijeva, oba spola, dobnog uzrasta od 19 do 40 godina, koji su ispunili uključujuće kriterije i potpisali suglasnost za uključivanje. U istraživanju smo primijenili slijedeće mjerne instrumente: Upitnik za registraciju socijalno-demografskih karakteristika ispitanika, Bekov upitnik na depresiju i anksioznost i Skalu suočavanja sa stresom, u dva mjerenja prije uključivanja u terapiju i nakon završetka terapije. Svi ispitanici su imali po osam seansi tokom osam sedmica trajanja individualne KB psihoterapije, jednom sedmično u trajanju od sat vremena. Istraživanje je provedeno na Psihijatrijskoj klinici Kliničkog centra Univerziteta u Sarajevu (KCUS). Studija je prospektivna, longitudinalna, komparativna, analitička i deskriptivna. Rezultati i zaključak: rezultati našeg istraživanja su dokazali statistički značajnu efikasnost individualne kognitivno-bihevioralne psihoterapije na

cognitive-behavioral psychotherapy on reducing depression and anxiety in patients with chronic inflammatory bowel disease. According to the results of this study patients with chronic inflammatory bowel disease, after completion of individual cognitive-behavioral therapy are significantly less likely to use non-adaptable strategies for coping with stress, and use more adaptable strategies, which improves the quality of life and the acceptance of the treatment of the underlying disease. Disadvantages of the study relate to small sample of 30 patients and exclusion of other variables significant for the treatment outcome.

Key words: chronic inflammatory bowel disease, cognitive-behavioral psychotherapy

INTRODUCTION

Inflammatory bowel disease is characterized by chronic inflammation of the gastrointestinal system, or some of its parts, which etiology has remained unexplained. Clinical and post-mortem studies defined two large entities: Crohn's disease and Ulcerative Colitis. Both diseases are idiopathic, given that the pathogenesis is not fully understood. In Crohn's disease (CD) changes can affect all parts of the gastrointestinal system, from the mouth to the anus. Crohn's disease is characterized by frequent exacerbations, with various clinical manifestations, and chronic unpredictable course of the disease. Ulcerative colitis (UC) is an inflammatory bowel disease characterized by recurrent episodes of inflammation limited to the colon mucosa. Almost always includes the final part of the colon, rectum, and can be extended to other parts of the colon (1,2). Psychotherapy is still not applied in routine treatment protocols despite numerous studies on the positive effects of psychotherapy and clinician's impressions on personality disorders of patients with chronic inflammatory bowel diseases (3). Studies show that properly indicated and properly implemented, psychotherapy can be a useful complement to the primary therapy. Studies also indicate that adapted cognitive behavioral psychotherapy (CBT) for patients with chronic inflammatory bowel diseases is effective in improving quality of life and alleviating the psychopathological symptoms, such as anxiety and depression associated with chronic inflammatory bowel disease, as well as changing opinions, attitudes and behavior in relation to the knowledge of the existence of chronic inflammatory bowel disease. In individual and group setting CBT initiate use of adaptable strategies to cope with the disease, such as problem solving and avoiding maladaptive coping strategies such as escape from disease and avoiding behavior, which is of benefit for patients' adherence to protocols for the treatment of primary chronic disease (4-6). Due to the low quality of life of these patients, beside basic therapy, a psychological support is also needed. According to previous study results there is a necessity to increase the quality of life of patients with chronic inflammatory bowel disease, especially Crohn's disease, and poor quality of life is not only a consequence of the disease, but the risk for deterioration and relapse (7,8).

Goal

To examine the effectiveness of individual CBT in patients with chronic inflammatory bowel disease, to reduce anxiety, depression and use strategies to cope with stress.

redukciju depresivnosti i anksioznosti kod ispitanika sa hroničnom upalnom bolešću crijeva. Prema rezultatima našeg istraživanja ispitanici sa hroničnom upalnom bolešću crijeva, nakon završetka individualne kognitivno-bihevioralne terapije značajno manje koriste neadaptabilne strategije suočavanja sa stresom, a značajno više koriste adaptabilne strategije suočavanja sa stresom, što poboljšava kvalitetu života i prihvatanje liječenja osnovne bolesti. Nedostaci istraživanja su mali uzorak od 30 ispitanika i ne uključivanje u ispitivanje i drugih varijabli, od kojih ovisi ishod terapije.

Ključne riječi: hronične upalne bolesti crijeva, kognitivno-bihevioralne psihoterapije

MATERIALS AND METHODS

The study included 30 patients diagnosed with ulcerative colitis or Crohn's disease, of both sexes, aged between 19 and 40 years. Inclusion criteria for the study were relative remission of the underlying disease based on the clinical judgment of the physician, performed psychiatric interview, which excluded psychosis, mental retardation, dementia, anti-social and addictions to alcohol and other psychoactive substances, and indications for inclusion in the individual cognitive-behavioral therapy with written consent to participate in the research. The following measurement instruments were used for testing the effectiveness of therapy:

1) Questionnaire for registration of socio-demographic characteristics of patients, prepared by the study authors.

2) Beck Depression Scale (9) which is a diagnostic questionnaire used as an instrument to diagnose and differentiate diagnostic groups, and is also suitable for the research. It contains 21 questions with four response options graded from 0 to 4. The total score on the scale is obtained by simply adding all the responses received from the first to the twenty-first question. Severity of the disorder is presented as numerical value. Assessment refers to the past seven days, including the day when the instrument was applied. The scale is highly correlated in case of retest and has very high research congruence with research diagnostic criteria (10). Results are interpreted in the following severity ranges: score of 0-9 relates to a state without depression; score of 10-15 relates to mild depression; score of 16-19 indicate mild to moderate depression; score of 20-29 indicate moderate to severe depression and the score of 30-63 indicate expressed depression.

3) Beck anxiety scale (11) is the questionnaire used as an instrument to assess anxiety. There are 21 questions pertaining to symptoms of general anxiety. Every question is answered by assessing the intensity of symptoms on a Likert-type scale from 0 (not present) to 3 (very pronounced). The sum of all responses represents the intensity of the general anxiety symptoms. The instrument has good psychometric properties. Questionnaire was used to evaluate the intensity of general anxiety, but not for the diagnosis of anxiety disorder. In this regard, a questionnaire can be used in conjunction with other techniques to identify less prominent anxiety, planning and monitoring of treatment, assessment of treatment outcome and evaluation of functioning at the end of treatment (12).

4) Scale of stress coping strategies by Folkman and Lazarus (13) is used for registration of strategies to cope with stress. It consists of 39 items, divided into eight subscales: seeking social support, coping with the problem, distancing, self-control, positive assessment, planned

problem solving, escape/avoidance, and acceptance of responsibility. Coping strategies that have a significant positive correlation with all kinds of psychological symptoms are maladaptive coping strategies, and those which have significant negative correlation with all kinds of psychological symptoms represent adaptive coping strategies. The maladaptive coping strategies are lacking strategy for planning problem solving and seeking social support, and the list of adaptive coping strategies lacking strategy of escape/avoidance.

A sample of 30 patients, who met the inclusive criteria for the study were tested by these instruments prior to introduction of individual cognitive-behavioral (CBT) psychotherapy. The treatment for each participant lasted eight weeks, while sessions took place once a week for an hour. After the initial eight sessions of individual CBT, all subjects were re-tested with the same instruments. The results were statistically analyzed using standard statistical methods and presented in tables and text. The study was conducted at the Psychiatric Clinic of the CCUS. The study was a prospective, longitudinal, comparative, analytical and descriptive.

RESULTS

Socio-demographic characteristics of the sample.

Table 1
Characteristic of the sample by gender.

Gender	Frequency	Percentage (%)
Male	11	36.7
Female	19	63.3

Majority of patients (63.3%) were women, while men were present in 36.7% of the sample.

Table 2
Characteristic of the sample by age.

Age	Frequency	Percentage
19 - 30 years	15	50%
31 - 40 years	15	50%

According to age, 50% of the patients were aged 19-30 years and 50% were aged 31 to 40 years.

Table 3
Representation according to clinical diagnosis.

Diagnosis	Frequency	Percent
Crohn's disease	12	40%
Ulcerative colitis	18	60%

Crohn's disease was present in 40%, and ulcerative colitis in 60% of patients.

Table 4 Descriptive statistical values for depression questionnaire.

Question	DEPRESSION			
	Test		Retest	
	Mean	Standard deviation	Mean	Standard deviation
The feeling of sorrow	1.85	0.66	1.11	0.71
The feeling of discouragement	1.66	0.82	1.15	0.82
The feeling of failure	1.69	0.84	1.12	0.76
The feeling of satisfaction	2.22	0.76	1.22	0.65
The feeling of guilt	1.34	0.73	0.85	0.92
The feeling of punishment	1.70	0.81	1.08	1.10
Disappointment	1.48	0.61	1.05	0.72
Self-accusation	1.68	0.81	0.82	0.91
Suicide attempt	0.75	0.64	0.31	0.45
Crying	1.36	0.54	0.82	0.74
Feelings of edginess (agitation)	1.90	0.55	1.00	0.51
Loss of interest	1.73	0.72	1.00	0.61
Difficulties in decision making	1.59	0.62	1.12	0.82
Physical appearance	1.49	0.58	0.38	0.72
Working ability	1.97	0.84	1.35	0.68
Sleeping	2.18	0.72	0.83	0.77
Fatigue	1.65	0.68	1.26	0.99
Appetite	1.21	0.72	0.39	0.70
Weight loss	0.80	0.84	0.46	0.66
Sense of anxiety	1.51	0.92	0.85	0.84
The desire for sexual activity	2.17	0.65	1.39	0.76

Table 4 shows the average and standard deviation of all items in questionnaires for depression during the test and retest.

Table 5 Descriptive statistical values for the anxiety scale.

Question	ANXIETY			
	Test		Retest	
	Mean	Standard deviation	Mean	Standard deviation
Numbness or tingling	1.47	0.71	1.02	0.76
Feeling the heat	1.42	0.67	0.88	0.72
Uncertainty in the legs	1.47	0.75	1.02	0.94
Inability to relax	2.10	0.62	1.04	0.92
Fear of the worst happening	2.13	0.61	1.37	0.80
Vertigo or haze	1.12	0.45	1.02	0.70
The pounding or fast heartbeat	1.53	0.49	0.80	0.77
Instability	1.34	0.72	1.22	0.62
Horrified	1.73	0.69	0.97	0.92
Nervousness	2.20	0.61	1.08	1.06
Feeling of suffocation	1.33	0.81	1.24	0.76
Shaking hands	1.57	0.67	0.51	0.90
Feeling shaky	1.44	0.77	0.85	0.91
Fear of losing control	1.45	0.72	0.89	0.93
Breathing difficulties	1.50	0.62	0.81	1.06
Fear of dying	1.59	0.76	0.50	0.91
Feeling scared	2.09	0.70	1.02	0.94
Poor digestion or stomach problems	1.99	0.90	1.32	1.02
Faint	1.10	0.47	0.46	0.82
Facial flushing	1.28	0.75	0.60	0.69
Unduly sweating	2.08	0.80	0.83	0.60

Table 5 shows the average values and standard deviation of all particles of the questionnaire for anxiety during the test and retest.

Table 6 Descriptive statistical values of the variables in two measurements.

Pairs		Mean	Standard deviation
Pair 1	Depression before treatment	1.54	0.42
	Depression after treatment	0.86	0.48
Pair 2	Anxiety before treatment	1.56	0.58
	Anxiety after treatment	0.88	0.71

Table 6 shows that after the treatment there was a decrease in the mean values for depression (1.54 test and 0.86 retest) and anxiety (1.56 test and 0.88 retest).

Table 7 Descriptive statistical values of the variables in two measurements.

Pair	t-value	Significance level (p)
Initial depression - depression retest	7.682	p<0.01**
Initial anxiety - anxiety retest	7.024	p<0.01**

Table 7 shows that between two measurements statistically significant differences existed in the level of depression and anxiety, specifically that there was a significant reduction in depression and anxiety at the end of treatment compared to baseline, at a level of significance, p<0.01.

Table 8 Maladaptive coping strategies during test and retest.

	N	Mean	SD	Significance level p<0.05
Test	30	11.80	2.38	
Retest		6.55	3.36	

Table 8 shows that there was a statistically significant difference between the initial measurements, before introduction and after completion of cognitive behavioral psychotherapy in the retest. Patients significantly less use maladaptive strategies for dealing with stress, at the level of significance, p<0.05.

Table 9 Adaptive strategies of coping with stress during test and retest.

	N	Mean	SD	Significance level p<0.05
Test	30	14.34	4.78	
Retest	30	18.96	7.12	

Table 9 shows that there was a statistically significant difference

between the initial measurements, before introduction of the CBT, and after completing CBT. The subjects used more adaptable strategies for dealing with stress, at the level of significance, $p < 0.05$.

DISCUSSION

The sample of 30 patients with chronic inflammatory bowel disease comprised 36.7% of men and 63.3% of women. Based on the age, 50% of patients were between 19 and 30 years of age and 50% were in a group of 31-40 years of age. Patients with Crohn's disease were represented in the sample of 40%, and with ulcerative colitis in the sample of 60%. After completion of individual CBT, the mean scores values were reduced for both depression (1.54 test and 0.86 retest) and anxiety (1.56 test and 0.88 retest).

In two measurements, initially and after eight weeks of KBT psychotherapy, statistically significant differences were obtained in the levels of depression and anxiety, which means that there was a significant reduction in depression and anxiety at the end of treatment compared to baseline, at the level of significance, $p < 0.01$. With regard to the use of maladaptive strategies to cope with stress there was no statistically significant difference between the initial measurements, before introduction of CB psychotherapy and after CB psychotherapy during the retest. Patients significantly less used non-adaptable strategies for dealing with stress, at the level of significance, $p < 0.05$.

Also, there was a statistically significant difference between the initial measurement and retest in the use of adaptable strategies to cope with stress. The subjects used more adaptable strategies for dealing with stress, at the level of significance, $p < 0.05$. Based on the results of randomized controlled studies in eighteen of nineteen papers published in 2012, psychotherapy has little effect on anxiety, depression, quality of life and disease progression, although it indicates a decrease in pain, fatigue, the rate of relapse and hospitalization, and improve adherence to medication for underlying disease, thus reducing medical costs.

Given that based on the conducted studies, the effects of psychotherapy on chronic inflammatory bowel disease are mixed, studies suggest that future studies should focus on examining whether the individual psychotherapy is superior over others. Studies recommend computerized cognitive-behavioral therapy, which is regarded as highly acceptable and low cost (14). As the results of our research are not in accordance with the review of previous studies, we believe that the disadvantage of our study is a small sample.

CONCLUSION

The results of our research showed statistically significant efficacy of individual cognitive-behavioral psychotherapy on reducing depression and anxiety in patients with chronic inflammatory bowel disease. According to the results of our survey, patients with chronic inflammatory bowel disease, after the completion of individual cognitive-behavioral therapy are significantly less likely to use non-adaptable strategies to cope with stress, and significantly more often use adaptable strategies to cope with stress, which improves their quality of life and acceptance of treatment for the underlying disease.

Disadvantages of the study relate to small sample of 30 patients and exclusion of other variables significant for the treatment outcome.

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Morphometric measurement of the mandible angle: determination of gender and age

Morfometrijsko mjerenje mandibularnog ugla: određivanje pola i dobi

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ABSTRACT

When it comes to skeletal remains, mandible has an extremely important role. Often, in the examined material, the best preserved bone whose morphological characteristics and measures can contribute to the easy identification of victims. The main goal set in this paper was to determine how age and gender influence the value of the mandibular angle (GA). As a material for this work, 200 mandibles (100 mandibular males and 100 females), aged 35 to 65 years old, were excluded from the osteological collection of the Department of Anatomy of the Faculty of Medicine in Sarajevo, divided into six groups with five-year age intervals. The mandibular angle is measured as the angle between the lower edge of the mandibular body and the back edge of the ramus using a specially designed software program Ellips (ViDiTo, Zoltan Tomoris, Kosice, Slovak Republic, tomori@saske.sk.). SPSS software version 18 was used for statistical analysis of the obtained data. Measurements show higher mandibular angle of mandibular females than males, and statistical analysis showed that mandibular angle is not a reliable parameter for determining sex based on skeletal remains.

Key words: mandible, mandibular angle, morphometry, sexual dimorphism

SAŽETAK

Kada je riječ o skeletnim ostacima, mandibula ima iznimno važnu ulogu. Često je to, u ispitivanom materijalu, najbolje očuvana kost čije morfološke karakteristike i mjere mogu doprinjeti lakoj polnoj identifikaciji žrtava. Osnovni cilj postavljen u ovom radu je bio ustanoviti kako dob i pol utiču na vrijednost mandibularnog ugla (GA). Kao materijal za izradu ovog rad korišteno je 200 mandibula (100 mandibula muškog i 100 ženskog pola), starosne dobi od 35 do 65 godine, izuzete iz osteološke zbirke Katedre za anatomiju Medicinskog fakulteta u Sarajevu, podeljene u šest grupa sa petogodišnjim starosnim intervalima. Mandibularni ugao je mjereno kao ugao između donjeg ruba tijela mandibule i stražnjeg ruba ramusa, pomoću specijalno dizajniranog softverskog programa Ellips (ViDiTo, Zoltan Tomoris, Kosice, Slovačka republika, tomori@saske.sk.). Za statističku analizu dobijenih podataka korišten je SPSS softver verzija 18. Mjerenjem su zabilježene veće vrijednosti mandibularnog ugla kod mandibular ženskog pola u odnosu na mandibule muškog pola, a statistička analiza je pokazala da mandibularni ugao nije pouzdana parametar za određivanje pola na osnovu skeletnih ostataka.

Ključne riječi: donja čeljust, mandibularni ugao, morfometrija, seksualni dimorfizam

INTRODUCTION

The mandible is the second most durable bone of the body and is the most resilient in unfavorable conditions (1). The angle of mandible is formed by the tangent lines of the posterior border of the ramus and the base of the mandible (2,3). It is an important landmark of the mandible and is commonly used in forensic science for age, race and sex identification (4). However, controversy exists with regard to the use of the angle of mandible in forensic science, specifically in age and sex determination (4). The literature revealed that the angle of mandible may be an accurate cephalometric tool for population-specific age and sex identification (5). Conversely, Oettlé et al. and Upadhyay et al. reported the angle of mandible to be an inefficient tool for the determination of age and sex, since there are countless factors that influence its development, viz. diet, dentition and population-specific characteristics (1,4). Furthermore, the literature reviewed has documented that the size of the angle

of mandible decreased from birth to adulthood and increased from adulthood to old age (6, 7). On the contrary, Leversha et al. reported a steady increase in the size of the angle of mandible with advancement in age (8).

MATERIALS AND METHODS

The material used for the study contained 200 human mandibles of known sex obtained from Department of Anatomy, Medical Faculty, University of Sarajevo. The bones collected were free from any pathological lesions or fractures. The human mandible (100 men and 100 women) aged between 29 and 69 years distributed into 6 age groups of ten-year age period each. All mandibles were photographed using a Nikon D5200 (AF-S 18-105MM VR KIT LENS) digital SLR camera. All photos were transferred in the specially designed software program Ellips (ViDiTo, Zoltan Tomoris, Kosice,

Slovak Republic, tomori@saske.sk.). Mandibular measurements were performed using Linea system program.

Statistical Analysis

Comparisons of the mean gonial angle values were made between different age groups and also between both sexes. The data obtained from comparisons was subjected to statistical analysis using SPSS software version no. 18. Mann-Whitney tests were used for inter- and intra-age-group comparisons. Unpaired T-Test was used to ascertain the presence of sexual dimorphism.

RESULTS

The range of gonial angle values recorded for females in the present study was shown in Table 1. In females, the mean gonial angle values ranged from $114.8^\circ \pm 8.341$ to $122.3^\circ \pm 8.722$. There was a uniform trend of increase in values of mean gonial angle with increase in age in females. The highest mean gonial angle value in females was reported in group 6 (61-65 years) while the lowest mean gonial angle was reported in group 1 (35-40 years).

Table 1 Gonial Angle (GA) values (in $^\circ$) in males and females in different age-groups.

Group	Age (years)	Females		Males	
		Range (in $^\circ$)	Mean GA (in $^\circ$) \pm S.D.	Range (in $^\circ$)	Mean GA (in $^\circ$) \pm S.D.
1	35 - 40	108.5 - 127.0	114.8 ± 8.341	113.5 - 122.5	113.4 ± 3.782
2	41 - 45	111.5 - 121.0	115.0 ± 3.889	110.5 - 123.5	114.7 ± 5.227
3	46 - 50	109.5 - 124.5	115.2 ± 5.696	116.0 - 122.0	117.9 ± 2.382
4	51 - 55	110.0 - 131.0	118.7 ± 11.015	113.5 - 128.5	121.5 ± 6.171
5	56 - 60	106.0 - 125.5	120.0 ± 8.047	117.0 - 126.0	122.0 ± 3.536
6	61 - 65	114.0 - 131.5	122.3 ± 8.722	118.5 - 131.5	122.6 ± 5.413

The range of gonial angle values recorded for males in the present study was shown in Table 1. In males, the mean gonial angle values ranged from $113.5^\circ \pm 3.782$ to $122.6^\circ \pm 5.413$. There was a uniform trend of increase in values of mean gonial angle with increase in age in males. The highest mean gonial angle value in males was reported in group 6 (61-65 years) while the lowest mean gonial angle was reported in group 1 (35-40 years).

Significant positive correlations ($p < 0.05$) between age and mean gonial angle were recorded for both males and females. Value of the correlation coefficient was greater in females than in males.

However, the inter-age-group comparisons showed statistically insignificant differences for both males and females when the mean gonial angle values were compared separately for successive age groups, in both the sexes (Table 2).

Table 2 Comparison of the mean Gonial Angle (in $^\circ$) between different age groups (Mann-Whitney Tests).

Age groups compared			
Group No.	Age (years)	Males	Females
1 & 2	35-40 & 41-45	$p > 0.05$	$p > 0.05$
2 & 3	41-45 & 46-50	$p > 0.05$	$p > 0.05$
3 & 4	46-50 & 51-55	$p > 0.05$	$p > 0.05$
4 & 5	51-55 & 56-60	$p > 0.05$	$p > 0.05$
5 & 6	56-60 & 61-65	$p > 0.05$	$p > 0.05$
1 & 6	35-40 & 61-65	$p > 0.05$	$p > 0.05$

Mean gonial angle values were found to be higher in females when compared to males in all age groups except for group 2 (41-45 years) where the female mean gonial angle value was less than that of males. However, all these differences were found to be statistically insignificant ($p > 0.05$) (Table 3).

Table 3 Age-wise comparison between male and female mean Gonial Angle (GA values (in $^\circ$)) (Mann-Whitney Tests).

Group	Age (years)	Mean Female GA ($^\circ$) \pm SD	Mean Male GA ($^\circ$) \pm SD	p-value
1	35 - 40	114.8 ± 8.341	113.4 ± 3.782	$p > 0.05$
2	41 - 45	115.0 ± 3.889	114.7 ± 5.227	$p > 0.05$
3	46 - 50	115.2 ± 5.696	117.9 ± 2.382	$p > 0.05$
4	51 - 55	118.7 ± 11.015	121.5 ± 6.171	$p > 0.05$
5	56 - 60	120.0 ± 8.047	122.0 ± 3.536	$p > 0.05$
6	61 - 65	122.3 ± 8.722	122.6 ± 5.413	$p > 0.05$

Even though the overall female mean GA value was greater than its male counterpart (Fig. 4), this difference was calculated as statistically insignificant ($p > 0.05$) by the unpaired T- test. Hence, no sexual dimorphism was observed.

DISCUSSION

The observations made in the present study implied that a strong association existed between gonial angle size and age, but the effect of sex on the gonial angle was relatively limited.

A significant positive correlation was observed between gonial angle and age in both sexes in our study, same as that observed by Ohm and Silness (9) in Norwegian population. A trend of increase in mean gonial angle values with increasing age was seen in both sexes, similar to that seen by Ohm and Silness (9) and Shamout et al. (10) in Jordanian population.

The explanation for this trend is the combined effect of senile alterations in the morphology of the basal bone of mandible and the reduced density and activity of masticatory muscles due to aging. With advance in age, the mandibular process and reinforcing bone are weakened by the osteoclastic activity which results in the formation of a silhouette, very similar to the process of development and growth of the mandibular condyle seen in the initial years of life. Due to this remodeling, the angle again opens out as in childhood. Also, the decreasing contractile power of masticatory muscles inserting in the mandibular angle region (masseter and medial pterygoid) has a widening effect on the gonial angle.

The male femean gonial angle values of present study were similar to the ones recorded by Xie and Ainamo (11) in Finnish population. Whereas, higher values have been observed by Shamout et al. (10) and Ohm and Silness (9). Male mean gonial angle values of our study were closest to those recorded by Xie and Ainamo (11) but were lower than those presented by Shamout et al. (10) and Ohm and Silness (9).

These variations in the values of gonial angle reported by different authors are attributed mainly to the racial or ethnic differences which exist in various populations of the world for any morphometric measurement. Besides that, the other factors which are responsible

are the nonuniformity of the sample size used independent studies, different morphometric techniques used and genetically acquired racial differences predisposing to biomechanical and physiological variations existing among different groups of people.

Taking into consideration the limitations of sample size and the non-availability of information such as tooth to tooth contact and chewing habits, the present study suggests a multifactorial approach for future research. All these factors which can possibly contribute to variations in the mandibular morphology should be explored further to firmly establish the effect of age and gender related changes of bone tissue resulting in altered mandibular basal bone morphological characters.

CONCLUSION

The results of the present study concluded that age had a significant influence on the gonial angle but sex affected the gonial angle only to a certain extent. Gonial angle size presented a significant positive correlation with age in both the sexes, but sexual dimorphism was not observed.

Conflict of interest: none declared.

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Monitoring of CD molecules specific expression in HIV positive patients

Praćenje specifične ekspresije CD molekula kod HIV pozitivnih pacijenata

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ABSTRACT

Objective: to determine correlation between CD16+56+ NK cells count and CD4+/CD8+ ratio. Natural killer (NK) cells with their typical phenotype CD3-CD56+CD16+ are significant part of the innate immunity which plays an important role in the prevention of HIV infection and AIDS progression. Materials and methods: in this investigation, we analyzed 40 selected samples of HIV positive patients under the therapy by flow cytometry. Flow cytometric immunophenotyping is one of the most important tools for diagnosing, staging and therapy monitoring of HIV positive patients. It is possible to determine the changes, relations and aberrations of B lymphocytes, T lymphocytes and NK cells by conducting specific flow cytometric analyses. Results: NK cells count has positive correlation with CD4+ T cells count and negative correlation with CD8+ T cell count. Numerous opportunistic infections occur when CD4+ T cell number is less than 200 cells/mm³. From the total of 40 specimens 28 showed significantly lower CD4+ T cells count, and 17 samples showed significantly higher count of CD8+ T cells. Calculated CD4+/CD8+ ratio was under referral values in over 72% of total specimens. Majority of serum specimens (87.5%) showed normal count of CD16+56+ NK cells which means that these cells compensate the loss of CD4+ cell function. Conclusion: HIV virus infects and destroys CD4+ T and NK cells. NK cells kill virally infected cells and in some cases compensate for CD4+ T cells function. This type of research has multiple usage in terms of therapy, monitoring efficiency and selecting patients for re-treatment.

Key words: flow cytometry, immunophenotyping, NK cells, CD4+/CD8+ ratio, HIV virus, antiretroviral therapy

SAŽETAK

Cilj: utvrđivanje korelacije između broja CD16+56+ NK ćelija i CD4+/CD8+ omjera. Urođeno ubilačke (NK) ćelije sa svojim tipičnim fenotipom CD3-CD56+CD16+ predstavljaju važan dio urođene imunosti koja ima značajnu ulogu u prevenciji HIV infekcija i njene progresije u AIDS. Materijali i metode: u ovom istraživanju analizirano je 40 odabranih uzoraka HIV pozitivnih pacijenata pod terapijom pomoću protočnog citometra. Imunofenotipizacija protočnim citometrom je jedna od najvažnijih tehnika za dijagnozu, prognozu i praćenje antiretroviralne terapije. Sprovedenjem specifičnih analiza pomoću protočnog citometra moguće je ustanoviti promjene, relacije i aberacije kod B limfocita, T limfocita i NK ćelija. Rezultati: broj NK ćelija je u pozitivnoj korelaciji sa brojem CD4+ T ćelija dok je u negativnoj korelaciji sa brojem CD8+ T ćelija. Kada broj CD4+ T ćelija padne ispod 200 ćelija/mm³ dolazi do brojnih oportunističkih infekcija kod pacijenata. Od ukupno 40 uzoraka, 28 njih je imalo značajno nizak broj CD4+ T ćelija, dok je 17 uzoraka pokazalo znatno visok broj CD8+ T ćelija. Izračunati omjer CD4+/CD8+ bio je ispod referentne vrijednosti kod više od 72% od ukupnih uzoraka. Većina uzoraka pokazala je normalne vrijednosti za CD16+56+ NK parametar što znači da ove ćelije donekle kompenziraju za gubitak funkcije CD4+ T ćelija. Zaključak: HIV virus inficira i uništava CD4+ T ćelije i NK ćelije. NK ćelije ubijaju druge virusom inficirane ćelije i u nekim slučajevima kompenziraju funkciju CD4+ T ćelija. Ovaj tip sprovedenog istraživanja ima brojne koristi u smislu praćenja efikasnosti terapije i selektiranja pacijenata za ponovnu antiretroviralnu terapiju.

Ključne riječi: protočna citometrija, NK ćelije, CD4+/CD8+ omjer, HIV virus, antiretroviralna terapija

INTRODUCTION

Numerous investigations have shown specific CD (cluster of differentiation) molecules expression of NK and T lymphocytes in serum specimens taken from HIV positive patients. Monitoring of

this expression can be successfully performed by flow cytometry, immunophenotyping for diagnostic and therapeutic purposes. NK cells, as the most important component of innate immunity, can recognize both foreign and own infected cells, without presence of antibodies or MHC molecules. NK cells activated only three days

after HIV infection. Otherwise, NK cells express specific CD16 (FcγRIII, the fragment crystallizable region, RIII), CD56 molecules and receptors for activation of an inhibition and their defense potential depend on signal balance transferred by these receptors. The NK cell activating receptors (Ly40, NCR, CD94, NKG2) can recognise different surface protein structures of cells exposed to stress, while the main inhibitor receptors are KIR (killer cell immunoglobulin-like receptor), CD94 and NKG2. The main phenotype of NK cells is CD3-CD16+CD56+. CD56+ is adhesive molecule responsible for target cells connection. There are two subpopulation of NK cells: CD3-CD56dim and CD3-CD56bright. CD3-CD56dim cytotoxic NK cells have lower CD56 molecules number and represent the largest NK cell subpopulation in peripheral blood of healthy individuals, while CD3-CD56bright have higher number of CD56 antigens and they are responsible for immunoregulatory functions. The expression of KIRs and CD57 molecules is most important feature of CD3-CD56dim cells. Also, CD3-CD56dim NK cells subpopulation have numerous granules with proteolytic enzymes for fast cytotoxic activity and high level expression of CD16 activating receptors. Immunoregulatory CD3-CD56bright NK cells subpopulation have lower CD16 expression and cytotoxicity (11-11).

The NK CD56dimCD16+ cells also express CD57 molecules. However, there are three subfraction of these cells, specifically: CD57-, CD57dim and CD57bright. In HIV positive patients the number of CD57bright cells is increasing in comparison to decrease of CD57dim cells (11-14).

Viremic HIV positive patients showed high frequency of dysfunctional CD16+/CD56- NK cell subpopulations and CD8+ T lymphocytes, but decreased CD4+ T cells. The molecular mechanisms for that are not clear yet. The main features of NK cells after HIV infection are: high activation status, differential expression of activation and inhibiting receptors, lower interaction level with dendritic cells, and finally decreased number of CD56dimCD16+ NK cells. The number of increasing CD57 molecules on CD56dimCD16+ NK surface cells is sign of NK cells final maturation and virus infection regarding the high cytotoxicity of these cells. There are also information about CD4+CD16+CD56+CD3- NK cells responsibility for the persistent HIV infections due to expression of HIV coreceptor molecules CCR5 i CXCR4 (15-19).

HIV positive patients show regular decrease of CD4+ and increase of CD8+ T lymphocytes. Total CD3+ number remains constant. Some investigations pointed to large importance of CD4+/CD8+ immunophenotypization parameter in both clinical and therapeutical sense. It can be reliable parameter of the disease progression and increased risk of the disease.

With regard to all above mentioned facts, we investigated the correlation between two very important immunophenotypization parameters in HIV positive patients: CD3-CD16+CD56+NK cells and CD4+/CD8+ ratio.

MATERIALS AND METHODS

Blood collection

Whole blood was collected into EDTA Vacutainer tubes and transported to the Flow Cytometry Laboratory of the Department of

Clinical Immunology of the Clinical Centre University of Sarajevo.

Flow cytometry

Flow cytometry is multiparametric analysis of morphological, biochemical and functional cell features with diameter range of 0,2-150 μm. The flow cytometry may determine the frequency of T lymphocytes (CD3+, CD4+, CD8+, CD4+/CD8+ ratio), B lymphocytes (CD19+), NK cells (CD16+CD56+), activated lymphocytes (CD8+CD38+) and absolute number of CD4+ T and CD8+ T lymphocytes (20-23).

Cell immunophenotyping was carried out by a standard method of sample preparation. After lyses of erythrocytes, the leukocytes of peripheral blood were analyzed for the expression of specific leukocyte markers using a panel of monoclonal antibodies and flow cytometry (flow cytometer - BD FACS Canto II). 10.000-50.000 events were recorded per tube and analyzed using the BD FACSDiva™ software. The best results are achieved if analysis of the cells on the flow cytometer is performed as soon as possible.

Monoclonal Antibodies

Combinations of surface markers that are determined by monoclonal antibody conjugated with FITC (i.e. fluorescein isothiocyanate), PE (i.e. phycoerythrin) and PerCP (i.e. Peridinin-chlorophyll-protein complex) or APC (i.e. alofokocianin)

RESULTS

Immune status of HIV positive patients under corresponding antiretroviral therapy must be monitored by CD4+ T cells counting. Decrease of this cell number under critical referral values is the sign that the antiretroviral therapy must be included or modified.

We analyzed 40 selected HIV positive patients under therapy. In this investigation special attention was given to the following immunophenotypization parameters: CD4+ T lymphocytes, CD4+/CD8+ ratio and CD16+/CD56+ NK cells. The absolute number of CD4+ and CD8+ T cells was calculated using the following formula:

$$CD4_{ap} = WBC \times \frac{\text{lymphocytes}}{100} \times \frac{CD4+}{100} \quad \text{and} \quad CD8_{ap} = WBC \times \frac{\text{Lymphocytes}}{100} \times \frac{CD8+}{100}$$

The obtained results were used for calculation of CD4+/CD8+ ratio for corresponding correlation analyses with CD16+56+ immunophenotypization parameter.

Table 1 Obtained results of immunophenotypization analyses for 40 samples.

	CD16+56+	Absolute number of CD4+ (CD4 ^{ap})	Absolute number of CD8+ (CD8 ^{ap})	CD4+/CD8+ ratio
Referral values	5.6 - 31%	0.6 - 1.6 x 10 ⁹ /l	0.3 - 0.9 x 10 ⁹ /l	0.9-3.6
Sample 1	22.8	0.53	1.19	0.45
Sample 2	12.3	0.83	0.96	0.86
Sample 3	10.9	0.35	1.19	0.29
Sample 4	6.7	0.47	0.56	0.84
Sample 5	4.0	1.00	0.81	1.23
Sample 6	11.0	0.36	0.34	1.06
Sample 7	6.7	0.47	0.56	0.84
Sample 8	10.0	0.65	0.60	1.08
Sample 9	16.8	0.54	0.55	0.98
Sample 10	10.5	0.14	1.15	0.12
Sample 11	22.5	0.35	0.82	0.43
Sample 12	8.7	0.51	0.95	0.54
Sample 13	10.5	0.13	0.82	0.16

Sample 14	19.9	0.77	1.01	0.76
Sample 15	18.2	0.98	0.85	1.15
Sample 16	19.7	0.20	0.44	0.45
Sample 17	5.3	1.05	1.96	0.54
Sample 18	20.3	0.27	0.67	0.40
Sample 19	7.3	0.35	1.32	0.27
Sample 20	30.0	0.71	0.68	1.04
Sample 21	14.9	0.28	0.86	0.33
Sample 22	5.0	0.45	0.97	0.46
Sample 23	24.3	0.49	1.86	0.26
Sample 24	10.5	0.64	0.63	1.02
Sample 25	8.0	0.22	0.84	0.26
Sample 26	24.8	0.21	0.27	0.78
Sample 27	13.0	0.48	0.43	1.12
Sample 28	13.8	0.83	0.51	1.62
Sample 29	6.7	0.48	1.17	0.41
Sample 30	19.6	0.74	0.73	1.01
Sample 31	9.2	0.46	0.66	0.70
Sample 32	5.4	0.44	1.66	0.26
Sample 33	19.7	0.17	0.71	0.24
Sample 34	9.4	0.58	1.29	0.45
Sample 35	10.3	0.39	1.88	0.21
Sample 36	19.4	0.50	1.93	0.26
Sample 37	9.8	0.72	0.70	1.03
Sample 38	5.9	0.69	1.48	0.47
Sample 39	16.0	0.55	0.65	0.85
Sample 40	2.5	0.32	0.98	0.41

Out of the total of 40 specimens, 28 (70%) showed significantly lower CD4+ T cells count. Significantly higher count of CD8+ T cells was recorded in 17 samples (42.5%). Calculated ratio CD4+/CD8+ was under referral values in more than 72% of the total specimens (Table 1). However, the majority of serum specimens (87.5%) showed normal count of CD16+56+ NK cells. NK cells count was in positive correlation with CD4+ T cells number, followed by decrease in HIV-RNA count (viral load). HIV infection caused CD4+ T cells and NK cells destruction. Seven samples (samples 8,15,20,24,28,30,37) or 17.5% of the investigated population showed normal values for all observed parameters (Figure 1). It was a significant percentage meaning that antiretroviral therapy in those patients showed satisfying degree of efficacy. Accordingly, those patients were excluded from further analysis for a certain period of time. High absolute number and percentage of CD4+ and CD16+56+ cells were associated with slower HIV disease progression. Three samples (samples 22,32,40) showed abnormally lower values for all observed parameters, especially for CD16+56+ and CD4+/CD8+ ratio (Figure 2). That was the sign that those patients had to be retreated with intensive antiretroviral therapy.

CD16+/CD56- NK cell subpopulation was not found in our samples which means that there was no determined viremic patients, given the good response on the antiretroviral therapy. NKT cell subpopulations were analyzed.

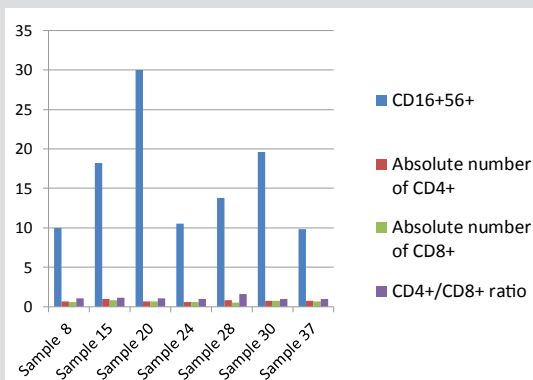


Figure 1 Seven samples with normal values for all observed parameters.

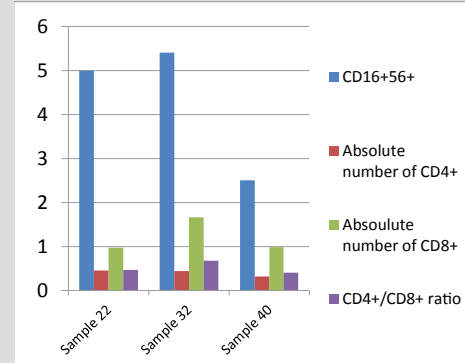


Figure 2 Three samples with abnormal values for all observed parameters.

Ten samples showed abnormal values for all observed parameters, except NK cell count (Figure 3). That means that NK cells still compensated for CD4+ T cells loss, but in limited time. Accordingly, those patients had to be treated with more aggressive or modified antiretroviral therapy.

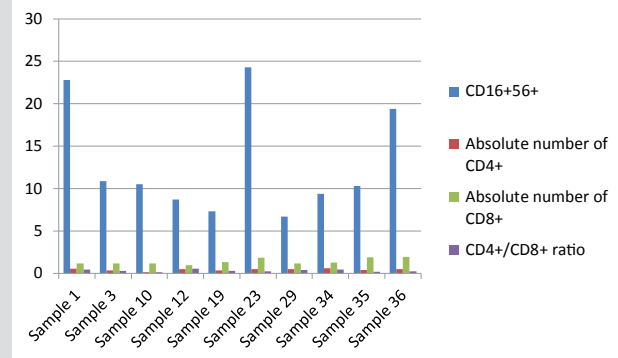
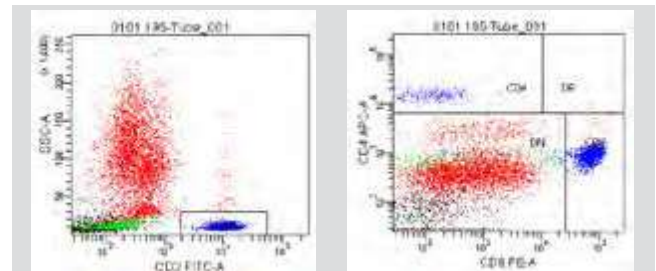


Figure 3 Ten samples with abnormal values for all observed parameters except NK cell count.

Flow cytometry is a technology which enables measuring and analysis of multiple physical characteristics of single cells including cell's size, internal complexity, granularity and fluorescence intensity. Given that our samples contained leucocytes, we had to restrict our analyses only to the lymphocyte by gating this subpopulation. A gate is graphical boundary used to select cells for further analyses. The main parameters in flow cytometry are FSC (forward scatter) and SSC (side scatter). Forward scatter is proportional to cell size while side scatter gives information about cell's inner complexity and granularity. By gating T lymphocytes subpopulation we can isolate CD4+ and CD8+ cells and get their correspondent presence by percentage (Figure 4). NK cells can be analyzed by the presence of CD16 and CD56 expression (Figure 5).



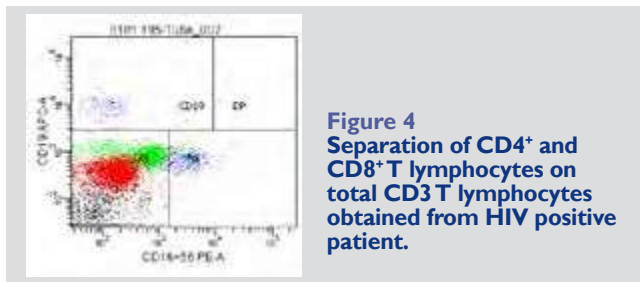


Figure 4
Separation of CD4⁺ and CD8⁺ T lymphocytes on total CD3⁺ T lymphocytes obtained from HIV positive patient.



Figure 5 Histogram representing CD16+56 and CD19 cells.

DISCUSSION

Natural killer (NK) cells large granular lymphocytes are important part of the innate immunity which plays an important role in prevention of HIV infection and AIDS progression. They account for up to 15% of peripheral blood lymphocytes and have ability to kill tumor and virus infected cells. Common NK cell phenotype is CD3-CD56⁺CD16⁺. HIV infection causes the number of NK cells decrease and their alterations as well. Beside NK cells, so called natural killer T cells (NKT) are determined within the T cell population. Their main feature is TCR (T cell receptor) molecules expression. Number of NKT cells in HIV positive patient remains constant (1-4).

NK cells can be divided into two subpopulations based on their functional and structural characteristics: cytotoxic CD3-CD56dim and immunoregulatory CD3-CD56bright subpopulation. CD56dim NK cells express high levels of FcγRIII (CD16), killer cell immunoglobulin-like receptors (KIR) and perforin which makes them effective mediators of natural cytotoxicity and antibody-dependent cellular cytotoxicity (ADCC). This population secretes cytokines at low levels. In contrast, CD56bright NK cells perform poor cytotoxicity and have important immunoregulatory role (11,14,15).

HIV infection is associated with CD56dimCD16⁺ NK cells decreasing and dysfunctional CD56-CD16⁺ NK cells increasing. Often, during HIV infection, NK cells can compensate CD4⁺ T cells function, given that these cells are the main target of HIV virus. The level of CD4⁺ T lymphocytes has prognostic value for AIDS prediction and development. Reduction of CD4⁺ T cell percentage and their absolute number, as well as decrease in CD4⁺/CD8⁺ ratio are the main characteristics of HIV infection and the basis for efficiency of therapy monitoring. It is recommended to monitor the level of these cells in HIV positive patients every 3 to 6 months (17,18,19).

Howsoever, the level of these cells depends on various factors such as hormones, environment, and temperature. During the day CD4⁺ T cells number is increasing. NK cell count remains constant throughout the day. This is very important to know when taking the

blood samples from patients for flow cytometry analysis (20,25).

During the progression of HIV infection a number of reduction of CD4⁺ T cells and increase of CD8⁺ T cells occur, while the total CD3 cells number remain constant. There is also decrease in CD4⁺/CD8⁺ ratio and reduction in CD56⁺16⁺ NK cells (26). Based on all these facts, it is important to examine all mentioned immunophenotypization parameters in HIV positive patients.

This study investigated the correlation between CD16+56⁺ NK cells count and CD4⁺/CD8⁺ ratio. In order to calculate CD4⁺/CD8⁺ ratio, we must determine CD4⁺ and CD8⁺ T lymphocyte percentage.

Based on the obtained results and usage of appropriate formula, we calculated absolute number of these parameters. Besides, we analyzed number changes of CD19 (B lymphocyte) and total lymphocyte (CD3).

Referral values of observed immunophenotypization parameters expressed in percentage are as follows:

- CD3⁺ 59-85%
- CD4⁺ 30-59%
- CD8⁺ 11-38%
- CD19⁺ 5-20%
- CD16⁺/CD56⁺ 5.5-31%
- CD4⁺/CD8⁺ ratio 0.9-3.6%
- CD4⁺ab 0.6 - 1.6 × 10⁹/l
- CD8⁺ab 0.3 - 0.9 × 10⁹/l

Out of the total of 40 analyzed specimens, only 3 showed abnormally lower values of all investigated immunophenotypization parameters, especially CD16+56 and CD4⁺/CD8⁺ ratio (22,32,40). In these cases there decreased number of cytotoxic CD4⁺ T lymphocytes and NK cells was established. It means that NK cells were not able to compensate the loss of CD4⁺ T lymphocytes or had been infected with HIV virus which started to destroy them.

In these two cases patients had to be urgently retreated with more intensive antiretroviral therapy. Using this approach of immunophenotypization parameters monitoring, it is possible to detect 4-5% of critical cases demanding therapeutical intervention. These results confirmed the main goals reached in the study.

Seven samples (samples 8,15,20,24,28,30 and 37) or 17.5% of the investigated population showed normal values for all investigated parameters. This significantly determined percentage confirms the effectiveness of corresponding antiretroviral therapy. Accordingly, those patients were excluded from further analysis for a certain period of time, after which they would require retesting. On the other hand, ten samples (samples 1,3,10,12,19,23,29,34,35 and 36) or 25% of them, had abnormal values of investigated parameters, except CD16+56⁺. Loss of these cells can cause development of opportunistic infections in the late stages of the disease. In order to prevent that, those patients were recommended to repeat antiretroviral therapy, especially those with CD16+56⁺ percentage near to lower reference value.

CONCLUSION

From all above mentioned results, we can derive general conclusion that this type of research have multiple usage in terms of efficiency of

the therapy monitoring. In this way, it is possible to select patients for re-treatment giving them a chance for longer and more quality life. Future immunophenotypization researches should include subpopulation of NKT cells since it is well known that CD3+/CD16+/CD56+ NKT cells counts increase in HIV positive patients which could be a reliable viremic marker in those patients.

Conflict of interest: none declared.

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International classification of functioning, disability and health (ICF) as a possible predictor of programme evaluation and outcome of treatment in rehabilitation of patients with stroke

Međunarodna klasifikacija funkcionisanja, onesposobljenja i zdravlja (ICF) kao mogući prediktor procjene programa i ishoda liječenja u rehabilitaciji kod pacijenata sa moždanim udarom

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ABSTRACT

Introduction: the International Classification of Functioning, Disability and Health (ICF) is a reference for the measurement of health and disability developed to ensure an internationally accepted and empirically established list of health related factors. Although in 2001 Bosnia and Herzegovina accepted the implementation of the ICF classification, the practical implementation has not been fully operational. The aim of this study is to compare all four components contained in the ICF classification („Body Function and Structure“, „Activity and Participation“, „Environment Factors“ and „Personal Factors“) with areas of evaluation and specific elements appearing in selected standardized estimates in rehabilitation (Barthel index, Functional Independence Measure). **Methods and patients:** this research is a clinical, prospective study. The sample is created out of 60 patients with stroke, hospitalized at the Clinic of Physical Medicine and Rehabilitation of the Clinical Centre University of Sarajevo (CCUS). **Results:** out of the total number, 32 were male and 28 female patients. The average Barthel (BI) value at admission was 13.16 ± 5.43 , and 16.01 ± 0.55 at discharge. The Chi-squared test showed significant difference compared to the Barthel Index at admission and discharge, $p=0.001$. Using the Paired t-test, a significant statistical difference in values of functional independence measure was established. The Pearson's correlation showed that values of Barthel index and Functional Independence Measure at discharge were lower in older respondents ($p=0.001$). **Conclusion:** International Classification of Functioning, Disability and Health implicitly support program evaluation and outcome of treatments in rehabilitation of patients with stroke.

Key words: ICF, stroke, activity, participation, rehabilitation

SAŽETAK

Uvod: međunarodna klasifikacija funkcionisanja, onesposobljenja i zdravlja (ICF) okvir je za mjerenje zdravlja i onesposobljenja koji je razvijen kako bi se osigurala međunarodno prihvaćena i empirijski utemeljena lista faktora povezanih sa zdravljem. Iako je Bosna i Hercegovina već 2001. godine prihvatila primjenu ICF klasifikacije, praktična primjena još nije u potpunosti zaživjela. Cilj ovog rada je usporediti sve četiri komponente sadržane u Međunarodnoj klasifikaciji funkcionisanja, onesposobljenja i zdravlja („Tjelesne funkcije“, „Tjelesne strukture“, „Aktivnosti i učestvovanje“, „Faktori okoline“) s područjima procjene i specifičnim elementima koji se pojavljuju u odabranim standardiziranim procjenama u rehabilitaciji (Barthel index, Funkcionalni test nezavisnosti). **Materijali i metode:** ovo istraživanje je klinička, prospektivna studija. Uzorak istraživanja je formiran od 60 ispitanika sa moždanim udarom, hospitaliziranih na Klinici za fizikalnu medicinu i rehabilitaciju Kliničkog centra Univerziteta u Sarajevu (KCUS). **Rezultati:** od ukupnog broja ispitanika, 32 je bilo muškog, a 28 ženskog spola. Prosječna vrijednost Barthel indexa na prijemu je iznosila 13.16 ± 5.43 , a na otpustu 16.01 ± 0.55 . Hi kvadrat test je pokazao značajnu razliku u odnosu na indeks ocjena po BI na prijemu i otpustu, $p=0.001$. Primjenom uparenog t-testa ustanovljena je statistički značajna razlika u vrijednostima Funkcionalnog testa nezavisnosti. Pearsonova korelacija je pokazala da su vrijednosti Barthel indexa i Funkcionalnog testa nezavisnosti na otpustu bile manje ukoliko je ispitanik bio stariji ($p=0.001$). **Zaključak:** Međunarodna klasifikacija funkcionisanja, onesposobljenja i zdravlja implicitno podržava program procjene i ishoda liječenja u rehabilitaciji.

Cljučne riječi: ICF, moždani udar, aktivnost, učestvovanje, rehabilitacija

INTRODUCTION

The International Classification of Functioning, Disability and Health (ICF) relates to classification of human functioning and disability. ICF is revised International Classification of Impairments, Disabilities and Handicaps (ICIDH), originally published by the World Health Organization for the purpose of study, 1980 (1). Created after systematic field studies and international consultations, it was accepted for international use on May 22, 2001 by the 54th World Health Assembly (WHA 54.21) (1).

The ICF is a reference for the measurement of health and disability developed to ensure an internationally accepted and empirically established list of health related factors (2). In rehabilitation, the emphasis is on the participation of special value to the ICF, activity, and on their connection to health. It ensures a standard language and framework in health and health related domains. The ICF is a multipurpose classification with conceptual bases for defining, measuring and formulating health and disability (1). From the physiatrist point of view, attention is directed towards the connection between health and occupation which is one of the highest values of the ICF (3). The activity component and participation in activity, which is new in health classification, has central position in working and therapeutic philosophy (4). Some authors believe that through ICF one better understands the effect of illness or disability on the individual and his/hers integration in real life, which also ensures the designing therapeutic strategy towards what is going to be a real problem to the individual during his/her integration into society. Although in 2001 Bosnia and Herzegovina signed the implementation of the ICF classification, the practical implementation has not been fully operational.

The ICIDH organizes information in two parts. Part 1: Functioning and Disability comprises two components, „Body Functions and Structures“ and „Activities and Participation“. Parts 2: consists of „Environment Factors“ and „Personal Factors“. Each component can be expressed by positive and negative terminology (1,2).

Parts	Function and disability			Contextual factors	
Components	b - Body functions	s - Body structures	d - Activity and participation	e - Environmental factors	Personal factors
Field	8	8	9	5	
Category*	in total 1424				
Qualification	Change of function	Change of structure	capacity performance	concessions barriers	
Quantifier	1	3	2	1	

* The basic unit of classification is a category

Figure 1 ICF Structure.

MATERIALS AND METHODS

The aim of this work is to compare all four components contained in the ICF with areas of evaluation and specific elements appearing in selected standardized estimates in rehabilitation (Barthel index, Functional Independence Measure, Visual analogue scale, Oswestry Low Back Disability Questionnaire, Back Depression Inventory). This research is a clinical, prospective study. The research sample is created out of 60 patients with stroke, hospitalized at the Clinic of Physical Medicine and Rehabilitation of the CCUS.

RESULTS

Table 1 Age of patients (n=60).

	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
Male	32	68.81	9.01	1.59	48.00	83.00
Female	28	66.53	11.91	2.25	35.00	84.00
Total	60	67.75	10.43	1.34	35.00	84.00

F=0.707; p=0.404

Out of the total number of respondents, 32 were male and 28 female. The average age was 67.75 ± 10.43 years (35-84) (Table 1.)

Table 2 Average values Barthel index at admission and discharge (n=60).

		Mean	N	Std. Deviation	SEM
Barthel index	admission	13.16	60	5.43	0.70
	release	16.01	60	4.28	0.55
Mean		Std. Deviation		SEM	
-2.85		2.13		0.27	
t		Df		p	
-10.365		59		0.001	

The average Barthel (BI) value at admission was 13.16 ± 5.43 , and 16.01 ± 0.55 at discharge (Table 2).

Table 3 Index rating by Barthel index (n=60).

		Admission		Discharge	
		No	%	No	%
Barthel index	Complete dependence	5	8.3	0	0
	Heavy dependence	17	28.3	13	21.7
	Moderate dependence	25	41.7	24	40.0
	Small dependence	4	6.7	7	11.7
	Independence	9	15.0	16	26.7
	Total	60	100.0	60	100.0

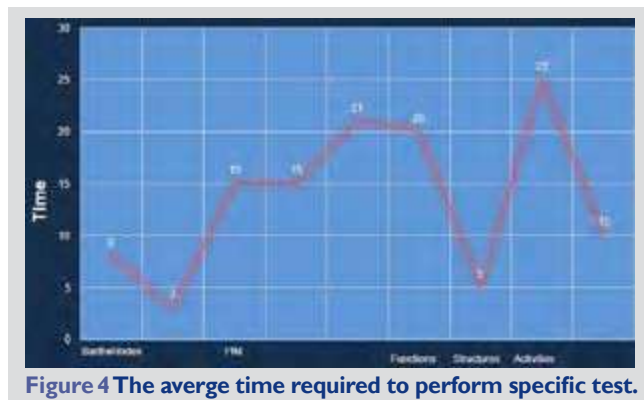
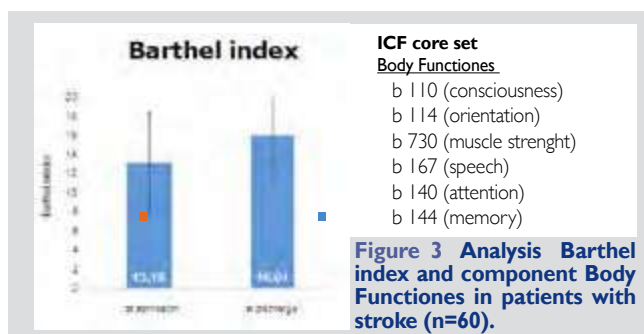
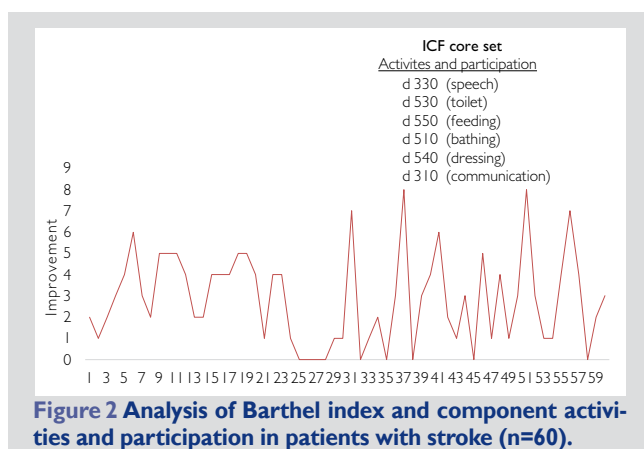
$\chi^2=81.764$; p=0.001

The Chi-squared test showed significant difference compared to the Barthel Index at admission and discharge, $p=0.001$. Using the Paired t-test, a significant statistical difference in values of functional independence measure was established. The Pearson's correlation showed that values of Barthel index and Functional Independence Measure at discharge were lower in older respondents ($p=0.001$).

Table 4 Barthel index values in muscle weakness at admission and discharge.

BI	Muscle strenght	N	Mean	SD	SEM	Minimum	Maximum
A.	Weakness	37	16.94	2.26	0.37	14.00	20.00
	Paralysis	23	7.08	2.84	0.59	4.00	14.00
		F=221.055; p=0.001					
R.	Weakness	37	18.83	1.21	0.19	17.00	20.00
	Paralysis	23	11.47	3.43	0.71	5.00	17.00
		F=142.460; p=0.001					

The average value of Barthel index in respondents with muscle weakness at admission was 16.94 ± 2.26 , and 18.83 ± 1.21 at discharge. In respondents with paralysis at admission BI was 7.09 ± 2.84 , and 11.47 at discharge. Accordingly, a significant difference in BI values was established in relation to type of muscle weakness at admission and at discharge ($p<0.05$) (Table 4).



The average time required for the analysis of the components contained in the ICF was 60 minutes. For component "Body functions and structures" and "Activity and participation" an average of 25 minutes was required, whereas the analysis of the environmental factors components took 10 minutes. Analysis of specific elements appearing in the standard estimates in rehabilitation showed that average time for Barthel index was 8 minutes, and for Functional Independence Test 5 minutes (Figure 4).

DISCUSSION

The ICF as a new classification in the professional literature of the last decade is recognized as a need. The aim of this study was to compare the components of "Body Function and Structure", "Activity and Participation", "Environment Factors" and "Personal Factors" as components contained in the ICF from the areas of assessment and specific elements appearing in the selected standardized assessments

in rehabilitation (Barthel index, Functional Independence Measure). Based on the literature, PubMed data base research and other Internet sources a significant number of studies based on new classification has been published so far. Unfortunately, not a single study was found which compared the ICF elements with areas of assessment and specific elements appearing in the selected standardized assessments in rehabilitation. Research review showed the benefit of the ICF application for the purpose of more clear establishment of the rehabilitation team role (5, 6). If used in practice, it can encourage standardization of therapeutic assessment which results were clear and intelligible team members, as well as the quality of planning therapeutic interventions, and monitoring changes over time (7). In addition, it is useful in stimulating cooperation between health professionals in planning intervention objectives and in expected outcomes (8). Thanks to ICF, the rehabilitation team has complete insight into all aspects of promotion of subjects and thus can adequately carry out further evaluation of programs and outcomes of treatment in rehabilitation (9, 10). Results of this study are consistent with the above mentioned studies, and they show all advantages and disadvantages of new classification in the practice. Gender structure of the sample in the study shows that there was slightly higher number of male patients (68.8%). These results are in accordance with the expected incidence of developing the above stated disorders (11). The average age of patients with stroke was 67.75 ± 10.43 years (35-84). The analysis of the male (68.81 ± 9.01) and female (66.53 ± 11.91) age of patients from the same group, using ANOVA test, showed that there was no statistically significant difference, $F = 0.707$; $p = 0.404$. The paired t-test showed a statistically significant difference in the values of Barthel index. The average value of the Barthel index at admission was 13.16 ± 5.43 and 16.01 ± 0.55 at discharge. The average difference at admission and discharge was 2.85 ± 2.13 ($t = -10.365$; $df = 59$; $p = 0.001$). The paired t-test showed a statistically significant difference in the Functional test of independence. The average value of the Functional test of independence at admission was 73.25 ± 34.31 , and 96.20 ± 31.01 at discharge. The difference in the average admission and discharge value was 22.95 ± 14.54 ($t = -12.219$; $df = 59$; $p = 0.001$).

This research allowed us to have insight into the time required to perform the evaluation as well as time required for the specific elements appearing in the selected standardized assessments in rehabilitation (Barthel index, Functional test of independence). The study showed that analysis of the components contained in the ICF takes five times longer than the analysis and evaluation of specific elements appearing in the standard estimates of the rehabilitation.

The benefits of the International Classification of Functioning, Disability and Health are significant, which was pointed out by many authors in their research. International Classification of Functioning, Disability and Health - ICF represents biopsychosocial model classifications developed with the aim of more efficient and more complete insight into the problem of health and functioning of the individual in the external environment, and the data obtained by algorithm method can significantly contribute to better overcome the above stated problems in patients with stroke.

CONCLUSION

The International Classification of Functioning, Disability and Health implicitly support program evaluation and outcome of

treatments in rehabilitation of patients with stroke.

Conflict of interest: none declared.

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NIHSS score predicts a post-stroke depression severity

NIHSS skor predviđa težinu depresije nastale nakon moždanog udara

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ABSTRACT

Introduction: post-stroke depression (PSD) is a very common disorder following acute cerebral stroke (ACS). The incidence of PSD is between 6% to 61% patients after ACS. PSD is associated with impaired recovery in cognitive function and activities of daily living and increases mortality. The mechanism of PSD is not well known. In this study we have compared occurrence of PSD after ACS regarding the discharge patient's status. **Materials and Methods:** this was a single-center prospective observational clinical cohort study conducted from February 2015 to August 2015. Inclusion criteria were: patients 18 to 75 years old and ACS diagnosed with CT or MRI. Exclusion criteria were as follows: history of psychiatric diseases and uncertain diagnosis of ACS. We correlated the discharge National Institutes of Health Stroke Scale score (NIHSS) of patients with Beck Depression Inventory (BDI) score a month after discharge. **Results:** the total of 70 patients, 30 women and 40 men, were included in the study. The mean age of patients was 59.21 ± 10.76 years. Using BDI we found that more than 70% of patients had moderate or severe post-stroke depression. We found a positive statistical correlation between NIHSS score at the discharge and BDI, $r = 0.425$, this correlation was statistical significant, $p < 0.01$. **Conclusion:** one of the predictive parameters of possible PSD after acute stroke is a NIHSS score at discharge, where patients with higher NIHSS had more severe PSD.

Key words: acute cerebral stroke, post-stroke depression, NIHSS, BDI

SAŽETAK

Uvod: depresija nastala nakon moždanog udara je veoma česta. Njena incidence iznosi od 6% do 61%. Ona je povezana sa otežanim oporavkom svakodnevnih kognitivnih funkcija i sa povećanom smrtnosti. Tačan mehanizam njenog nastanka nije poznat. U ovoj studiji mi smo uporedili natanak depresije nakon moždanog udara sa kliničkim stanjem pacijenata na otpustu. **Materijali i metode:** ovo je bila studija sprovedena u jednom centru, dizajnira kao prospektivna observacionalna klinička kohorta. Studija je sprovedena od februara do avgusta 2015. godine. Kriteriji uključivanja su bili: pacijenti su morali biti stari između 18 i 75 godina i sa dijagnosticiranim moždanim udarom sa CT ili MRI tehnikom. Kriteriji isključivanja su bili: historija psihijatrijskih poremećaja i nesigurna dijagnoza moždanog udara. Usporedili smo National Institutes of Health Stroke Scale score (NIHSS) vrijednost skora na otpustu sa Beck Depression Inventory (BDI) skorom mjesec dana nakon otpusa. **Rezultati:** ukupno je bilo 70 pacijenata, 30 ženskih i 40 muških pacijenata. Prosječna dob pacijenata je bila 59.21 ± 10.76 godina. Koristivši BDI pronašli smo da više od 70% pacijenata ima težu ili tešku depresiju nakon moždanog udara. Pronašli smo pozitivnu korelaciju između NIHSS skora na otpustu i BDI skora, $r = 0.425$, koja je bila statistički signifikantna, $p < 0.01$. **Zaključak:** jedan od prediktivnih parametara mogućeg nastanka depresije nakon moždanog udara je NIHSS skor na otpustu, gdje su pacijenti sa višim NIHSS skorom imali teže oblike depresije.

Ključne riječi: akutni moždani udar, depresija nakon moždanog udara, NIHSS, BDI

INTRODUCTION

Acute cerebral stroke (ACS) is the third leading cause of death in developed countries, after myocardial infarction and cancer, and second leading cause of death in global world (1). Acute stroke depression is one of the most frequent neuropsychiatric disorders, being present in 6%–52% of acute stroke patients. Depression is one of the strongest predictors of quality of life in stroke survivors; it persists in 34% of elderly patients after 20 months of ACS (2). The post-stroke depression (PSD) can start in early after stroke time (week) or later after months or years. In meta-analysis of Hackett ML et al., community, hospital and rehabilitation based studies found that 33% of stroke survivors experience depression at some point

during the acute, medium and long-term phases of recovery. After ACS, PSD is associated with impaired recovery in cognitive function and activities of daily living and increases mortality (3). Furthermore PSD is associated with increased disability, increased cognitive impairment, increased mortality, and increased risk of falls and with worse rehabilitation outcome (4). It is known that antidepressants such as selective serotonin reuptake inhibitors improve stroke outcome, beyond they antidepressant function, they improve motor recovery (3). The neurobiological mechanism of PSD is not well known until yet. Many different factors including neurobiological, behavioral and social have been researched as factors related to all kinds of PSD, but there is no obvious mechanism of pathogenesis. The early diagnosis and treatment of PSD is challenging and ACS patients should be

routinely screened for depression. Tracking depressive symptom change should start in the hospital and after discharge (5).

This study shows our institutional experience with PSD. We compared occurrence of PSD after ACS regarding the discharge patient's status. The diagnosis of PSD was estimated using Beck Depression Inventory.

MATERIALS AND METHODS

This was a single-centre study conducted at the Clinic of Neurology of the Clinical Center University of Sarajevo in the period from February to August 2015. The study was designed as a prospective observational clinical cohort study. Patients admitted for ACS were included in the study. Inclusion criteria were: patients 18 to 75 years of age and ACS diagnosed with CT or MRI. Exclusion criteria were as follows: history of psychiatric diseases and uncertain diagnosis of ACS. The National Institutes of Health Stroke Scale (NIHSS) was used at the admission and discharge to objectively quantify the impairment caused by stroke. All patients were evaluated for PDS with Beck Depression Inventory (BDI) one month after discharge. BDI is a psychometric test for measuring the severity of depression; it has 21 questions related to how the patient felt in the last week. Each answer has a range of severity from 0 to 3 points. Total score is compared to a key to determine the depression's severity. The standard cut-off scores are as follows:

- 0-9: indicates minimal depression
- 10-18: indicates mild depression
- 19-29: indicates moderate depression
- 30-63: indicates severe depression (6).

The higher total score indicates more severe depression. We correlated the discharge NIHSS score and age of patients with BDI. The statistical analyses were performed with SPSS version 18 (SPSS Inc., Chicago, Illinois, USA). We used descriptive statistics, t-test, Chi-square, and Pearson correlation for data analysis. Probability value of < 0.05 was considered statistically significant.

RESULTS

The total of 70 patients, 30 women and 40 men, were included in the study. The mean age of patients was 59.21 ± 10.76 years, where most male and female were between 51 and 65 years of age, 48.6%. There was no statistical significant difference between male and female patients, $t=0.260$; $p=0.742$. The average hospitalization time was 25.11 ± 11.96 days, with no significant difference between male and female patients, $t=0.098$; $p=0.756$. Less than 30% of patients had an educational level below high school. Out of the total number of patients 45.7% were work active and 47.1% were married. A large number of patients had a ischemic stroke, specifically 90% of all patients (Table 1). The most common symptoms of stroke at the admission were headache and gait disturbance (Table 2). A hemiparesis was present at the admission in 57.1% of patients (Table 1), without significant statistical difference between male and female patients, $\chi^2=1.944$; $p=0.12$. At admission, 48.6% of patients had NIHSS score between 5 and 15, with no statistically significant difference between male and female patients, $\chi^2=3.176$; $p=0.365$ (Table 3). At the time

of discharge from our department 55.7% of patients had NIHSS score between 5 and 15, with no statistically significant difference between male and female patients, $\chi^2=1.13$; $p=0.769$ (Table 3). After the Beck Depression Inventory procedure it was established that more than 70% of patients had moderate or severe depression (Table 4). There was no statistically significant difference between male and female patients, $t=0.005$, $p=0.942$. Using Pearson's correlation we found that the age of patients and BDI were positively correlated, where elderly patients had higher BDI scores $r=0.043$, but this correlation was not statistically significant, $p>0.05$. We found a positive statistical correlation between NIHSS score at discharge and BDI, $r=0.425$, and this correlation was statistically significant, $p<0.01$.

Table 1 Stroke etiology and neurological signs of stroke.

		Sex		Total
		Male	Female	
Etiology	Hemorrhagic	N 5	2	7
		% 12.5	6.7	10
	Ischemic	N 35	28	63
		% 87.5	93.3	90
Total	N	40	30	70
	%	100	100	100
		Sex		Total
		Male	Female	
Sign	Hemiparesis	N 20	20	40
		% 50	66.7	57.1
	Hemiplegia	N 20	10	30
		% 50	33.3	42.9
Total	N	40	30	70
	%	100	100	100

Table 2 Symptoms at admission.

		Sex		Total
		Male	Female	
Headache	N	37	27	64
	%	92.5	90	91.4
Seizures	N	17	7	24
	%	42.5	23.3	34.3
Sensibility disturbance	N	28	16	44
	%	70	53.3	62.9
Visual disturbance	N	22	16	38
	%	55.5	53.3	54.3
Speech disorder	N	24	14	38
	%	60	46.7	54.3
Gait disturbance	N	36	26	62
	%	90	86.7	88.6
Incontinence	N	14	6	20
	%	35	20	28.3

Table 3 NIHSS score at admission and discharge.

		Sex		Total
		Male	Female	
NIHSS at admission	1-4	N 5	6	11
		% 12.5	20	15.7
	5-15	N 22	12	34
		% 55	40	48.6
	16-20	N 8	10	18
		% 20	33.3	25.7
	21-42	N 5	2	7
		% 12.5	6.7	100
Total	N	40	30	70
	%	100	100	100
		Sex		Total
		Male	Female	
NIHSS at discharge	1-4	N 8	9	17
		% 20	30	24.3
	5-15	N 24	15	39
		% 60	50	55.7
	16-20	N 6	4	10
		% 15	13.3	14.3
	21-42	N 2	2	4
		% 5	6.7	5.7
Total	N	40	30	70
	%	100	100	100

Table 4 Beck Depression Inventory results.

			Sex		Total
			Male	Female	
BDI [®]	0-9	N	6	5	11
		%	15	16.7	14.7
	10-15	N	2	3	5
		%	5	10	7.1
	16-19	N	1	2	3
		%	2.5	6.7	4.3
	20-29	N	11	6	17
		%	27.5	20	24.3
	30-63	N	20	14	34
		%	50	46.7	48.6
	Total	N	40	30	70
		%	100	100	100
* Beck Depression Inventory					

* Beck Depression Inventory

DISCUSSION

This study showed that in cerebral stroke patients higher NIHSS at discharge predicts higher Beck Depression Inventory score, indicating a more severe post stroke depression.

PSD is a common neuropsychiatric disorder after cerebral stroke with incidence ranging from 18% to 61%, depending on patients' selection (7). In practice a very small number of patients is diagnosed with PSD and even fewer are treated, because some symptoms overlap due to stroke itself (3,4). Symptoms of PSD include: depressed mood, suicidal thoughts, vegetative disorders, apathy/loss of interest, anxiety, catastrophic reaction, increased emotionalism and anhedonia (3). Diagnosis of PSD is only objective when score systems are used. There are many score systems, but no one clearly stood apart from the others (8). Using BDI score system we established that more than 70% patients had moderate or severe PSD. This high rate of PSD occurred among young patients with mean age 59.21 ± 10.76 years. These young patients needed a special follow up given that PSD can slow their recovery from ACS and increase mortality (9). A systematic review of 71,131 patients has shown that PSD is highly prevalent in both sexes, but appears to be slightly more common among women than men (10). In our study we did not find statistically significant difference in the occurrence between male and female patients, which can be attributed to a small size sample. One of the most important steps in reducing the incidence of PSD and its effect to the recovery to the patient is the early follow up of suspected patients for developing PDS. One of the predictive parameters of developing PDS is a higher NIHSS score (11). NIHSS is a reliable and valid test which can predict long-term occurrence of PDS. Our study revealed that the discharge NIHSS score was positive correlated with BDI score. Patients with higher NIHSS score need a special care and follow up, and routine screened for PDS. The screening should be performed early and more common in younger female patients (9). Pharmacological prevention of PSD was researched and one meta-analysis summarized the effects of it. They showed that the likelihood of developing PSD was reduced among subjects receiving active pharmacologic treatment, especially following a 1-year treatment, and with the use of selective serotonin reuptake inhibitors (SSRI) (9). But this kind of prevention is not very commonly used because of many unsolved questions of mechanisms, short follow up and cost-benefit thereof. The best treatment choice for PSD is a pharmacological treatment and today

SSRIs are the recommended pharmacotherapy of PSD for their favorable tolerability profile (4).

Our study had some limitations given that it was conducted in a short time, the follow up of patients was short, it was a single center study, and we did not include patients with previous neuropsychiatric disease.

CONCLUSION

PSD is a common neuropsychiatric disease after ACS, which leads to worse rehabilitation outcome and increased mortality. All stroke patients should be routinely screened for depression given that early diagnosis and early treatment can lead to better rehabilitation outcome and mortality decrease. One of the parameters of possible PSD is a NIHSS score at discharge where patients with higher NIHSS have more severe depression. Another parameter is routine screening for PSD with BDI score. We need to conduct a long term study for PSD with BDI screening.

Conflict of interest: none declared.

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The significance of T2 3D Constructive Interference in Steady State (CISS) sequence use in magnetic resonance imaging (MRI) of lumbosacral spine

Značaj korištenja T2 3D Constructive Interference in Steady State (CISS) sekvence kod magnetno rezonantnog oslikavanja (MRI) lumbosakralne kičme

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ABSTRACT

Introduction: the aim of this study was to compare the quality of lumbosacral spine image using the standard T2 TSE sequences and T2 3D CISS sequences, to prove the significance of applying T2 3D CISS sequence which so far has not been used for the imaging of this segment of vertebral column. **Materials and methods:** the survey was conducted at Clinic of Radiology of the Clinical Center University of Sarajevo, as a prospective analytical comparative study, which included 80 patients of both sexes and different age structure. It was conducted in the period from January 2016 to November 2017. The study included patients referred for lumbosacral spine imaging. Following the imaging, reformatting of 3D CISS sequence into planes was done on the reconstructive station of the device, with slice thickness and spacing used in T2 TSE sequence. The obtained data were anonymized and as such sent to two radiologists who graded the quality of the obtained data. **Conclusion:** based on the qualitative analysis of relationship between IV disc and nerve roots within the intervertebral foramen and cauda equina we can say that T2 CISS sequence provides reliable results, and it should be used in routine lumbosacral spine imaging protocol.

Key words: lumbosacral spine, CISS, MRI, imaging

SAŽETAK

Uvod: cilj rada je komparirati kvalitet prikaza lumbosakralne kičme korištenjem standardnih T2 TSE sekvenci i T2 3D CISS sekvence, te dokazati važnost primjene T2 3D CISS sekvence, koja se do sad nije koristila za snimanje ovog segmenta kičmenog stuba. **Materijali i metode:** istraživanje je izvršeno na Klinici za radiologiju Kliničkog centra Univerziteta u Sarajevu, kao prospektivna analitička komparativna studija, koja je obuhvatila 80 pacijenata oba spola i različite starosne strukture i koja je provedena u periodu od januara 2016. do novembra 2017. godine. U studiju su uključeni pacijenti koji su imali uputnicu za snimanje lumbo-sakralne kičme. Nakon završenog snimanja na rekonstruktivnoj stanici aparata rađen je reformat 3D CISS sekvence u planove, debljine sloja i razmake koji su korišteni kod T2 TSE sekvenci. Svi dobijeni podaci su anonimizirani, te kao takvi poslani na ocjenu kod dva radiologa koja su ocjenjivala kvalitet dobijenih podataka. **Zaključak:** kvalitativnom analizom prikaza odnosa IV diska i nervnih korjenova unutar intervertebralnog foramena i kaude ekvine možemo reći da T2 CISS sekvenca daje dobre rezultate, te bi se trebala koristiti u rutinskom protokolu snimanja lumbosakralne kičme.

Ključne riječi: lumbosakralna kičma, CISS, MRI, oslikavanje

INTRODUCTION

New technologies in lumbosacral spine diagnostics have been rapidly developed, expecting to enhance the comprehension of pathophysiology of the disease and help us to allay the pain and uneasiness in patients (1). Magnetic resonance imaging (MRI) uses a great number of sequences, which are mostly two-dimensional (2D). Conventional 2D sequences require individual acquisitions for every plan of the imaging. Three-dimensional (3D) sequences are able to collect volumetric data sets that can be reformatted after the acquisition and thus show any required plan (2). Additionally, Constructive Interference in Steady State (CISS) sequences use strong T2 balanced three-dimensional gradient echo technique that generates high resolution isotropic images. Due to high

T2/T1 ratio water and fat have hyper signal. T2 three-dimensional Constructive Interference in Steady State (T2 3D CISS) sequences enables excellent contrast between the cerebrospinal fluid and other structures. Because of this CISS sequences have been very useful for the evaluation of the structures surrounded by cerebrospinal fluid. Magnetic resonance imaging (MRI), as a choice modality for the survey of suspicious intraspinal pathology, using T2 3D CISS sequences with high space resolution provides further improvement of the scanning quality (3). T2 3D CISS sequence has not been used so far in standard lumbosacral spine imaging protocol, but due to the relationship between the intervertebral disc and surrounding structures, with the emphasis on the relationship of the nerve roots within the intervertebral foramen, and given the described characteristics of this sequence in the

literature, we consider it can provide good results for the showing the relationship between the given structures that enter the composition of this vertebral column segment.

The aim of this study was to compare the quality of the lumbosacral spine display using standard T2 turbo spin echo (T2 TSE) sequences and T2 3D CISS sequences, to prove the importance of the appliance of T2 3D CISS sequence, not used so far for imaging of this segment of the vertebral column.

MATERIALS AND METHODS

The survey was conducted at Clinic of Radiology of the Clinical Center University of Sarajevo, as a prospective analytic comparative study, which included 80 patients of both sexes and different age structure. The study was performed in the period from January 2016 to November 2017. Patients referred for the lumbosacral spine imaging were included in the study. All patients underwent scanning in accordance with the following protocol:

- T2 tse sag: TR 3370 ms, TE 110 ms, FOV 320 mm, slice thickness 4 mm, slice number 11, distance factor 10%;
- T1 tse sag: TR 552 ms, TE 12 ms, FOV 320, slice thickness 4 mm, slice number 11, distance factor 10%;
- T2 tirm sag: TR 3200 ms, TE 78 ms, TI 160 ms, FOV 320 ms, slice thickness 4 mm, slice number 11, distance factor 10 %;
- T2 tse cor: TR 5610 ms, TE 180 ms, FOV 320 mm, slice thickness 4 mm, slice number 19, distance factor 10 %
- T2 tse tra msma (multi slice, multi angle): group slice number 3, slice number by the group 5, TR 3200-4000 ms, TE 104 ms, FOV 210, slice thickness 4 mm, distance factor 10%;
- T2 3D CISS sag: TR 1156 ms, TE 5,78 ms, FOV 280 mm, slice thickness 0,7 mm, distance factor 20 %.

Following the imaging, reformatting of 3D CISS sequence into planes was done on the reconstructive station of the device, with slice thickness and spacing used in T2 TSE sequence.

All obtained data were anonymized and as such sent to be graded by two radiologists who evaluated the quality of the obtained data with grades from 1-4 (1 - not visualised, 2 - badly visualised, 3 - visualised, 4 - perfectly visualised).

RESULTS

Table 1 Grading the quality of the relationship between the IV disc and nerve roots within the intervertebral foramina and cauda equine.

Level	Grade	T2 TSE	CISS
L3/L4	1- not visualized		
	2- badly visualised	2	3
	3- visualised	16	
	4- perfectly visualised	62	77
L4/L5	1- not visualized		
	2- badly visualised	2	1
	3- visualised	17	2
	4- perfectly visualised	61	77
L5/S1	1- not visualized		
	2- badly visualised	1	1
	3- visualised	20	2
	4- perfectly visualised	59	77

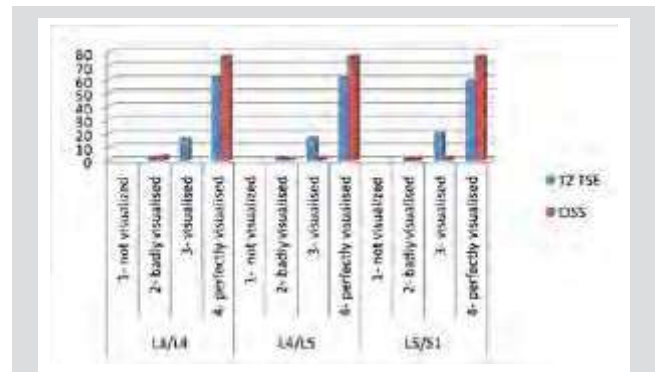


Figure 1 Comparative quality review of the relationship between the IV disc and nerve roots within the intervertebral foramina and cauda equine.

Table 2 Wilcoxon Signed Rank Test of the quality of relationship between the IV disc and nerve roots within the intervertebral foramina and cauda equine.

Wilcoxon Signed Ranks Test			
	L3/L4 CISS – L3/L4 T2 tse	L4/L5 CISS – L4/L5 T2 tse	L5/S1 CISS – L5/S1 T2 tse
Z	-3.500 ^b	-3.900 ^b	-4.243 ^b
Asymp. Sig. (2-tailed)	.000	.000	.000

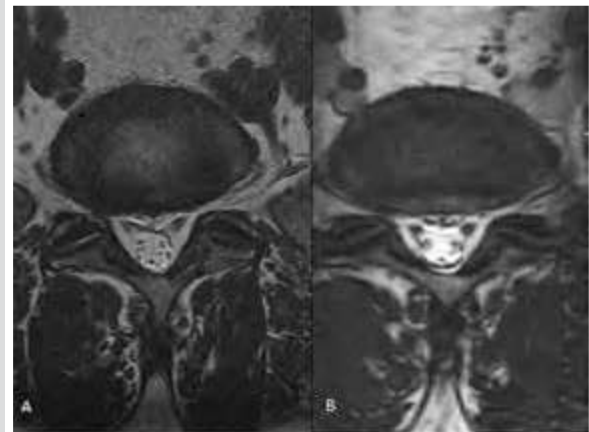


Figure 2 A T2 tse tra, B T2 CISS tra reconstructed in axial cross section.

DISCUSSION

More frequent occurrence of the pain in the lower part of the back, development of the chronicity and the great influence on the working ability imply the need for the improvement of the precise diagnostics system.

After the analysis of the results and the qualitative grading of the relationship between the intervertebral disc and nerve roots within the intervertebral foramina, and the relationship between the intervertebral disc and cauda equina we can see that grade 4 - perfectly visualised for levels L3/L4, L4/L5 and L5/S1 occurred in 77 cases for CISS sequence on all levels, while the T2 TSE sequence varies, for the level L3/L4 it occurred in 62 cases, L4/L5 in 61 case, and for the L5/S1 in 59 cases.

By Wilcoxon test statistically notable significance was confirmed on the level $p < 0,001$, which shows the significance of using T2

CISS sequence for the analysis of the afore mentioned segments of lumbosacral spine.

Owing to high space resolution, 3D CISS sequences demonstrate the continuity of cauda equina in lumbosacral area. Findings obtained in this way are accurate and show redundant nerve roots in cauda equina syndrome (4).

Using this sequence high space resolution can be achieved and T2 CISS sequence provides detailed information on anatomical structures. Confusing and subtle abnormalities of the vertebral column can be presented more clearly by using T2 CISS sequences (5,6).

MRI scanning with high resolution T2 CISS sequence can precisely determine disc herniation. This method of scanning ought to be used when the optimal preparation for the surgical operation is required. Neurosurgeons and radiologists both ought to be aware of the 3D high resolution CISS sequence potential in demanding cases (7).

CISS sequences with high imaging quality provide data necessary for determining the degree of lumbar disc hernia. Compared to routine MR scanning CISS sequence shows 89% sensitivity and 100% specificity for all disc hernia in lumbar spine, as well as 100% sensitivity and specificity for sequestered and extruded disc hernia (8).

CONCLUSION

By qualitative analysis of the relationship between the IV disc and nerve roots within the intervertebral foramen and cauda equina we can say that T2 CISS sequence provides good results and should be used in routine lumbosacral spine imaging protocol.

Conflict of interest: none declared.

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The most common reasons for hospitalization of haemato-oncological patients in pediatric intensive care unit

Najčešći razlozi hospitalizacije hematoonkoloških pacijenata u pedijatrijskoj jedinici intenzivnog liječenja

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ABSTRACT

Introduction: according to pediatric oncology literature, 1 out of 600 children between birth and 15 years of age suffer from malignant tumors. Modern multidisciplinary treatment including chemotherapy, radiation and surgery, has increased 5-year survival rate to 70-75% depending on the type of tumor. A large number of children suffering from malignant diseases require treatment in intensive care unit (ICU) due to complications of the disease. Complications include infections, respiratory, cardiovascular and renal failure, and may require long-term and expensive treatment. Aim of this article was to show the most common causes of hospitalization of haemato-oncological patients in the Intensive Care Unit of the Pediatric Clinic, Clinical Center University of Sarajevo, in the period from April 2009 to April 2017. **Materials and methods:** the article has retrospective and descriptive character. Data was taken from medical histories of patients hospitalized in Intensive Care Unit of the Pediatric Clinic. **Results:** during the mentioned period, 1665 patients were hospitalized in Intensive Care Unit of the Pediatric Clinic, of which 67 were haemato-oncological patients with total of 75 hospitalization, with the following diagnoses: acute lymphoid leukemia in 16 patients, acute myeloid leukemia in 2 patients, hemophilia in 2 patients, spherocytosis in 1 patient, 7 patients with lymphomas (Hodgkin's lymphoma 3, Non-Hodgkin's 1, Burkitt 2, large intestinal lymphoma 1 patient), myelodysplastic syndrome was diagnosed in 1 patient, Rohhad syndrome in 1 patient and histiocytosis in 1 patient. Tumors were present in 36 patients - 10 patients with CNS tumors, 15 with Wilms' tumor, 7 with neuroblastoma and 1 patient was diagnosed with rhabdomyosarcoma, pancreatoblastoma, retinoblastoma, hepatoblastoma, and teratoma ovary respectively. Out of the total number of patients 11 (16.6%) died. **Conclusion:** in practice, there are various combinations of multiple organ dysfunctions and multiple organ failures in haemato-oncological patients which require individual, multidisciplinary approach to each patient. The results of the treatment are not in correlation with individual efforts, funds and technical support possibilities, but are in majority of cases the reflection of severity and number of organ dysfunction.

Key words: malignant tumors, complications, child, pediatric oncology intensive care unit

SAŽETAK

Uvod: prema podacima pedijatrijske onkologije procjenjuje se da će jedno od 600 djece, između rođenja i 15-te godine života oboljeti od malignog tumora. Moderni multidisciplinarni pristup liječenju koji uključuje kemoterapiju, zračenje i kirurško liječenje omogućava 5-godišnje preživljavanje od 70-75% neovisno o tipu tumora. Veliki broj djece oboljele od malignih bolesti zahtijeva liječenje u jedinici intenzivnog liječenja (JIL-u) zbog komplikacija osnovne bolesti. Komplikacije uključuju infekcije, respiracijsku, kardiovaskularnu i bubrežnu insuficijenciju, i mogu zahtijevati dugotrajno i skupo liječenje. Cilj: prikazati najčešće razloge hospitalizacije hematoonkoloških pacijenata u Jedinici intenzivnog liječenja Pedijatrijske klinike KCUS u periodu od travnja 2009. do travnja 2017. godine. **Materijali i metode:** radi se o retrospektivnoj studiji u kojoj su korišteni podaci iz istorija bolesti pacijenata hospitaliziranih u Jedinici intenzivnog liječenja Pedijatrijske klinike. **Rezultati:** tokom navedenog perioda u Jedinici pedijatrijskog intenzivnog liječenja ukupno je hospitalizirano 1665 pacijenata, od toga 66 hemato-onkoloških pacijenata sa ukupno 75 hospitalizacija. Od toga 16 pacijenata je imalo akutnu limfatičnu leukemiju, dva pacijenta akutnu mijeloičnu leukemiju, dva pacijenta sa hemofilijom, jedan sa sferocitozom. Kod 7 pacijenata dijagnosticiran je limfom od toga tri Hodgkin-ova limfoma, 1 non Hodgkin limfom, 2 Burkitt lymphoma i 1 pacijent sa velikostaničnim limfom crijeva. Mijelodisplastični sindrom je imao jedan pacijent, i po jedan pacijent sa Rohhad sy i histiocitozom. Različite vrste tumora imalo je 36 pacijenata: 9 pacijenata sa tumorima CNS-a, Wilmsov tumor 15 pacijenata, 7 pacijenata sa neuroblastomom i po jedan pacijent rhabdomyosarkomom, pankreatoblastomom, retinoblastomom, hepatoblastomom i teratomom ovarija. Ukupno je umrlo 11 pacijenata (16.6%). Najčešća komplikacija koja je dovela do smrtnog ishoda bila je sistemska infekcija - sepsa kod 8 pacijenata (72.7%), sa razvojem sindroma multiorganskog zatajenja, krvarenje i razvoj diseminirane intravaskularne koagulacije zbog razvoja hepatične insuficijencije kod preosjetljivosti na metotrexat, zatajenja centara u produženoj moždini usljed malignog edema mozga i encefalitisa. **Zaključak:** u praksi se svakodnevno susrećemo s različitim kombinacijama višestruke disfunkcije organa i multiorganskog zatajenja kod hematoonkoloških pacijenata što iziskuje individualni, multidisciplinarni pristup svakom bolesniku. Rezultati liječenja uglavnom ne ovise o uloženom trudu, potrošenim sredstvima i tehničkim mogućnostima potpore, nego su najčešće odraz težine i broja disfunkcije organa.

Ključne riječi: hematoonkološka oboljenja, komplikacije, dijete, jedinica intenzivnog liječenja

INTRODUCTION

With modern chemotherapy in combination with cytostatics, surgery, radiation, hematopoietic stem cell transplantation (HSCT), the total of five-year survival rate after diagnosis for all pediatric malignancies is 70-75%. Children with malignant diseases are treated in intensive care unit (ICU) for several reasons (1,2). For example after high-risk surgery, due to primary complications of life-threatening tumors, but also due to complications related to the treatment of malignant disease itself. The prognosis of treatment in ICU after the surgery is usually good and short. Treatment in ICU due to therapeutic complications of malignant disease is longer, less successful, and requires the use of expensive and complicated therapeutic procedures (1,3,4). Treatment of children with malignant diseases includes treatment that damages healthy organs, impairs immunological protection of the organism, and thus increases the possibility of developing the infection, and life-threatening complications such as sepsis, shock, cardiorespiratory insufficiency, liver and kidney failure. The cause of fatal outcome in these patients is usually not the primary malignant disease, but the complications of treatment resulting in multiorgan failure (1,2,3,4).

Aim

The aim of this paper is to present the most common reasons for hospitalization of hematology-oncology patients in Intensive Care Unit of the Pediatric Clinic, Clinical Center University of Sarajevo (CCUS) in the period from April 2009 to April 2017.

MATERIALS AND METHODS

In the period from April 2009 to April 2017, a total of 1665 patients were hospitalized in Intensive Care Unit of the Pediatric Clinic, of which 66 (3.96%) were hematology-oncology patients. This is a retrospective study using data from the history of patients hospitalized in Intensive Care Unit of the Pediatric Clinic. Patients were monitored according to the type of hemato-oncological disease and signs which occurred as a result of the underlying disease or consequences of the administered therapy.

RESULTS

Out of the total number of patients 66 were hematology-oncology patients (16 patients had acute lymphoblastic leukemia, 2 patients had acute myeloid leukemia (AML), hemophilia A was diagnosed in 2 patients, spherocytosis in 1 patient and 7 patients were diagnosed with lymphomas (Hodgkin's lymphoma in 3 patients, Non-Hodgkin lymphoma in one, Burkitt lymphoma in 2, large bowel lymphoma in one patient). Myelodysplastic syndrome was present in one patient, Rapid-onset Obesity with Hypothalamic dysfunction, Hypoventilation and Autonomic Dysregulation (ROHHAD syndrome) was present in one patient, and one patient had histiocytosis. Different types of tumors were present in 36 patients - 9 patients had tumors of central nervous system, 15 had Wilms' tumor, 7 patients had neuroblastoma, 1 patient had rhabdomyosarcoma, 1 patient suffered from pancreato-

blastoma, 1 patient from retinoblastoma, 1 patient had hepatoblastoma, and 1 patient had teratoma. According to the gender distribution, there were 37 boys and 29 girls. The youngest patient was a female newborn whom in the first day of life, due to the development of global insufficiency, chest X-ray showed a mediastinal tumor mass, and whom following the MRI of the thorax, surgery and histopathologic examination teratom was confirmed. The oldest patient was 14 years and 10 months old. The average age of patients was 7 years and 6 months. Out of the total number of patients hospitalized at the Pediatric Intensive Care Unit, 41 patients were admitted after surgery, with surgical removal of tumor mass as part of multidisciplinary treatment which was the case in 36 patients or surgical treatment of complications in 5 patients where acute development of hydrocephalus required the insertion of a ventriculoperitoneal shunt. The most common reason for hospitalization of hematology-oncology patients who were on conservative treatment of the underlying disease was the development of the following complications of which one or more were associated with one patient: systemic infection and sepsis in 14 patients, epileptic status in 5 patients, pneumonia isolated or followed by global respiratory insufficiency in 8 patients, febrile neutropenia in 2 patients, encephalitis in one patient, 3 patients had intracranial hemorrhage, 2 patients had severe enterocolitis with hypovolaemic shock and gastrointestinal bleeding, 4 patients had relapses of the underlying disease- acute lymphoblastic leukemia and there were 2 relapses of the tumor -neuroblastoma. Out of the total of 11 deceased patients, 6 were surgically treated patients who developed postoperative complications in the sense of multiple organ failure as a systemic infection and sepsis - five patients, and the death of one surgically treated patient occurred due to malignant brain edema after the brain tumor surgery. In conservatively treated patients, death cause complications occurred in the total of five patients, of which three had sepsis with the development of multiple organ failure, one had encephalitis with malignant brain edema, whereas one patient died from the development of hepatic insufficiency as a hypersensitivity reaction on methotrexate.

The most frequent cause of systemic infection-sepsis was *Klebsiella pneumoniae* which was isolated in the blood cultures of 8 patients, of which in 5 patients with lethal outcome (Figure 1). *Pseudomonas aeruginosa* was isolated in 3 patients, of which in one with a lethal outcome, in one blood culture *Escherichia coli* was isolated, and in two blood cultures *Candida glabrata* with lethal outcome in both patients.

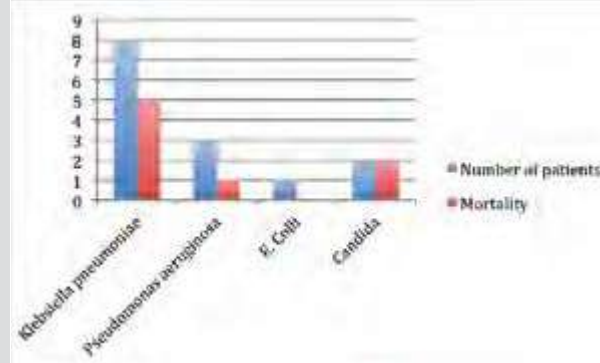


Figure 1 Isolated cause and outcome of sepsis.

DISCUSSION

Intensive Care Unit of the Pediatric Clinic admitted patients with life-threatening conditions, including hemato-oncological patients who experienced complications due to primary illness or consequences of the administered therapy. In cases of admission of patients following the surgery, the prognosis is usually good, and the treatment is usually short-term. Treatment due to therapeutic complications of malignancies is longer, less successful, and requires the use of expensive and complicated therapeutic procedures. The most frequent reason for admission to the Intensive Care Unit was the postoperative care of hematology-oncology patients, the total of 41 patient (62.1%). Complications in conservatively treated hematology-oncology patients who had indications for admission to the ICU were sepsis, epileptic seizures, and respiratory depression in the sense of developing pneumonia with or without development of respiratory insufficiency. The most frequent cause of systemic infection-sepsis was *Klebsiella pneumoniae* which was isolated in the blood cultures of 8 patients, of which in 5 patients with lethal outcome. *Pseudomonas aeruginosa* was isolated in 3 patients, one of which with lethal outcome. *Escherichia coli* was isolated in one blood culture and *Candida glabrata* in two blood cultures, with lethal outcome in both patients.

CONCLUSION

This paper describes the reasons for hospitalization of hematology-oncology patients in Intensive Care Unit of the Pediatric Clinic, CCU Sarajevo. In practice, various combinations of multiple organ dysfunction and multiple organ failure are encountered on daily basis, which requires multidisciplinary approach to each patient, combination of procedures and therapeutic measures applied according to the severity of pathophysiological events. Treatment of children with malignant diseases includes treatment that damages healthy organs, impairs immunological protection of the organism, and thus increases the possibility of developing infections, and life-threatening complications such as sepsis, shock, cardiorespiratory insufficiency, liver and kidney failure. The cause of death in these

patients is usually not the primary malignant disease, but the complications of treatment resulting in multiple organ failure. The results of the treatment are not in correlation with individual efforts, funds and technical support possibilities, but are in majority of cases the reflection of severity and number of organ dysfunction.

Conflict of interest: none declared.

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Treatment of tibial pseudoarthrosis by Ilizarov method

Liječenje pseudoartroza tibije metodom ilizarova

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ABSTRACT

The aim of this paper was to analyze the treatment of tibial pseudoarthrosis by utilizing the distraction-compression method by Ilizarov. The surgical treatment was based upon the resection of pseudoarthrosis with the slanted resection of fibula. The healing process was monitored through laboratory tests, ultrasound, radiologically, and clinically, on a sample of 40 patients, of whom 97.5% were presented with unilateral, and 2.5% with bilateral pseudoarthrosis. In terms of patients' sex, 82.5% were male and 17.5% female, with right-hand side domineering over the left side with 57.5% versus 42.5%. Most of the patients were middle-aged (37.5%), with the average duration of the apparatus application being 140.85 days, while patients were able to return to their daily activities after 161.75 days. The results were assessed by means of the ASAMI scoring and INDEKS rehabilitation. A 21-day physical rehabilitation was prescribed during the treatment.

Key words: pseudoarthrosis, Ilizarov method

INTRODUCTION

The external fixation is a method by which bone fragments or segments of a skeleton are fixed by pins or wires through the skin, soft tissues, and bone, while attached to the outer frame (1,2,3).

The external fixator is an apparatus used in bone-joint surgery, and its purpose is to fix bone fragments by means of pins through the segments of a skeleton. On the outer part, it is attached to the frame. This method helps stabilize fragments of the injured bone and maintain them in the desired position. By utilizing the fixator, we can achieve the following processes regarding bone fragments: neutralization, dynamisation, distraction, angulation, rotation, osteotaxis, ligamentotaxis, elastic fixation, and biocompression (4).

The dynamic distraction-compression external fixation by Ilizarov was introduced in 1951, and was founded on experiments and theoretical concepts which proved the influence of compression on the speed of regeneration of spongiouse and compact bone (5,6).

The distraction osteogenesis is a mechanical induction of a new bone after a corticotomy between gradually distracted bone surfaces. The biological bridge between the bone surfaces is created from local revascularization, and it encompasses the entire cross section of the surface treated (7,8).

SAŽETAK

Cilj rada je analizirati liječenje pseudoartroza potkoljenice distrakciono-kompresionom metodom po Ilizarovu. Operativni pristup liječenju zasnivao se na resekciji pseudoartroze uz kosu resekciju fibule. Zarastanje je praćeno laboratorijski, UZV, radiološki i klinički. Praćeno je 40 pacijenata. Od toga 97.5% sa unilateralnom, a 2.5% sa bilateralnom pseudoartrozom. Muškaraca je bilo 82.5%, a žena 17.5%. Desna strana kod 57.5%, a lijeva kod 42.5%. Najveći procenat srednje životne dobi je 37.5%. Prosječna dužina nošenja aparata je 140.85 dana, a prosječno vrijeme vraćanja normalnim životnim aktivnostima je 161.75 dana. Rezultati cijenjeni ASAMI scoringom i INDEKS-om rehabilitacije. Tokom liječenja provedena je fizikalna rehabilitacija u trajanju od 21 dan.

Ključne riječi: postoperativna rehabilitacija, operacija vratnog diskusa

If a gradual mechanical distraction is performed, the live bone is fully regenerated, enabling the segmental bone transport by means of callus distraction. In that way, an unlimited amount of live bone between vascular surfaces separated by corticotomy can be produced (9,10).

The aim of this paper was to analyze tibial pseudoarthrosis treatment by utilizing the open distraction-compression method by Ilizarov at the Clinic of Traumatology of the University Clinical Center of the Republic of Srpska, over a five-year period.

MATERIALS AND METHODS

We analyzed the results of tibial pseudoarthrosis treatment by bloody reposition and application of the Ilizarov circular apparatus in the period from 2012 to 2016.

The surgical approach to the treatment was based on the resection of tibial pseudoarthrosis with a slanted resection of fibula. It was performed in multiple ways: by resection of pseudoarthrosis with apparatus compression, wedge resection of pseudoarthrosis with apparatus distraction, longitudinal resection with the apparatus distraction, the Docking-type resection of pseudoarthrosis with the apparatus compression. In all these cases a slanted resection of fibula

(1-6 cm) was performed.

The healing process was monitored:

By ultrasound, radiologically, through laboratory tests (values of alkaline phosphatase), and clinically.

By ultrasound - through the increase of waves speed through the location of fracture during the first month of post-operative rehabilitation. The linear sonde of 7MHz, 10MHz, and 12MHz respectively on the LOGIQ 5 2002 apparatus was used.

The radiological monitoring was based on the verification of endosteal and periosteal callus, and determination of interfragmentary diastasis in millimetres.

The clinical signs of healing were confirmed by the absence of pain at the location of pseudoarthrosis, the disappearance of swelling and pathological mobility at the location of pseudoarthrosis.

The results of the survey were analysed and presented by means of descriptive statistics and application of adequate statistical tests through the SPSS package (Originally: Statistical Package for the Social Sciences, later modified to read Statistical Product and Service Solutions), version 20.

RESULTS

Over the period from January 1, 2012 to December 31, 2016, 40 patients were treated at Clinic of Traumatology of the University Clinical Center of the Republic of Srpska by means of utilising the open distraction-compression method by Ilizarov. Out of this number, 39 patients (97.5%) were treated for unilateral pseudoarthrosis, while 1 patient (2.5%) was treated for bilateral pseudoarthrosis.

The previous treatment, in most cases, was either non-operative or a combination of operative and non-operative.

Table 1 Previous treatment of patients.

DC-plate	9(22.5%)
Sarmiento plaster	15(37.5%)
External fixator and Sarmiento plaster	16(40%)
Overall	40

The distribution of patients regarding the type of pseudoarthrosis, sex, side of the extremity, and age was versatile.

Table 2 Type of pseudoarthrosis, sex, and age.

Unilatera pseudoarthrosis	39(97.5%)
Bilateral pseudoarthrosis	1(2.5%)
Men	33(82.5%)
Women	7(17.5%)
Right lower leg	23(57.5%)
Left lower leg	17(42.5%)
Age	10-75 years
Overall	40

In the majority of cases, the patients were middle-aged men, with right lower leg injuries domineering.

As far as the resection types are concerned, the most frequent related to complete resection of pseudoarthrosis with apparatus

compression, and the least performed was the Docking-type with apparatus compression and the angular resection with apparatus distraction.

Table 3 Type of pseudoarthrosis resection.

Resection of pseudoarthrosis with compression	28(70%)
Docking-type resection with compression	2(5%)
Resection angularis with distraction	3(7.5%)
Longitudinal corticotomy with distraction	7(17.5%)
Overall	40

The values of alkaline phosphates were determined before the surgery, two days after the surgery, and on the thirtieth day after the surgery. They showed rising tendencies, which were in correlation with the ultrasound and radiological verification of callus.

By applying the Wilcoxon test, a statistically significant difference ($z=-7.788$, $p=0.000$) of alkaline phosphates was obtained in the second reading as opposed to the first one, and a similar result was obtained when the third reading was compared to the second one ($z=-7.771$, $p=0.000$).

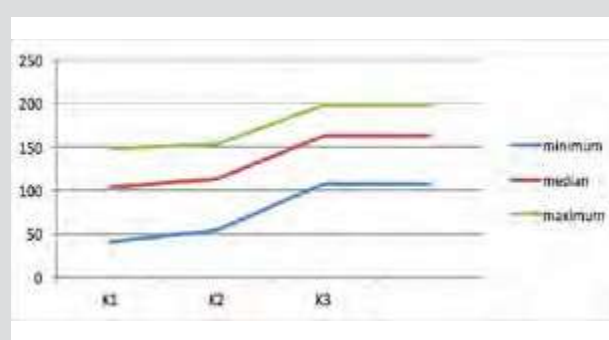


Figure 1 Alkaline phosphatase median.

The average duration of the apparatus application was 140.85 days, and all patients were prescribed a 21-day physical rehabilitation. In all patients, pseudoarthrosis was improved, while patients could return to their daily activities after 161.95 days on average.

The assessment of bone results was performed by the ASAMI-scoring, and the functional ones by the INDEKS rehabilitation.

Table 4 Bone results of the Association for Study and Application of the Method of Ilizarov - ASAMI.

Excellent	Union, absence of infection, deformity <7°, limb length discrepancy < 2.5cm
Good	Union + any two of the following (no contractions, no bone atrophy), absence of infection, deformity <7°, limb length discrepancy <2.5cm
Fair	Union + any one of the following (contractions or severe pain), absence of infection, deformity <7°, limb length discrepancy <2.5cm
Poor	Non-union/refractured/ union and infection + deformity >7° + difference in extremity length > 2.5cm

The overall assessment of results was obtained by summing up the ASAMI-scoring and INDEKS-rehabilitation.

Table 5 Functional results of INDEKS rehabilitation.

Clinical signs	3	2	1	0
Bone integrity	normal bone	not complete	bone atrophy	ruined
Deformation	absent	5-10°	11-20°	more than 21°
Shortening	absent	1-3cm	4-6cm	more than 7cm
No visible damage	absent	light, occasional	pronounced at effort	heavy, permanent
Supportive function	full	gradually increasing	partial, relief needed	absent
Joint moves	full range	within normal limits	half-normal	absent
Extremity function	intact	gradually increasing	intact but possible	absent
Pain	absent	mild at effort	permanent at normal function	pronounced at rest
Working ability	full	temporary inability	partial inability	Complete inability

Table 6 Results of ASAMI-scoring and INDEKS rehabilitation.

ASAMI	EXCELLENT	12 (30%)
INDEKS	EXCELLENT	
ASAMI	GOOD	16 (40%)
INDEKS	GOOD	
ASAMI	GOOD	1 (2.5%)
INDEKS	FAIR	
ASAMI	FAIR	11 (27.5%)
INDEKS	FAIR	
OVERALL		40

DISCUSSION

The Ilizarov method is a challenge in terms of therapy. Apart from proper indication, it demands extreme precision at fixing the apparatus, in order for possible corrections to be performed on the apparatus itself during the infirmity post-operative treatment (11,12). It is a sovereign method in dealing with pseudoarthrosis, which is supported by other authors as well.

The process of osteogenesis is followed by checking the values of alkaline phosphatase during the treatment. The zero value was registered two days before the surgery, the maximum on the thirtieth day after surgery in all patients. A statistically significant difference of the increase in value of alkaline phosphatase in relation to zero value ($z=-7.771$, $p=0.000$) was obtained. This increase was followed by the increase in volume of periosteal callus and decrease of transparency from 3mm to 1mm, measured radiologically and by ultrasound.

Farley J. et al. (13) in their research in vitro, as well as Martin M. et al. (14) in their research in vivo, established that the activity of alkaline phosphatase was in proportion with the size of callus.

The overall assessment of the ASAMI-INDEKS results was: excellent 12 (30%), good 16 (40%), and fair 11 (27.5%). With 1 (2.5%) patient, the ASAMI was good and the INDEKS was fair, because, in that particular case, there was a good union, but accompanied with 2.5cm extremity shortenin.

The results of Lalic I. et al. from the Clinical Centre of Vojvodina, Novi Sad (Serbia) show that 23 (59%) patients had an excellent result, 9 (23%) good, 5 (13%) fair, and 2 (5%) poor (15).

As for Mukesh N. Shah et al. at the Medical Centre in Ahmedabad (India), the ASAMI-scoring was excellent in 14 (66,67%) patients, good in 6 (28,57%), and fair in 1 (4,76%) patient (16).

With regard to our study, good results were registered in 16 (40%) patients, those who scored fair gained the maximum number of points for the most part - in 7 patients, with no poor results.

CONCLUSION

Tibial pseudoarthrosis is highly frequent in all long bone fractures. Previous treatments usually proved to be long-term and inefficient in terms of non-healing, bone defects, and deformities. If properly indicated, the Ilizarov method offers the greatest possibility for deformity corrections, compensation of defects, and a shorter healing period, as well as a returning to daily activities, so we recommend it as a method of choice in the treatment of tibial pseudoarthrosis.

Conflict of interest: none declared.

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The incidence of the pathological spermogram findings in patients with testicular germ cell tumors

Učestalost patološkog nalaza spermograma kod pacijenata sa germinativnim tumorima testisa

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ABSTRACT

Malignant tumors of the testicle are rare neoplasms that account for approximately 1% of all tumors in men. These are tumors that occur at a younger age, between 18 and 35 years of age. In the given age group, they represent the most common solid malignant tumor, which makes this malignancy one of the top oncological problems. Treatment is very complex and requires a multidisciplinary approach. In patients diagnosed with malignant testicular tumors, semen analysis (spermogram) and semen cryopreservation are required before a specific oncological treatment, due to possible fertility problems caused by chemotherapy or radiotherapy. Our study included 42 patients with testicular carcinoma (seminoma and non-seminoma), by numerous parameters. After a detailed anamnesis, all patients underwent an oncological clinical examination, laboratory analyses including tumor markers (alpha-fetoprotein-AFP, human chorionic gonadotropin-HCG and lactate dehydrogenase-LDH). After orchiectomy, semen analysis was performed for all patients. In 19 patients (45%), numerous anomalies were found in the findings, ranging from reduced mobility of spermatozoa and reduced sperm count, to total absence of spermatozoa (azoospermia), which was found in 5 patients (11.9%). No significant incidence of the pathological spermogram findings was observed for the histological type of tumor.

Key words: germ cell tumors, pathological semen analysis

SAŽETAK

Maligni tumori testisa su rijetke neoplazme i čine oko 1% svih tumora kod muškaraca. Ovo su tumori mlađe životne dobi, između 18 i 35 godina i u toj dobi čine najčešći solidni maligni tumor, što ovaj malignitet svrstava u sam vrh onkološke problematike. Liječenje je vrlo kompleksno i zahtijeva multidisciplinarni pristup. Kod pacijenata kojima se dijagnosticuje maligni tumor testisa potrebno je uraditi spermogram i krioprezervaciju sperme prije započinjanja specifičnog onkološkog liječenja, zbog mogućih problema sa fertilitetom uzrokovanih hemioterapijom ili radioterapijom. U našem radu obradili smo 42 pacijenta sa karcinomom testisa (seminomi i neseminomski tumori), po brojnim parametrima. Svim pacijentima je nakon detaljne anamneze, urađen onkološki klinički pregled, laboratorijske analize uključujući i tumorske markere (alfa fetoprotein-AFP, humani horionski gonadotropin-HCG, laktat dehidrogenaza-LDH). Svim pacijentima je nakon orhiektomije urađen spermogram. Kod 19 pacijenata (45%) uočene su brojne anomalije u nalazima, od smanjene pokretljivosti i broja spermatozoida do potpunog izostanka spermatozoida (azoospermija) - kod 5 pacijenata (11.9%). Nije primijećena značajnija učestalost patološkog nalaza spermograma prema histološkom tipu tumora.

Ključne riječi: tumori germinativnih ćelija, patološki spermogram

INTRODUCTION

Germ cell tumors occur in younger men, usually between 18 and 35 years of age (1). Approximately 98% of all patients with testicular cancer have germ cell tumors. Incidence rates of testicular cancer differ throughout the world. The highest incidence rates are recorded in Denmark and Norway - 12/100,000, whereas the incidence was lowest among black males in Africa - 0.5/100,000 (2,3). From decade to decade, the number of patients is increasing in the western countries. It is important to note that mortality has been significantly reduced due to the discovery and application of platinum-based chemotherapy since the mid - 1970s. Thanks to this discovery, testicular tumors are one of the highly curable diseases, with five-year survival of approximately 89% (4).

However, the use of cytostatic agents in various combinations according to protocols leads to various side effects and unintended consequences. Among other, there is a detrimental effect on spermatogenesis. Also, it has been observed that the disease itself, before the oncological treatment, is sometimes associated with gonadal function disorders and infertility. A specific oncological treatment in men can cause disorders in terms of ejaculation, erectile function and sexuality and general. Patients are advised to have hormone receptor status testing done for testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Sperm banking should be performed before orchiectomy in all men with testicular cancer independent of disease stage. TESE (testicular sperm extraction) is recommended in fertility preservation management

for patients who fail to bank semen samples or those with severe spermatogenesis impairment. However, it is never too late to perform sperm banking after orchiectomy, but before adjuvant therapy. Sperm banking must be maintained until the complete fulfillment of parental objectives. This is critical for patients with bilateral testicular cancer, anejaculation, or azoospermia but can also be recommended for patients with unilateral testicular cancer. Indeed, these men have only one testicle, which may potentially be exposed to another disease, such as cancer recurrence, trauma, or infection (5).

Spermatogenesis is the process of maturation of spermatozoa in the testicles. Normal semen analysis processes several parameters. Normal semen volumen is 2ml or more (usually 2-4 ml per ejaculation). Total sperm count is more than 40 milion spermatozoa per ejaculat, with semen PH of 7.2-8.0. Sperm morphology - more than 30% of the sperm have normal shape and structure, and more than 50% of the sperm show progressive movement (sperm motility). Vitality is 75% or more live. There are less than 1 million/ml white blood cells.

Morphological and functional disorders of spermatozoa are the following:

- Aspermia - complete lack of ejaculate
- Asthenozoospermia - reduced spermatozoa mobility
- Azoospermia - complete lack of spermatozoa in the ejaculate (secretory and excretory)
- Hyperspermia - increased sperm count in the ejaculate
- Normospermia - normal sperm count 20 milion per mililitre (40 – 120 milion)
- Hypospermia - decreased sperm count in the ejaculate (20 – 60 milion/ml)
- Oligospermia - decreased sperm count in the ejaculate (less than 20 milion/ml)
- Necrozoospermia - dead or immobile spermatozoa
- Teratozoospermia - spermatozoa with abnormal morphology.

Causes of spermatogenesis disorders can be different and they are classified as pre-testicular, testicular and post-testicular. Secretory azoospermia may be caused by genetic disorders (Klinefelter syndrome, Down syndrome, cystic fibrosis, Y chromosome microdeletion), non-genetic disorders of testicular development (bilateral testicular atrophy, bilateral cryptorchidism,...), inflammation of the testicle, hormone deficiency (FSH - follicle-stimulating hormone secretion disorder), radiotherapy or chemotherapy (6,7).

The aim of this study was to determine the association of testicular germ cell tumors with various types of spermatogenesis disorders and to establish whether there was a higher incidence of pathological spermogram findings in our patients in relation to the pathohistological (PH) findings.

MATERIALS AND METHODS

The study represents a retrospective study which examines 42 patients with testicular cancer, who were treated at the Oncology Clinic of the University Clinical Centre of the Republic of Serpska and the Healthcare Institution "S.tetik" Banja Luka in the period from 2008 to 2014. All patients were processed by age, anamnestic data (cryptorchism, previous trauma, malignancy in the family), histological

type of tumor and clinical stage at the time of diagnosis. Tumor marker values (AFP, HCG, LDH) have been analysed. These were presented with S category, with TNM classification. Spermogram was done for all patients after orchiectomy and the findings were analysed in detail.

RESULTS

The average age of the patients was 27 years (19 to 33 years). There were 17 (40%) patients with seminomatous tumor and 25 patients (60%) with non-seminomatous tumor (NSGCT) (Figure 1).

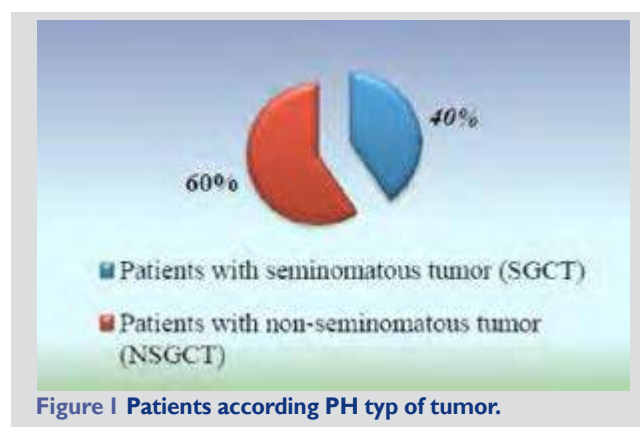


Figure 1 Patients according PH typ of tumor.

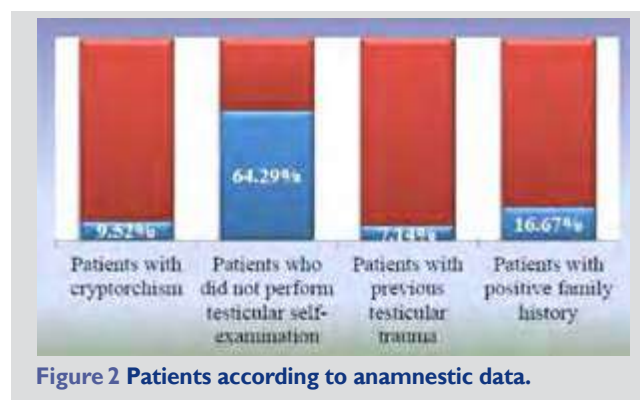


Figure 2 Patients according to anamnestic data.

For NSGCT, the incidence of individual histological variants was as follows: mixed tumor (seminoma, embryonal carcinoma, yolk sac tumor) in 8 patients, mixed tumor (teratoma immaturum, embryonal carcinoma, seminoma) in 6 patients, mixed tumor (choriocarcinoma, embryonal carcinoma, yolk sac tumor, seminoma, teratoma) in 3 patients, mixed tumor (embryonal carcinoma, choriocarcinoma, seminoma) in 3 patients, mixed tumor (choriocarcinoma, embryonal carcinoma, yolk sac tumor, seminoma) in 5 patients.

Anamnestic data suggest that there were 4 patients (9.5%) with cryptorchism. There were 27 patients (65%) who provided anamnestic data that they did not perform testicular self-examination. Previous testicular trauma (a blow during sport activities) was indicated by 3 patients (7%), while 7 patients (17%) reported positive family history (close relatives suffering from various malignancies) (Figure 2).

There were no patients who indicated that their brothers suffered from the same disease. There were also no patients with contralateral testicular tumor. Patients with seminoma were in the clinical stage I A and II B. Patients with NSGCT were in the clinical stadium I B – III A.

In all patients with seminoma, the S0 category was observed, while in 2 patients with NSGCT the S1 stage was observed (status of tumor marker values in the serum). Spermogram was analysed for patients with seminomatous and non-seminomatous tumors separately, without an analysis based on histological tumor subtypes (NSGCT). When it comes to patients with seminoma, azoospermia was found in 2 patients (11.7%), oligospermia in 5 patients (29.4%), with different degrees of reduced mobility of spermatozooids in 6 patients (35.2%) (in 5 patients with oligospermia and in one patient with a proper number of spermatozoa). In patients with seminomatous testicular tumor (SGCT) - pathological spermogram findings were observed in a total of 8 patients (47%). For NSGCT, there were 11 patients (44%) with pathological spermogram findings; namely, azoospermia was found in 3 patients (12%), oligospermia in 7 patients (28%), with mobility disorders (asthenozoospermia) in a total of 5 (20%) patients (in 4 patients with oligospermia and in one patient with a proper number of spermatozoa).

There is no statistically significant difference in these findings between patients with SGCT and patients with NSGCT (Table 1).

Table 1 Type of pathological finding of semen analysis in different PH type of tumor.

Type of pathological finding of semen analysis	SGCT		NSGCT		Pearson χ^2 test
	n	%	n	%	p
Azoospermia	2	11.76	3	12.00	0.644
Oligospermia	5	29.41	7	28.00	0.803
Asthenozoospermia	6	35.29	5	20.00	0.269
- with oligospermia	5 (83.33%)		4 (80.00%)		0.520
- with proper number of spermatozoa	1 (16.67%)		1 (20.00%)		
Total	8	47.06	11	44.00	0.845

DISCUSSION

All patients with testicular carcinomas should be advised to have a quality sperm sample preserved in the so-called sperm banks, thus ensuring the progeny if a spermatogenesis disorder occurs as a result of treatment. It is well known that chemotherapy and radiation may cause fertility disorders. However, in a certain number of patients with testicular carcinoma, at the time of diagnosis, the spermogram shows pathological findings, making the sperm cryopreservation uncertain. In our subjects, various spermogram disorders were found in 19 patients (45%), namely in 8 patients (47%) with seminomatous tumor and in 11 patients (44%) with NSGCT.

Numerous data from literature also suggest that testicular dysgenesis is often associated with the occurrence of malignant neoplasms of the testicle.

Guminska A. et al. in their study found the results that indicate that neoplastic lesions appear only in testes with disturbed spermatogenesis. Worsened condition of spermatogenesis is associated with the presence of other dysgenetic features, but neoplastic lesions appear more frequently in testes with the less advanced features of testicular dysgenesis (8).

There is more and more evidence that the CIS cell is a gonocyte with stem cell potential, which explains why an adult man can develop a non-seminoma, which is a neoplastic caricature of embryonic growth. We consider the possibility that CIS cells may lose their stem cell potential with ageing. Along these lines, a seminoma is regarded a gonocytoma where single gonocytes have little or no

stem cell potential. The Sertoli and Leydig cells activated postnatally and during and after puberty, may play a crucial role for both the development of the CIS gonocyte and progression of the neoplasm to invasiveness. The reported increase in testicular cancer is not the only sign that male reproductive health is at risk. There are reports that undescended testis and hypospadias have become more common. Also, semen quality has deteriorated, at least in some countries. The epidemiological evidence suggests that environmental factors may play a role. Recent genome-wide association studies (GWAS) have identified susceptibility alleles for adult testicular GCT (TGCT). They test whether these SNPs are associated with GCT in pediatric and adolescent populations. They observed associations between SNPs in *SPRY4*, *BAK1*, and *GAB2* and GCTs. This analysis suggests there may be common genetic risk factors for GCT in all age groups (9).

Raman et al. have proved that infertile men with abnormal semen analyses have a 20-fold greater incidence of testicular cancer compared to the general population. Patients and physicians should be aware that one of the causes of infertility could be cancer, particularly testicular cancer (10).

Skakkebaek et al. were exploring the association between testicular dysgenesis syndrome (TDS) and testicular neoplasia. They identified 20 patients who fulfilled the histological criteria for testicular dysgenesis, 9 of whom were diagnosed with uni- or bilateral testicular germ cell neoplasia, and the remaining ones with subfertility. The majority of cases had oligozoospermia or azoospermia. Their reproductive hormone profiles correlated with the results of semen sampling and testicular histology. This study of 20 patients with various reproductive abnormalities provided evidence that TDS is a real clinical entity. They speculate that most of these abnormalities are caused by adverse environmental effects rather than specific gene mutations (11).

It should be noted that the suggestion is to do a spermogram before orchiectomy, since it is considered that the cryopreservation material obtained in this way is of better quality. There are studies that support this thesis. Semen quality was poor at diagnosis and deteriorated further after orchiectomy compared with pretreatment values. Findings of Petersen et al. indicate that in some patients the most appropriate time for cryopreservation of semen is before orchiectomy. Androgen production was maintained by increased luteinizing hormone stimulation after orchiectomy (12).

According to data from the references, it is clearly established that testicular cancer is associated with reduced semen quality and decreased fertility.

CONCLUSION

In our studies, we have established a significant association of various types of spermatogenesis disorders with testicular germ cell tumors. A higher incidence of pathological spermatogenesis depending on the PH tumor type has not been observed. Bearing in mind the association between TDS (testicular dysgenesis syndrome) and the occurrence of testicular carcinoma, special attention should be paid to patients with this problem in order to detect the disease early and to provide adequate treatment. Cryopreservation of sperm is recommended before orchiectomy whenever possible. Given the

fact that this organ is accessible for self-examination, the population needs to be educated and awareness of the general population about the possibilities of early detection of this disease should be raised.

Conflict of interest: none declared.

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The impact of Diabetes Mellitus Type 2 on occurrence of colorectal anastomosis dehiscence

Uticaj Dijabetes melitusa tip 2 u nastanku dehiscencije kolorektalne anastomoze

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ABSTRACT

Colorectal anastomosis is very specific procedure in surgery of digestive tract, in sense of constructing, healing and occurrence of complications. Healing of anastomosis is a complex mechanism that depends on several mutually related factors. Those factors may be classified as systemic, local and technical ones. Diabetes mellitus type 2 (DM2T) is one of important factors affecting healing of colorectal anastomosis that may cause anastomosis dehiscence and related complications. This study observed 86 patients divided into two study groups. The first group had 52 patients who had surgery due to rectal cancer and no verified DM2T. The second group consisted of 34 patients who also had surgery due to rectal cancer, but had verified DM2T. Both groups were monitored for the occurrence of colorectal anastomosis dehiscence, as one of the most important post-operative complications. Values of procalcitonin and C reactive protein (CRP) were examined in both groups as predilection factors for colorectal anastomosis dehiscence.

Key words: Diabetes mellitus type 2, rectal cancer, colorectal anastomosis complications

SAŽETAK

Kolorektalna anastomoza je veoma specifična procedura u digestivnoj hirurgiji kako u formiranju, zarastanju tako i u nastanku komplikacija. Zarastanje anastomoze je složen mehanizam koji zavisi od više međusobno povezanih faktora. Na proces zarastanja kolorektalne anastomoze utiču mnogobrojni faktori koji se mogu podijeliti na sistemske, lokalne i tehničke faktore. Jedan od značajnih faktora koji utiče na zarastanje kolorektalne anastomoze i koji može dovesti do dehiscencije anastomoze i pratećih komplikacija je dijabetes melitus tip 2 (DM2T). U ovoj studiji posmatrano je 86 pacijenta koji su podijeljeni u dvije studijske grupe. Prva grupa obuhvatala je 52 pacijenta koji su operisani zbog karcinoma rektuma a kod kojih nije verifikovano postojanje DM2T. Druga grupa obuhvatala 34 pacijenta koji su operisani zbog karcinoma rektuma a kod kojih je verifikovano i potvrđeno postojanje DM2T. Kod obje studijske grupe praćeno je pojavljivanje dehiscencije kolorektalne anastomoze kao jedne od najvažnijih postoperativnih komplikacija. U obje studijske grupe ispitivane su vrijednosti prokalcitonina i C reaktivnog proteina (CRP) kao predilekcionih faktora za nastanak dehiscencije kolorektalne anastomoze.

Ključne riječi: Diabetes mellitus tip 2, karcinom rektuma, komplikacije kolorektalne anastomoze

INTRODUCTION

Colorectal cancer is the third leading cause of cancer-related deaths in the world (1). Majority of surgical treatments of colon and rectum are resections that require reconstruction of digestive tract continuity and introduction of colorectal anastomosis. Rectal anastomoses are performed manually, with several available sewing techniques and use of stapler (2). The use of stapler significantly eased construction of low colorectal anastomoses and it is extensively used in surgery at present. However, anastomosis dehiscence still presents a big problem in spite of all advancements in surgical technique and pre-operative care. The more distal the anastomosis is, the greater the possibility of dehiscence (3). Dehiscence of anastomosis occurs more often with anterior low rectal resections than in other types of colon or rectum resections. Several big multi-institutional studies performed on several thousand surgically treated patients found colorectal anastomosis dehiscence in 10-12% of patients (4). De-

hiscences of colorectal anastomoses usually manifest five to eight days after the surgery. Colorectal anastomosis dehiscence may be caused by numerous factors like patient's age, sex, preoperative radiotherapy and chemotherapy, treatment with corticosteroids, the height of constructed anastomosis and presence of diabetes mellitus type 2 (DM2T). They jeopardize patient's life and often require urgent surgery and prolonged complex and costly treatments, and in some cases they may be fatal for the patient (5). Diabetes mellitus is certainly a very important factor that may cause the dehiscence of colorectal anastomosis. DM2T may lead to numerous chronic complications that are mainly caused by pathological influence of hyperglycemia on blood vessels. Diabetes is also related to high blood lipid levels, hypertension and changes of blood vessel walls (6). Also, it causes changes of blood itself, like increased activation of platelets and blood coagulation disorders. Changes of blood vessels caused by diabetes mellitus may affect small blood vessels (microangiopathy) and large ones (macroangiopathy) (7).

MATERIALS AND METHODS

This research was planned as retrospective-prospective study that lasted from January 2016 to December 2017 and included 86 patients who were hospitalized and surgically treated for rectal cancer that was verified by endoscopy and histopathology. All patients were examined by the oncology medical advisory board and based on disease stadium and tumor localization, the surgical treatment was indicated without preoperative radio- or chemotherapy. Patients were divided into two study groups. The first study group included 52 patients who were surgically treated for rectal cancer and colorectal anastomosis was constructed during the surgery, but who did not have verified DM2T. The second study group included 34 patients with colorectal cancer and surgically constructed colorectal anastomosis who suffered from DM2T. All data for the study came from the history of illness, surgery protocols and clinical examinations of patients who were surgically treated at our Clinic.

Both groups of patients were subjected to preoperative preparations after admission, and patients from the second group with verified diabetes mellitus were transferred to therapy with crystalline insulin. Both study groups had identical surgical treatment that included preparation, high clamping and resection of lower mesenteric blood vessels. Then, adequate preparation of rectum and total mesorectal excision was performed using thermal cautery and LigaSure™ sealing system, while the resection of the bowel was performed using the linear TA stapler. In both groups, the rectal anastomosis was constructed using circular stapler. All surgeries were performed by open procedure – medial laparotomy above and below the umbilicus.

For patients with DM2T, regular checks of blood sugar were performed before and after the surgery and the internist corrected the insulin therapy accordingly. During the postoperative period, patients of both study groups were monitored for the occurrence of anastomosis dehiscence by monitoring their levels of CRP and procalcitonin, examination by abdominal ultrasound, inspection of abdominal drains contents and clinical examination of patients.

RESULTS

The study included 86 patients, mean age 66.5 – the youngest patient was 41, while the oldest was 78.

Out of the total number of patients included in the study, there were 56 males and 30 females. Results of χ^2 test ($\chi^2=7.860$; $p=0.005$) showed statistically significant difference ($p<0.05$) in the number of patients between male and female patients. Male patients were more represented than female ones (Table 1).

Table 1 Distribution of patients by sex.

Sex	N	%
Male	56	65.12
Female	30	34.88
Total	86	100.0

There were 34 patients suffering from DM2T in this study, which represented 39.53 % of the total number of included patients. The aim of the study was to determine the connection of diabetes mel-

litus and colorectal anastomosis dehiscence. Based on the results of Fisher's exact test ($p=0.026$), it was concluded that there was statistically significant ($p<0.05$) inter-dependence of dehiscence and diabetes and that patients suffering from diabetes mellitus had statistically significant increase in number of dehiscence compared to patients without diabetes (Table 2).

Table 2 Distribution of patients by dehiscence and DM2T.

		Diabetes (DM2T)		Total
		Yes	No	
Dehiscence	Yes	N 7	2	9
		% 20.6	3.8	10.5
	No	N 27	50	77
		% 79.4	96.2	89.5
Total		N 34	52	86
		% 100.0	100.0	100.0

However, this study did not determine if there was statistically significant connection between the sex of the patient and occurrence of dehiscence of colorectal anastomosis.

Also, this study observed the connection of procalcitonin and CRP as predilection factors for colorectal anastomosis dehiscence. Procalcitonin levels were increased in all patients with dehiscence, while they remained normal in patients without it. Based on the results of Fisher's exact test ($p=0.000$), it was concluded that there was statistically significant ($p<0.05$) connection between dehiscence and increased level of procalcitonin, and that increased level of procalcitonin implied the occurrence of anastomosis dehiscence (Table 3).

Table 3 Distribution of patients by dehiscence and increased procalcitonin levels.

		Increased procalcitonin		Total
		Yes	No	
Dehiscence	Yes	N 9	0	9
		% 100.0	0.0	10.5
	No	N 0	77	77
		% 0.0	100.0	89.5
Total		N 34	52	86
		% 100.0	100.0	100.0

CRP level was increased in 17 patients – in all patients with anastomosis dehiscence and in another 8 patients who had skin infection of surgical wound. Results of Fisher's exact test ($p=0.000$) showed that there was statistically significant ($p<0.05$) connection of dehiscence and increased CRP in patients, i.e. that occurrence of anastomosis dehiscence caused the increased level of CRP (Table 4).

Table 4 Distribution of patients by dehiscence and increased CRP level.

		Increased CRP		Total
		Yes	No	
Dehiscence	Yes	N 9	0	9
		% 52.9	0.0	10.5
	No	N 8	69	77
		% 47.1	100.0	89.5
Total		N 17	69	86
		% 100.0	100.0	100.0

DISCUSSION

Dehiscence of anastomoses is one of the most severe complications in colorectal surgery and despite the advancement of surgical techniques and treatment methods, it still occurs in significant percentage (8). Dehiscence of colorectal anastomosis occurs more frequently in rectal surgeries with preservation of sphincter. Occurrence of dehiscence significantly prolongs the treatment and increases the costs, while often causes the death (9).

Some studies state that numerous factors may cause the anastomosis dehiscence, classifying them into preoperative, intraoperative and postoperative (10). This study examined the connection of DM2T and occurrence of colorectal anastomosis dehiscence.

This study determined that DM2T represents the comorbidity that significantly affects the occurrence of colorectal anastomosis dehiscence. It was also determined that dehiscence occurs more often in patients who suffered from poorly regulated form of diabetes before the surgery.

DM2T leads to progressive and diffuse narrowing of small blood vessels (microangiopathy) and damage of large blood vessels (macroangiopathy) (11). In their study Jung et al. confirmed that macroangiopathy was characterized by atherosclerotic changes of blood vessel walls caused by high levels of blood sugar, but also high levels of blood lipids, which is a regular finding in patients suffering from DM2T. Also, increased atherosclerotic pressure causes the rupture, thrombosis and occlusion of blood vessels. Microangiopathic changes occur in small vessels, and together with macroangiopathic changes they lead to reduced perfusion of tissues and organs (12). Such disturbances of tissue perfusion often prevent healing of constructed colorectal anastomoses and cause their dehiscences.

Onodera et al. determined in their study that diabetes significantly causes colorectal anastomoses dehiscences compared to patients without DM2T. They determined that diabetes reduced the production of collagen I and III, and by that it directly affected the strength of colorectal anastomoses, which was especially pronounced on 7th day after the surgery, when dehiscence of colorectal anastomosis may occur (13).

Deng et al. performed retrospective study on 471 patients in the period 2014-2017 and researched the effects of several risk factors on occurrence of colorectal anastomosis dehiscence after the surgery performed by Dixon procedure. This study confirmed that among all risk factors diabetes mellitus had the strongest effect on healing of colorectal anastomoses and occurrence of dehiscence. Due to the stated reasons, it is considered that in patients with diabetes mellitus and other risk factors, an assessment of protective colostomy should be done after the construction of colorectal anastomosis (14).

The study of Zeng J. et al. determined that DM2T was one of important risk factors for disturbed healing of newly-constructed anastomosis and for occurrence of colorectal anastomosis dehiscence. Anastomosis dehiscence occurred in average around 7th postoperative day. Diagnostic confirmation of dehiscence was performed by CT scan (15).

Our study that was conducted on 86 patients classified into two groups – patients with and without diabetes, showed that in 34 patients with diabetes, anastomosis dehiscence occurred in 7 patients, while in the group of 52 patients without diabetes it occurred in 2

patients. Significant predictors of anastomosis dehiscence in our study were levels of procalcitonin and CRP, which were determined on third and fifth postoperative day.

Studies showed that concentrations of total collagen were nearly equal in patients with and without diabetes, but there was disturbance in quantity and quality of newly-formed collagen, especially in the synthesis of type I collagen. Such concentration of type I collagen was lower around day 7 after the surgery, but the level became normal around day 14 after the surgery. Due to these reasons, the risk of anastomosis dehiscence occurrence is especially pronounced around postoperative day 7 (13).

In our study, analyses of procalcitonin and C-reactive protein (CRP) levels as predictive factors of dehiscence were performed in all patients who were treated surgically, on the first and the third day after the surgery. All patients with anastomosis dehiscence had increased levels of procalcitonin and CRP above reference values on the third postoperative day. However, increased levels of CRP were found also in patients who had local infections of wound and other inflammatory changes, but no anastomosis dehiscence. Procalcitonin had much higher specificity as a marker in diagnosing of colorectal anastomosis dehiscence.

In their study, Hayati et al. analyzed the levels of procalcitonin as the predictor of dehiscence of colorectal anastomosis. They determined that procalcitonin was a reliable biochemical marker in diagnostics of dehiscence of colorectal anastomosis with sensitivity of 100% and specificity of 85%. In their study, levels of procalcitonin in patients with dehiscence were five times the normal value (16).

Also, in their multicentric observational study, Giaccaglia et al. tested procalcitonin as early predictive marker of dehiscence of colorectal anastomosis. The study included 504 patients who were surgically treated for rectal cancer, out of whom 28 had anastomosis dehiscence. Control values of procalcitonin and CRP were checked on the third and the fifth postoperative day. Obtained values had high specificity for dehiscence on both the third and the fifth postoperative day, so they may be regarded as early and reliable markers that significantly improve diagnosis of colorectal anastomosis dehiscence (17).

CONCLUSION

This study confirmed that DM2T represents significant risk factor for anastomosis dehiscence in patients with low resection of rectum. Because of this fact, such patients should be assessed for protective ileostomy in order to protect constructed colorectal anastomosis. This study also determined that levels of procalcitonin and CRP on the third postoperative day were multiple times higher in patients with anastomosis dehiscence compared to normal values. Due to its high specificity, procalcitonin may be used as significant predictive factor of dehiscence of colorectal anastomosis.

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Vascular structures relevant in facial dermal filler injections and their complications

Vaskularne strukture relevantne kod injekcija intradermalnih filtera i njihove komplikacije

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ABSTRACT

Intradermal filler injections have become increasingly popular in aesthetic medicine given the change in the face beauty philosophy from two- dimensional to three- dimensional face perception. Intradermal fillers are considered as safe non-surgical procedure although serious adverse effects have been reported. Vascular structures are particularly important in the pathophysiology and anatomy of those complications. We made a systematic review of English literature and extracted a total of 21 different vascular structures of which 15 related to arteries and 6 to veins. We narratively reviewed the most occurring structures and provided clinical advice for intradermal filler injections in the region of those structures. Understanding of clinical anatomy and correct technique are the most important variables in successful filler injection due to imperfect nature of the filler material itself. The glabellar complex structures (supratrochlear and supraorbital artery), the dorsal nasal artery and the facial artery together with its branches, the superior and inferior lip arteries are the most cited arteries in the literature and should be particularly studied by the aesthetic surgeon.

Key words: intradermal fillers, vascular anatomy, face, complications

SAŽETAK

Intradermalni fileri su sve popularniji u estetskoj medicine jer su se filozofije u pogledima ljepote lica promijenili od dvodimenzionalnih ka trodimenzionalnim shvatanjima lica. Injekcije intradermalnih filtera se smatraju sigurnim nehirurškim procedurama iako su ozbiljne komplikacije prijavljene. Vaskularne strukture su posebno bitne u patofiziologiji i anatomiji tih komplikacija. Napravili smo sistemski pregled literature na engleskoj jeziku i izvukli smo ukupno 21 različitu vaskularnu strukturu od kojih je 15 arterija i 6 vena. Napravili smo narativni pregled najčešće spomenutih struktura i pružili kliničke savjete za injekcije filtera u tim regijama. Razumijevanje kliničke anatomije i ispravna tehnika su najbitnije varijable za injekcije intradermalnih filtera shodno nesavršenoj prirodi materijala za filere. Strukture glabularnog kompleksa (supratrohearna i supraorbitalna arterija), dorzalna nazalna arterija i facijalna arterija zajedno sa svojim granama gornjom i donjom usnom arterijom su najčešće spomenute arterije u literaturi i trebale bi biti posebno proučene od strane estetskog hirurga.

Ključne riječi: intradermalni fileri, vaskularna anatomija, lice, komplikacije

INTRODUCTION

Injectable fillers are one of the most popular trends in aesthetic medicine and general medicine alike together with their predecessors neuromodulators (1,2,3). The popularity of injectable fillers is perceived to be the result of outstanding clinical value and popularity of botulinum toxin injections together with a more refined understanding of the aging face mainly due to the fact that a two-dimensional approach to anti-aging procedures is no longer appreciated and that a three-dimensional approach that encompasses facial volume is a prerequisite to adequate aesthetic treatment (2,4,5). The perceived characteristics of a youthful face are not merely a difference in philosophical values but also a clinical matter seeing that the look of the glabellar and perioral wrinkles requested

by the patients cannot be obtained by tension of the forehead and cheek. Therefore injectable fillers are needed, either standalone or combined with face lifting (5). The emergence of injectable fillers is also due to botulinum toxin popularity (2). Although botulinum toxin injections are used as measures against aging effects on the face, their usage is limited mainly to dynamic wrinkles. Seeing that the aging face goes through morphologic and physiologic changes including volume and fat depletion, skin atrophy and wrinkles are also discussed in this context (2,3). Wrinkles can be either dynamic or static, depending on the mechanism of their occurrence. Dynamic wrinkles are due to the mimical muscle actions and static ones are usually due to loss of volume either because of lipodystrophy or loss of water binders i.e. collagen. Botulinum toxin is limited to dynamic wrinkles whilst injectable fillers are used for static wrinkles, as either intradermal or

subcutaneous injections depend on the depth of the wrinkles (2). The location of the wrinkles is also important. The useful simplification is that the botulinum toxin injections are used for upper face aesthetic management and injectable fillers are most appreciated in the lower face (1,2,3). Although injectable fillers have been around for some 200 years, new materials for injectable fillers are another reason for their increased popularity (1). Fat injections are usually regarded as the oldest filler material although it is known that Carl von Reichenbach, a chemist, used paraffin as the first modern filler material (1,2). Gersuny and Corning also appreciated paraffin, although in different contexts (1). Paraffin proved itself unacceptable as injectable filler, after a period of overwhelming popularity complications arised, particularly granulomas dubbed as paraffinomas. Liquid silicone which popularity rose in the middle of the 20th century was made popular by James Hyde, another chemist. Liquid silicone had a fate similar to the paraffin- craze in the 1800s, given that adverse effects appeared after a period of grace, they too were due to late cellular reactions of the fourth type resulting in granulomas or siliconomas. Liquid silicone was banned by the FDA in 1992 (1). Although recent evidence shows that it can be useful as a non- biodegradable filler with proper technique and experience of the surgeon injecting the material (1,6). Nowadays, injectable fillers are classified as either non-biodegradable or biodegradable or permanent and non-permanent fillers (2,6). Generally non-biodegradable fillers are followed by late onset complications (1 year after injection) and are more susceptible to physician errors and require devoted choice of patients (7). Liquid silicone and polymethylacrilate are the most popular permanent fillers (1,2). Biodegradable fillers are generally more favorable with regard to complications of the fillers and are mostly responsible for early onset complications (less than 1 year after injection). The materials, usage and indications vary for different types of non- permanent fillers summarized in Table 1. The ideal filler is regarded as biocompatible, hypoallergenic, non-migratory, non-carcinogenic, non-toxic, resistant to infection with consistent and foreseeable reactions (1). Although the search for such filler has varied throughout these 200 years of filler usage, proper technique and knowledge of injections sites will be prerequisite no matter how ideal the filler is (4). The anatomy relevant for facial intradermal injections is most commonly featured as anatomy of upper, mid and lower face with layers of the face, muscles, vascular structures and nerves as basic components, although topography of the skin, fat pockets and aponeuroses also play a vital part of anatomy relevant for the aforementioned injections (4). The basic layers of the face from outer to inner are: skin, subcutaneous tissue, SMAS (superficial musculoaponeurotic system), retaining ligaments and tissues, periosteum and deep fascia. The fat tissue throughout these layers is seen as superficial, intermediate and deep. Superficial fat is the specific, although unrestricted to different regions and can be widespread throughout the subcutaneous plane (4). The deep fat is demarcated by fibrous tissue, either capsules or retaining ligaments from the superficial fat and is different in color and histological characteristics. Intermediate fat is embedded in the SMAS and serves as contour for the skin and connective medium for facial muscles (2,4). The layers of the face are particularly relevant in facial filler injections because of the aforementioned static wrinkles and their different depths (8). Nerves of the face are mostly relevant in the context of nerve blocks. The vascular structures of the face are of utmost importance for the aesthetic surgeons seeing that most

the aforementioned complications arise due to vascular involvement either as part of their etiology or pathophysiology (4,9). The purpose of this review article is to gather data, meta-analysis and narratively review the vascular anatomy relevant to filler injections and their complications.

MATERIALS AND METHODS

This review is a systematic review adhering to PRISMA rules. Eligibility criteria are either randomized controlled studies or other reviews due to the fact that randomized controlled trials are scarce in anatomic studies. The studies include mentioning or describing relevant vascular structures and complications related to those structures, either directly or through unspecific patomechanisms. The target studies included the indications and types of the used fillers. Studies that do not include filler alone and mention them in an auxiliary fashion like augmentation materials and anatomy relevant for botulinum toxin injections were reviewed and conforming data was extracted. Only the literature written in English was included in the study. The searched databases were MedLine and Cochrane database with the keyword: anatomy, filler, vascular, complications. The literature cited in the selected articles was reviewed for missed articles and books on the subject of aesthetic plastic surgery or particularly injectable fillers published in the last 5 years (2012-2017) were also reviewed. Three reviewers followed the PRISMA 2009 Flow Diagram and used the exclusion criteria to select the studies for reviewing criteria. The exclusion criteria were as follows: studies not mentioning injectable fillers, studies mentioning injectable fillers but not the relevant anatomy, studies mentioning the injectable fillers and their anatomy but not mentioning the complications related to them, studies not mentioning the vascular anatomy although included other relevant anatomy for filler injections i.e. fat anatomy, layers, skin topography. Studies related to animal experiments were also excluded. The following data was extracted from the selected studies: study characteristics (not published in this article), vascular structure, expected or related complication, indication for filler injections and type of filler used. The data was divided based on location (upper, middle and lower face) and also based on type of vascular structure (arteries or veins). A total of 43 articles were found using the database and 49 other after reviewing references and books. After initial screening and exclusion followed by analysis of the articles a total of 28 articles was eligible for data extraction. Due to the fact that both randomized clinical trials and systematic reviews were included based on the specificity of the matter a partial statistical analysis was performed and the focus was shifted to the narrative review of relevant structures thus a hybrid approach was deemed as optimal.

RESULTS

The total number of studies left after exclusion of studies that met the exclusion criteria was 28. A total of 94 vascular structures were mentioned in the context of intradermal filler injection complications of which 84 were arterial and 10 venous structures. A total of 21 particular structures were identified of which 15 were arteries and 6 were veins. The dorsal nose artery was most common (15.95%) fol-

lowed by supratrochlear (13.82%) and supraorbital artery (12.76%). Angular artery was mentioned 11 times (11.70%) and the facial artery 8 times (8.51%) as was the temporal superficial artery (8.51%). Superior (5.31%) and inferior labial arteries (4.25%), the lateral nasal, central retinal, submental and infraorbital were all mentioned twice (2.12%). The veins had 6 particular structures of which the angular vein was mentioned 4 times (4.25%) and the medial temporal vein twice (2.12%). The other veins were mentioned once each and included superior ophthalmic vein, the supraorbital and supratrochlear veins and intercanthal vein (1.06%). The ophthalmic artery was ubiquitous seeing that most complications arise from retrograde emboli and lead to ocular disturbances, the aforementioned artery was excluded from the statistics as it was present in all of the reviewed articles. As in the case with the ophthalmic artery the ubiquitous complication was blindness due to the fact that all of the distal branches of the ophthalmic artery can serve as pathways for retrograde embolism and resulting blindness (1,3,4,10,11,12,13). Although majority of authors state blindness as the complication other authors prefer the term ocular event due to the fact that blindness is not the only complication that can arise from retrograde emboli causing ischemia, blepharospasms, chemosis and even oculomotor nerve paralysis (14,15,16). The other common stated complications were skin necrosis whether generalized or specific like lip necrosis, alar necrosis or Nicolau syndrome (1,2,3,4,5,17,18,19,20). Cerebral events, mainly ischaemic strokes were described due to strong current retrograde embolisms into the middle cerebral artery (4,5,6,10,11,12). Other specific events include non-thrombotic lung embolism from filler injections into the middle temporal vein and chewing discomfort due to facial artery occlusions (12,19). Clinical anatomy rather than complications were implied in the case of globe perforations and arterial haemorrhage after attempted hyaluronidase injections to treat ocular events due to hyaluronic acid embolisms after intradermal injections (11). Other generalized complications associated with blood vessels pathophysiologically but not anatomically are rashes, edema, skin bleaching and granulomatosis and as such were not included in the summary tables. The indications were not described in most cases but are structurally bounded and as such were described in the summary tables for specific vessels. In most cases hyaluronic acid was the filler material of choice although autologous fat injections are responsible for most of described complications. Other mentioned filler materials were collagen and hydroxylapatite. In the rest of the results section we provide a narrative review of the relevant structures extracted from the data.

Supratrochlear artery

The supratrochlear artery is a skin bound branch of the ophthalmic artery and is frequently described together with the supraorbital artery due to their similar topographic parts. The supratrochlear artery pierces through the orbital septum and ascends superiorly for 20 mm after which it branches into two branches, the superficial and deep branch of the supratrochlear artery (4,8). Although the superficial branch is always present, the deep branch is missed in most cases dividing the arterial patterns of the forehead into two types: Type I (with superficial branches of the supratrochlear and supraorbital artery, with the deep branch of the supraorbital artery) and Type II (with both superficial and deep branches of the supratrochlear and supraorbital artery). The types are further divided depending on

whether an central dorsal artery is present medial to the superficial branch of the supratrochlear artery (10,21,31,32). It is safe to say that 20 mm proximally to the upper brow margin the supratrochlear artery becomes superficial to the frontalis muscle or subcutaneous (8,32). The deep branch if present follows the superficial branch below the frontalis muscle. Topographically the supratrochlear artery is presented by the corrugator crease which is the landmark for dividing the medial and lateral corrugator department, there it lies superficial to both the deep branches of supratrochlear and supraorbital artery, naturally and superficially medial to the superficial branch of the supraorbital anatomy (4,21,31,32). The filler injections have several clinical implications with the supratrochlear artery since it is located in the glabellar region, it is not only frequently encountered due to popular procedures at the glabella but is also dangerous since it branches from superior and medial arcade of the ophthalmic artery and therefore anastomoses with the supraorbital and angular artery provide a retrograde pathway to ocular events due to filler material embolisms (11,14,21). Care should be taken to specify the type of procedure performed since superficial creases at the glabella benefit from intradermal botulinum toxin injections resulting in risks regarding superficial branches of the artery or deep creases which are better dealt with using filler injections above the periosteum risking damage to the deep branches of the supratrochlear artery (4,8,32). The filler should be injected either lateral to the frontalis muscle beneath the passage of the temporal superficial artery or distal to 20 mm above the upper orbital rim, optimally with a cannula 23-25 gauge (4). If the dorsal angle is sharp or other aesthetic implications are at order the injections is performed medial to the medial canthal border, medial to the corrugator crease into the medial corrugator department until the periosteum is reached and then directed medial but not above 20 mm proximal to the upper orbital rim (4).

Supraorbital artery

The supraorbital artery is a branch of the ophthalmic artery that runs through the orbita after the division of the ethmoid artery. It is directed superiorly and exits through the supraorbital foramen. The supraorbital foramen is found approximately 10 mm lateral to the medial epicanthal line or corrugator crease which is equivalent to the breadth of one finger tip (4,8,32). Specifically, the supraorbital foramen is located 10.8 ± 4.9 mm laterally to the medial epicanthal line and 22.1 ± 2.6 mm from lower palpebral rim (21,32). The supraorbital artery courses superolaterally and becomes superficial in three different areas from the arcade between the superficial branches of the supratrochlear, temporal superficial artery and itself. The mean of superficial exit above the frontalis muscle is 29.6 ± 4.1 mm lateral to the medial epicanthal line and 20.7 ± 5.1 mm above the upper orbital rim (21,32). The deep branches continue the path of the supraorbital artery superolaterally or are divided into medial and deep branches. Care should be taken in the same manner as with the supratrochlear artery for the same reasons. It should be noted that taking the lateral approach to filler injections the final destination is equivalent to the foremost injection point of the medial approach, specifically the filler should end above the periosteum and not intradermally only medial to the corrugator crease or the medial epicanthal line and lateral to the forementioned line the injection should not go deeper than the subcutaneous tissue.

Dorsal nasal artery

The dorsal nasal artery was the most commonly mentioned artery in our review. Since the review encompassed both related structures and complications it is understandable since less filler material is needed to create a filler embolus than in the other arteries mentioned in this study (22,23,24). Non-surgical rhinoplasty is limited to concavity fixing but is fairly popular in the Asian population due to their flat dorsums of the nose (4,25,26,27). The variations of the dorsal nasal artery are also problematic since the aforementioned complications have been reported by surgeons using meticulous techniques. The dorsal nasal artery is a skin branch of the ophthalmic artery that is found 8-12 mm proximolaterally in regard to the medial epicanthal tendon (4,8). It courses distally in the superficially fat layer of the nose until it reaches the upper third part of the nose where it continues above the deep fat layer. The dorsal nasal artery anastomizes with the lateral nasal artery and provides an anatomic basis for retrograde emboli traveling. Reports have been made about bilaterally missing dorsal nasal arteries, with a radix artery present as compensation, a branch submitted by the lateral nasal artery (4,13,14,15,22,23,24,25). The radix artery is deep and midline situated so that it interferes with the recommended techniques for proper filler injections (4,13). A single dorsal nasal artery can be large and tortuous but most importantly it can traverse the midline of the nose to create an anastomosis with the lateral nasal artery (4,8). The traversing of the nose dorsum has been dubbed as the oblique artery and creates a particular problem for the aforementioned proper techniques (4,13). Random distribution of the dorsal nasal artery is present in 38% of the time (13). Other relevant arteries in the nose region are the columella branches of the superior lip artery which supply the tip of the nose together with the aforementioned arteries. They are located just above the medial curs of the great alar cartilage and should be avoided due to the risk of alar necrosis. Generally, superficial injections should be avoided in the columella region because of vascular complication due to the filler material osmolality and small gauge canulae should be avoided due to their high pressure insertions (4,13,22,23,24,25,30). A proper technique for dorsum injections should have ideally large gauged canulae, small gauge canulae and sharp tip needles should be avoided for the above stated reasons (2,4,13). The insertion in the tip of the nose should be made preperiostally or prechondrally since subperiostal and subchondral injections are hard to perform and result in suboptimal results. Insertion points should be changed if a bony ridge is encountered since injectors tend to direct the needle to the superficial layers and compromise the vascular structures (4). Retrograde and slow insertion is prerequisite since blood aspiration can be uncertain (4).

Facial artery

The facial artery is a branch of the external carotid artery and is divided into neck and head divisions based on its topography. The head division of the facial artery is located approximately 10 mm in front of the masseter muscle which it crosses, the artery then courses proximally beneath the risourius muscle, above the buccinator after which it crosses beneath the zygomaticus minor and major muscles. The facial artery proceeds toward the lateral side of the nose and proximally turns in to the angular artery. In the vermilion border the artery gives the superior and inferior lip arteries. In no more than

10% of the cases, the facial artery ends as the superior and inferior lip arteries (24). The artery is located 11 mm laterally from the vermilion measured with the skin and 9 mm measured from the mucosa with a depth of 15 mm beneath the skin (11,24). With regard to the nasolabial fold the facial artery can run either medially or laterally to the nasolabial fold and send medial and lateral branches to transverse the fold either way. A specific problem is the detoured branch of the facial artery which coordinates are divided into three spots (4,24,28,29). The first spot is 39 mm lateral to the midline at the level of the nose tip and 35.2 mm inferior to the horizontal line that passes through the medial epicanthal tendon at the level of the lateral iris border (4). The second spot 34.8 mm lateral from the midline in the middle nose level and 28.8 mm inferior to the horizontal line at the center of the iris. The third spot is 17.4 mm lateral to the midline at the radix level and 12.8 mm inferior to the horizontal line at the medial epicanthal line (4). The main indication for injectable fillers around the facial artery is the augmentation of nasolabial fold (24,28). Since the facial artery shows unpredictable variations as a rule of thumb, the injections should be made 1 cm above and inferior to nasolabial fold if needed (4,28). The other danger zone is 1.5 cm in all directions around the chellion (4,24). Precautions should be the same as for the rest of the described fillers.

Superior and inferior lip arteries

The superior and inferior lip arteries can be affected due to compromise or intravascular infarction and as such should be minded for. The superior lip artery arises from the facial artery in a square space of 15 mm near the chellion, approximately the size of a finger tip (4,24). It courses superior following the vermilion border of the upper lip through the orbicularis muscle or between it and mucosa. The inferior lip artery branches earlier, 15 mm beneath the branching of the superior lip artery and mostly does not follow the lower lip vermilion since it passes between depressor anguli oris and depressor labii inferior (2,4,8). The artery projects superiorly in the midline to end in the mucosa of the lower lip. Lip filler injections are made mostly to increase volume, but can be used to accentuate the borders of the lips (4,11). When used to accentuate the border of the lips, the injection should follow the vermilion of the upper and lower lips. For volume increase the injections are made in the submucosa or intramuscular. Caution should be given to approach the space from the skin side of the lips since the arteries are located between the muscles and the wet mucosa (4). Lip infarction has been reported as a complication due to intravascular injection of filler material (4,10,11,28,29).

Middle temporal vein

The middle temporal vein is located in temporal fossa, just lateral to the deep temporal fascia and receives tributaries from the superciliary, lacrimal, zygomaticotemporal and the zygomaticofacial veins. It runs horizontally and changes direction preauricular to receive tributaries from temporal superficial vein after which we designate it as the retromandibular vein. The middle temporal vein is clinically relevant in intradermal injection fillers due to the increasing popularity in temporal hollowing augmentation (4,12). It has been reported that filler material emboli can cause non-thrombotic lung embolia a therefore measures to assure prevention have been sought. It has been postu-

lated that preauricular pressure during injection in the temporal fossa can prevent such complications (12).

DISCUSSION

This study used a hybrid mostly narrative based systematic review. Although we used the PRISMA rules as a guide, the heterogeneity of the cases reviewed demanded such an approach. Facial filler injections are regarded as a safe alternative to aesthetic surgery, although it is evident that in some indications or anatomic regions, surgery is the method of choice (1,2,3,4,10,11). The concern for abuse from non-certified physician using facial intradermal fillers is also a problem given that thorough approach to face aesthetics is needed including neuromodulators and surgery together with facial fillers. Such an approach has been deemed three-dimensional instead of the classic two-dimensional understanding of aesthetic medicine (5). The general rules to adhere to when using facial filler injections should be the understanding of facial anatomy, since the filler material can change and no ideal material has been found, but the understanding of underlying anatomy should be paramount (1,2,4). A technique using retrograde and slow injection ensures adequate filler dispersion with the minimal risk for intravascular injection or vascular compromise (1,6). Large gauge canulae should be used in most cases ensuring minimum vascular involvement. Small gauge canulae inject the fillers under high pressure resulting in vascular compromise and sharp tip needles have a natural risk of rupturing the vascular structures (2,4). Specific for the nose region is the appreciation of the variations of the dorsal nasal artery and the understanding the repositioning of the entrance point can be useful if bony obstacles are encountered (13). Jung et al. classified the vascular patterns of the nose arteries into four types based on the supply of the nose tip whether it was from ipsilateral or contralateral dorsal or lateral nasal arteries (33). Tansatit et al. found 5 patterns of which the absent dorsal nasal artery with random distribution was most common it 38% with a total number of 19 out of 50 (13). Bilateral dorsal nasal arteries with different sizes were it 34% with 17 total specimens found. The facial artery has numerous variations that affect the safety of the nasolabial fold augmentation. The most common complications during nasolabial fold augmentation were skin necrosis and Nicolau syndrome (4,16,17,24). Phumyoo et al. described coordinates for the facial artery with regard to the facial muscles (24). The facial artery can end as the superior or inferior labial artery in no more than 10% of the population. Koh et al. report the facial artery ends as the inferior labial artery in 5.5% of the Korean population (34). In the West Indies, Loukas found that the facial artery ends as the inferior labial artery in 1.4% (35). The diameter of the facial artery varies in different topographic regions. Qassemmyar found a diameter of 2.8 mm in the mandibular region and Pinar described it as 2.6 mm (36,37). Phumyoo et al. described a diameter of 3.5 mm (24). Numerous complication can occur during facial filler injections, the most serious of all are ocular events particularly blindness. Li proved the retrograde embolism mechanism in his studies and other studies on rabbit ears have also confirmed the possibilities and mechanisms regarding these adverse effects (9,38). Tansatit et al. conclude that in other methods like manual manipulation such as pressure increase for prevention of non-thrombotic lung embolism are a prerequisite for further advancement of aesthetic medicine (12).

CONCLUSION

Understanding of facial anatomy will always be a prerequisite for successful application of intradermal fillers since most serious complications can be prevented based on thorough knowledge of vascular anatomy. The dorsal nasal artery and vascular structures of the glabellar complex are particularly important and are appropriately seen as danger zones. The course and localization of the facial artery is also paramount to nasolabial fold filler injections. Other than vascular anatomy, standardized instructions should be implemented regarding adequate technique to prevent intravascular penetration or vascular compromise. Such techniques include retrograde and slow injection, rethinking of the entry point of the injection and fast recognition of the complications.

Table 1 Upper face arteries relevant in facial filler injections.

Structure	Indications	Complications
Supratrochlear artery	Forehead augmentation, glabellar complex procedures	Skin necrosis, ocular events, Blindness, Stroke
Supraorbital artery	Forehead augmentation, glabellar complex procedures	Skin necrosis, ocular events, Blindness, Stroke
Temporal superficial artery	Forehead augmentation, glabellar complex procedures	Skin necrosis, ocular events, Blindness, Stroke

Table 2 Midface arteries relevant for filler injections.

Structure	Indications	Complications
Dorsal nasal artery	Non-surgical rhinoplasty	Skin necrosis, Alar necrosis, ocular events, Blindness
Lateral nasal artery	Non-surgical rhinoplasty, nasolabial fold augmentation	Skin necrosis, ocular events, Blindness
Columella branches	Non-surgical rhinoplasty, nasal tip augmentation	Alar necrosis
Facial artery	Nasolabial fold augmentation, lip augmentation	Skin necrosis, Lip necrosis, Alar necrosis, Nicolau syndrome, ocular events, Blindness
Angular artery	Nasojugal groove drooping, Tear through, Hyaluronidase injection	Nicolau Syndrome, ocular events, Blindness

Table 3 Lower face arteries relevant for filler injections.

Structure	Indications	Complications
Superior lip artery	Lip volume augmentation, lip border augmentation	Lip necrosis
Inferior lip artery	Lip volume augmentation, lip border augmentation	Lip necrosis

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Risk factors for colonization and infections caused by carbapenemase-producing enterobacteria in hospitalized patients: a systematic review

Faktori rizika za nastanak kolonizacije i infekcija prouzrokovanih enterobakterijama koje produkuju karbapenemaze kod hospitalizovanih pacijenata: sistematski pregled

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ABSTRACT

Introduction: in recent years there has been an increase in the number of infections caused by carbapenemase-producing Enterobacteria. They appeared at the end of the 20th century and only a decade later became a global threat. In order to effectively implement measures to prevent colonization and infections by these germs, it is very important to know the risk factors for their appearance. The aim of this systematic review is to determine the risk factors for the infection occurrence and colonization of hospitalized patients induced by carbapenemase-producing Enterobacteria. Materials and methods: the Pubmed and Cochrane database search was limited to clinical studies and studies in English language published in the last 5 years. The analysis included four studies evaluating the risk factors for the occurrence of infections, colonization and carriage of various Enterobacteria producing carbapenemases (CPE or CRE). Results: out of four analyzed studies, one was retrospective observational study, one retrospective, case-case-control study, one was case-control and one retrospective cohort study. The studies were published in the period from 2013 to 2017. The most isolated Enterobacteria were *Klebsiella pneumonia* (59.3%, 84.7%, 13-23.6%), *Escherichia coli* (3.7%, 4.6%, 40-72.7%), *Enterobacter cloacae* (5.7%, 3.6%). Assessment of risk factors by univariate and multivariate analysis, established the following: the most common risk factors associated with colonization and infections by carbapenem-resistant Enterobacteria, according to univariate analysis, are: wide usage of Carbapenems ($p=0.04$, $p<0.001$, $p=0.001$, $p<0.001$), Vancomycin ($p<0.001$, 0.001, 0.024), Beta lactams ($p=0.007$, <0.001 , 0.001), excessive use of broad spectral antibiotics ($p=0.01$, $p=0.001$), length of hospitalization, admission to intensive care unit, mechanical ventilation, exposure to invasive procedures and associated comorbidities. The most common risk factor associated with colonization and infections by carbapenem-resistant Enterobacteria, based on multivariate analysis, is admission to intensive care unit ($p=0.04$, $p<0.05$). Conclusion: risk of infections induced by carbapenemase-producing Enterobacteria is influenced

SAŽETAK

Uvod: posljednjih godina bilježi se ekspanzija infekcija prouzrokovanih enterobakterijama koje produkuju karbapenemaze. One su se pojavile krajem 20 vijeka a samo deceniju kasnije postale su globalna prijetnja. Da bi se efikasno provele preventivne mjere u smislu sprečavanja kolonizacije i infekcije pacijenata ovim uzročnicima, jako je važno poznavanje riziko faktora za njihov nastanak. Cilj ovog sistematskog pregleda je utvrđivanje faktora rizika za nastanak infekcija i kolonizacije hospitalizovanih pacijenata uzrokovanih karbapenemaza produkujućim enterobakterijama. Materijali i metode: pretraživali smo Pubmed i Cochrane bazu podataka. Pretraživanje smo ograničili na posljednjih 5 godina, kliničke studije i studije na engleskom jeziku. Analizom smo obuhvatili 4 studije koje su evaluirale procjenu riziko faktora na nastanak infekcija, kolonizacije i kliconoštva uzrokovanih različitim enterobakterijama koje produkuju karbapenemaze (CPE ili CRE). Rezultati: od četiri analizirane studije, jedna je bila retrospektivna observaciona studija, jedna retrospektivna, case-case controlna studija, jedna case-control i jedna retrospektivna kohortna studija. Studije su objavljene u periodu od 2013.do 2017. godine. Najčešće izolovane enterobakterije bile su *Klebsiella pneumoniae* (59.3%, 84.7%, 13-23.6%), *E.coli* (3.7%,4.6%, 40-72.7%), *Enterobacter cloacae* (5.7%, 3.6%). Procjenom riziko faktora, univarijantnom i multivarijantnom analizom utvrđeno je sljedeće: najčešći riziko faktori koji se dovode u vezu sa kolonizacijom i infekcijom karbapenem rezistentnim enterobakterijama koristeći univarijantnu analizu su: široka upotreba karbapenema ($p=0.04$, $p<0.001$, $p=0.001$, $p<0.001$), Vancomycina ($p<0.001$, 0.001, 0.024), Beta laktama ($p=0.007$, <0.001 , 0.001), i uopšte, prekomjerna upotreba širokospektralnih antibiotika ($p=0.01$, $p=0.001$), dužina hospitalnog boravka, prijem u jedinicu intenzivne njege, mehanička ventilacija, izloženost invazivnim procedurama i pridruženi komorbiditeti. Najčešći riziko faktor, koristeći multivarijantnu analizu, je prijem u jedinicu intenzivne njege ($p=0.04$, $p<0.05$). Zaključak: rizik za nastanak infekcija uzrokovanim

by individual risk factors such as: length of hospitalization, exposure to invasive procedures and extended antibiotic therapy. However, the local epidemiological situation of the environment should not be neglected. Acquiring knowledge about risk factors can be useful for promotion of preventive measures and treatment of infections caused by these microorganisms.

Key words: enterobacteria, carbapenemases, risk factors, epidemiology

INTRODUCTION

Nowadays, the expansion of antibiotic resistant microorganisms is considered to be the largest public health problem, primarily because it is associated with increased morbidity and mortality (1). They appeared at the end of the twentieth century, and only a decade later became the biggest public health problem and the global threat. Carbapenemase-producing Enterobacteria among the others are perhaps the biggest threat because they cause a wide range of infections with a high mortality rate. Clinically the most important in this group of bacteria is *Klebsiella pneumoniae*, which has become endemic in many countries, and is therefore the most common in clinical studies. However, other enterobacterial species such as *Escherichia coli* and *Enterobacter* species may also be the cause of various infections with an equally high mortality rate. These Enterobacteria produce different types of carbapenemases. The most common are IMP, KPC, NDM, OXA-48 and VIM carbapenemases. The aim of this systematic review is to assess the risk factors associated with the infections and carriage caused by Enterobacteria producing carbapenemases.

Risk factors for colonization and infections caused by Enterobacteria producing carbapenemases

In order to effectively implement measures to prevent colonization and infection of patients by these Enterobacteria, it is very important to know the risk factors for their occurrence. Studies dealing with this issue are not numerous and up-to-date. After the first isolation of OXA-48 carbapenemase from *K. pneumoniae* in Turkey in 2001, the same bacterial strain caused more hospital epidemics in the country (2,3) but after 2008 the strain was found in other Mediterranean countries, and the gene encoding the OXA-48 carbapenemase had spread among the other bacteria. Today, North Africa and the Middle East are considered the reservoir of OXA-48 producing strains (endemic areas) (4). OXA-48 has become the most common carbapenemase in Belgium, France, Malta and Spain. In Croatia, six strains of Enterobacteria producing OXA-48 β -lactamase were isolated for the first time, while four OXA-48 producing Enterobacteria isolates were reported in 2013 (5).

The assessment of risk factor in this case is not easy, as the studies are mostly related to local communities, different in design and often involve a small number of respondents.

The occurrence of infection is usually preceded by colonization period. The mostly involved are gastrointestinal, respiratory and urinary tract. After the colonization, it may take several days to several months for an infection to develop.

In endemic regions, the average risk for colonization by these

enterobakterijama koje proizvode karbapenemaze je pod uticajem individualnih faktora rizika kao što su: dužina hospitalnog boravka, izloženost invazivnim procedurama i dugotrajna antibiotska terapija, ali se ne smije zanemariti ni lokalna epidemiološka situacija sredine. Sticanje znanja o riziko faktorima, može biti korisno za unapređenje preventivnih mjera kao i za liječenje infekcije uzrokovanih ovim mikroorganizmima.

Ključne riječi: enterobakterije, karbapenemaze, riziko faktori, epidemiologija

germs is estimated to about 5%, based on the results of the study involving over 1000 patients in the intensive care unit, surgery department and rehabilitation in two large New York hospitals (11). Furthermore, the prevalence of colonization is time-limited, as shown by recent study conducted on patients admitted in intensive care unit, where the prevalence at the admission was 6-7% and grew to 27%-59% during the hospitalization (6,7).

Independent risk factors for colonization by carbapenemase-producing Enterobacteria

Primary risk factors for colonization by Enterobacteria are the same as for other nosocomial microorganisms such as ESBL (extended spectrum beta lactamases), gram negative bacilli, Methicillin resistant *Staphylococcus aureus*, Vancomycin resistant *Enterococcus*, *Clostridium difficile*. Each extended stay in healthcare institutions, long-lasting antibiotic therapy, as well as sensitivity to infections, represents independent risk factors for colonization and infections by these microorganisms. The use of broad spectral antibiotics is considered to be the primary cause of carriage state. The use of beta-lactam antibiotics, cephalosporins, quinolones, aminoglycosides, metronidazole, vancomycin and carbapenems are independent risk factors for colonization by these agents. It should be noted that the effect of these antibiotics may come as a result of long-term therapy. Previous exposure to carbapenems is considered a risk factor for the colonization in the range from 15% to 75%. Also, the use of other medical equipment increases the risk of colonization. Trips to countries endemic to these enterobacteria and contact with health care facilities therein, significantly contribute to the risk of colonization and infection by these germs. Percentage of patients who after colonization will develop an infection depends on the invasiveness of the causative agent and sensitivity of the organism. Although the data is highly deficient, it is estimated that between 10% and 30% of the colonized patients develop the infection (8). The percentage varies considerably and is probably higher in immunocompromised patients and those with comorbidities. The length of antibiotic therapy and the use of invasive procedures such as mechanical ventilation and urinary catheter application are also significantly associated with a higher percentage of infected patients.

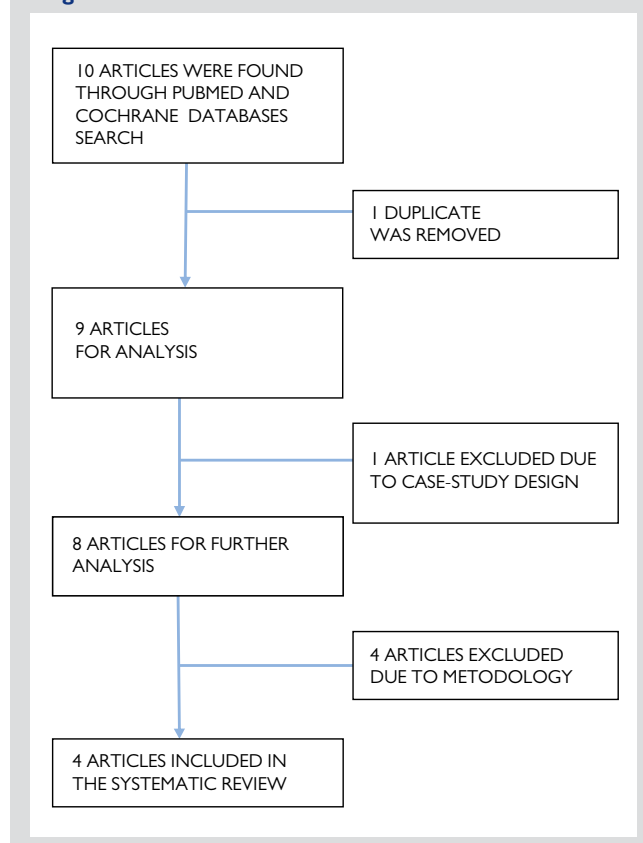
Given that the number of infected and colonized by carbapenemase-producing Enterobacteria grows, this is a matter of great importance globally. In order to prevent the occurrence of a massive CPE pandemic, it is necessary to coordinate the work of all health professionals and national authorities.

The aim of this systematic review is to determine the risk factors for the occurrence of infections and colonization of hospitalized patients induced by carbapenemase-producing Enterobacteria.

MATERIALS AND METHODS

The Cochrane and Pubmed databases were searched in the period from 4 to 15 April 2017. The PubMed search strategy was as follows: “(CRE or carbapenem-resistant Enterobacteriaceae or CPE or carbapenemase-producing Enterobacteriaceae) AND (Klebsiella or Enterobacter cloacae) AND (risk factors) AND (transmission or spread).” The search was also expanded to review of similar articles (similar articles). The filters used were: free full text, published in the previous five years and studies in English.

Diagram of studies selection



Selection of studies

This study included only studies investigating the influence of risk factors on colonization and infections of hospitalized patients caused by Enterobacteria producing carbapenemases. All case-studies and those primarily concerned with gene determination, genetic engineering, responsible for further horizontal transmission of resistance to antibiotic therapy were excluded. Therefore, we did not include studies that primarily dealt with molecular epidemiology.

Selected data

The selected data include the main characteristics of the study (name of the first author, year of publication, country, type of study, period of study, number of subjects), the most common risk factors estimated in univariate and multivariate analysis that result in colonization and infection by these Enterobacteria. Variables analyzed as risk factors included: 1 Demographic (gender), 2 Comorbidity (cere-

brovascular disease, chronic kidney disease, malignancy, chronic pulmonary disease), 3 Diabetes mellitus, 4 Hospitalization history (length of hospitalization, stay in the intensive care unit) 5 Exposure to invasive interventions (urinary catheter, mechanical ventilation, dialysis, surgical interventions), 6 Widespread use of antibiotics and types, 7 Presence of focal or generalized infection.

Table 1 Main characteristics of the study.

Name of first author	Country and year of publication	Study design and number of respondents	Study duration
Shanthi M. et al.	USA 2017.	Retrospective observational study 111	April 2010 - October 2010
Grace CL et al.	USA 2013.	Retrospective case-control study 104	June 2007 - June 2012
Swaminathan M. et al.	USA 2013.	Case-control 208	1 February 2009 - 31 January 2010
Gonzalez PT et al.	Mexico 2015.	Cohort study 330	1 January 2014 - 30 April 2014

Table 2 Risk factors for the CRE colonization and infection (Univariate analysis).

Risk factors for CRE infection	Shanthi M. CRE + N(%)	Shanthi M. CRE - N(%)	p	Grace C. Lee CRE vs. Control (OR 95% CI)	p	Mahesh S. Case-Control (OR 95% CI)	p	Pedro TG CRE + n/N(%)	CRE - n/N(%)	p
Male gender	50(66.7)	25(33.3)	0.05	0.55 (0.20-1.51)	0.25		0.99	114/275 (41.5)	32/55 (58.2)	
Length of hospitalization	33	22	0.93			1.034 (1.01-1.06)	0.001	8 (3-16)	15 (8-28)	<0.001
ICU admission	62(64.6)	34(35.4)	0.02					163/275 (59.3)	27/55 (49.1)	0.163
Mechanical ventilation	54(67.5)	26(32.5)	0.01	32.50 (1.55-4.04)	<0.001			230/275 (83.6)	48/55 (87.3)	0.499
Invasive devices			0.0111							
Urinary catheter				20.44 (5.53-75.62)	<0.001	2.82 (1.42-5.61)	0.003			
CVC				66.00 (14.4-301.6)	<0.001	6.6 (2.57-16.91)	0.001			
Diabetes mellitus	26(50)	26(50)	0.036	2.56 (0.86-7.62)	0.09	1.67 (0.88-3.16)	0.12			
COPD				6.47 (1.75-23.92)	<0.001	1.71 (0.89-3.31)	0.11			
Chr. renal disease				5.32 (1.87-15.09)	<0.001					
Cerebrovasc.				9.33 (0.98-88.56)	0.04					
Dialysis						2.67 (1.04-6.82)	0.04			
Malignancy						0.63 (0.36-1.09)	0.10	23/275 (8.3)	14/55 (25.4)	<0.001
Focal or generalized infection	49(69)	22(31)	0.013							
Surgery intervention	18(45)	22(55)				1.21 (0.66-2.22)	0.538			
Broad usage of antibiotics	40(71.4)	16(28.6)	0.01			67.25 (2.55-20.62)	0.001			
Carbapenem	36(70.6)	15(29.4)	0.04	236 (7.09-182.84)	<0.001	6.67 (2.83-15.72)	0.001	127/275 (46.1)	40/55 (72.7)	<0.001
β-lactams	13(40.6)	19(59.4)	0.007	18.86 (3.73-95.29)	<0.001	3.23 (1.73-6.02)	0.001			
Aminoglyco.				36 (7.09-182.84)	<0.001	2.25 (0.69-7.31)	0.18			
Vankomycin				42.67 (8.33-218.47)	<0.001	6.86 (3.10-15.15)	0.001	155/275 (56.4)	40/55 (72.7)	0.024
Previous stay in healthcare institution				14.46 (3.54-59.02)	<0.001	1.33 (0.76-2.35)	0.32	44/275 (16)	16/55 (29.1)	0.022

CRE – Carbapenem-resistant Enterobacteria
CVC – Central venous catheter
COPD – Chronic obstructive pulmonary disease
ICU – Intensive care unit

Table 3 Risk factors for CRE colonization and infection (Multivariate analysis).

Risk factors for CRE colonization and infection	Shanthi M. OR (95% CI)	p	Grace C. Lee Time at Risk-Adjusted CRE vs Controls OR (95% CI)	p	Mahesh S. Case-Control (OR 95% CI)	p	Pedro TG Prevalence (n=330) OR (95% CI)	p
Diabetes mellitus	0.613 (0.256-1.469)	0.272						
Admission to ICU	3.218 (0.609-17.000)	0.169	12.48 (1.14-136.62)	0.04			0.42 (0.20-0.88)	<0.05
Mechanical ventilation	1.502 (0.374-6.030)	0.566						
Invasive devices	0.459 (0.137-1.538)	0.207						
Focal or generalis Infection	2.013 (0.738-5.495)	0.172						
Carbapenems	0.762 (0.256-2.261)	0.624					2.54 (1.15-5.62)	<0.05
Surgical intervention	2.029 (0.765-5.386)	0.155						
Antibiotics exposure	0.560 (0.197-1.588)	0.275	1.47 (1.02-2.16)	0.04				
Dialysis					2.00 (0.33-122.4)	0.045		
Malignancy					0.82 (0.28-2.42)	0.72	4.02 (1.88-8.60)	<0.001
Cardiovascular diseases					1.13 (0.33-3.90)	0.85		
Pulmonary diseases					5.19 (1.07-25.31)	0.04		
Transfer from other healthcare institutions							2.16 (1.02-4.59)	<0.05
Invasive procedure							2.18 (1.10-4.32)	<0.05
Stay in the same room with colonis. or infected patient							3.0 (1.43-6.31)	<0.01

RESULTS

The results of the studies were published in the period from 2013 to 2017, and implemented in the period from 2007 to 2017. The studies included in this systematic review were retrospective observation study, case-control studies, and cohort studies comprising the total of 753 respondents. The most isolated Enterobacteria producing carbapenemases were: *Klebsiella pneumoniae* (59.3%, 84.7%, 13-23.6%) (10,11,12), *Escherichia coli* (3.7%, 4.6%, 40-72.7%) (10,11,12), *Enterobacter cloacae* (5.7%, 3.6%) (11,12) respectively, *Citrobacter freundii*, *Providencia rettgeri*, *Klebsiella oxytoca*. The most common localization of the obtained isolates was in blood, respiratory secretion, urine, exudative samples, sputum and wounds. Based on univariate analysis, the risk factors associated with colonization and infection by Enterobacteria producing carbapenemases are: male gender ($p=0.05$), admission to intensive care unit ($p=0.021$), mechanical ventilation ($p=0.013$), usage of invasive devices ($p=0.011$), diabetes mellitus ($p=0.036$), focal infection or sepsis ($p=0.013$), surgical interventions ($p=0.016$), consumption of broad spectral antibiotics ($p=0.007$), carbapenems ($p=0.042$). (9) The same study, using the multivariate analysis of the risk factors showed that none of them was associated with CRE. In the case-control study, based on the Univariate analysis, large number of patients had Charlson index of comorbidity 3 or higher (OR 6.61, 95% CI 2.27- 19.96), chronic obstructive pulmonary disease (HOBP) (OR 6.47, 95% CI 1.75 -23.29) and chronic renal disease (OR 5.32, 95% CI 1.87-15.09) as compared to controls. Respondents with isolated carbapenem-resistant Enterobacteria were more frequently admitted to the intensive care units as compared to controls (OR 49.61, 95% CI 11.24-218.90), had inserted central venous catheter (OR 66, 95% CI 14.40-301.6), were on mechanical ventilation (OR 2.50; 95% CI 1.55-4.04) and had urinary catheter inserted (OR 20.44 95% CI 5.53-75.62). The average exposure to health care institutions was significantly higher in the group with isolated CRE compared to controls (3 vs 1 $p < 0.001$). It was also established that larger number of positive CRE patients received beta-lactam antibiotics, fluoroquinolones, vancomycin, carbapenem and aminoglycosides compared to controls. CRE positive respondents had more antibiotic therapy days ($p < 0.001$) as compared to controls (10). Multivariate analysis in the same study showed that only two variables presented independent risk factors for acquiring CRE in hospitalized patients, namely: admission to ICU (OR 12.48, 95% CI 1.44-136.62) and total number of days on antibiotic therapy (OR 1.47; 95% CI 1.02-2.16).

In a case-control study of Swaminathan et al. (11), the Univariate analysis showed that respondents in the study group had longer hospital stay, which usually included insertion of central venous and urinary catheter, mechanical ventilation, more frequent antibiotic therapy (96 in the first and 71 in the control group, $p < 0.001$), and they received antibiotics of almost all classes. The average duration of antibiotic therapy was 53.9 vs. 14.4 days in the control group ($p < 0.001$). Factors independently associated with CRE include: pulmonary disease (OR 11.53), mechanical ventilation (OR 5.19), length of antibiotic therapy (OR 1.04), medium colonization pressure (OR 1.15). Multivariate analysis of two variables was significant for CRE infections: mechanical ventilation ($p = 0.02$), number of antibiotic therapy days ($p = 0.003$). In the cohort study (12), based on Bivari-

ate analysis, CRE carriers were mostly male of younger age, patients with transplanted organs, malignancy, and those moved from other healthcare institutions. Fecal carriers with carbapenem-resistant Enterobacteria were associated with intrahospital pneumonia, extended hospitalization, invasive procedures, treatment by carbapenems, vancomycin, ciprofloxacin, and piperacillin/tazobactam, or accommodation in the same room with CER carrier or infected patient.

Multivariate analysis showed that factors independently associated with CRE fecal carriers included carbapenem treatment (OR 2.54, CI 95% b1.15-5.62), transfer from other healthcare institutions (OR 2.16, CI 95% 1.02-4.59), hematological malignancy (OR 4.02, CI 95% 1.88-8.60), admission to intensive care unit (OR 0.42, CI 95% 0.20-0.88), invasive procedures (OR 2.18 CI 95% 1.10-4.32), stay in the same room with CRE carrier or infected patient (OR 3.0, CI95%, 1.43-6.31).

DISCUSSION

Studies investigating risk factors responsible for the spread of carbapenem-resistant enterobacteria are rare (9). In the 1990s, first carbapenem-resistant Enterobacteria were described, which resistance was mediated by chromosomally encoded β -lactamases called carbapenemases. At the beginning of the third millennium, we became a witness to the global crisis due to the rapid spread of multiple resistant *K.pneumoniae* strains that produce plasmids encoded carbapenemases. It is of great concern the fact that the occurrence and spread of gram-negative carbapenem-resistant bacteria, including *P. Aeruginosa*, *A. baumannii* and members of the Enterobacteriaceae family contribute to the increased morbidity and mortality of patients. The trend of spreading these β -lactamases between hospital and outpatient isolates is becoming a serious global threat. The problem is not only the endangering of effective clinical application of carbapenem, but also the generation of multiple resistant "super-bacteria". Because of the frequent placement of bla genes encoding carbapenemases on portable gene elements, infections caused by these strains often occur in the form of epidemics in hospital facilities, nursing homes, and palliative care facilities. Risk factor identification is very important in order to take effective strategies to reduce the spread of these strains. However, despite their expansions, available data on risk factors are very limited (10). In the studies included in this systematic review, broad use of antibiotics proved to be a significant risk factor for carriage and carbapenem-resistant Enterobacteria infection (9,10,11,12). Some studies, investigating risk factors, focused on exposure to antibiotic therapy (10). Proving the increased number of isolated CREs using carbapenem, fluoroquinolone, cephalosporin, imperatively emphasize the need to minimize the use of antibiotic therapy as well as the length of its use (10). It has also been shown that staying in the intensive care unit is an independent factor for carbapenemase-producing Enterobacteria infection (9,10). The transfer from other health institutions also contributes to the carriage of these strains. However, it should be taken into account that patients are moved from lower level institutions to a tertiary healthcare institution where they are treated. Most of them had complex medical conditions, already had prolonged treatment in the lower level institutions and most likely they had already been on therapy with broad spectral antibiotics (12). It

has also been proven that there are more independent risk factors for the occurrence of these infections such as use of mechanical ventilation and invasive procedures requiring the use of urinary catheter and central venous catheter (9,10,11). Previous research has shown that there are many risk factors for infection and carriage related to CPE: the severity of the disease, the intensive care unit stay, long-term antibiotic or immunosuppressive therapy, mechanical ventilation, foreign bodies (tubes, catheters). The migration of patients from endemic areas is also considered a risk factor for the infection and colonization of carbapenem-resistant Enterobacteria (13). In 2013, thirty-nine national experts throughout Europe followed the number and spreading of carbapenemase-producing Enterobacteria. 37 out of 39 stated that they were fully aware of the current epidemiological situation in their country. Three experts representing Island, Montenegro and Macedonia stated that there were no cases of carbapenemase-producing Enterobacteria in their countries. The sporadic cases are referenced by 21 experts. Representatives from three countries (Greece, Italy and Malta) reported that the CPE isolation was regular in most hospitals in accordance with the endemic situation. Thirty-three experts reported that *K. pneumoniae* was the most common enterobacterial species producing carbapenemases in their state. IMP, KPC, NDM, OXA-48 and VIM were the five most potent carbapenemases from Enterobacteria. Thirty-three experts reported that one or more of these most common carbapenemases could be isolated in their country. In five countries (Bosnia and Herzegovina, Estonia, Montenegro, Serbia and Macedonia), this data was not available (14).

Identification of risk factors associated with carbapenem-resistant infection assists in the empiric therapeutic decision-making process and also allows for early implementation of appropriate infection prevention measures (15,16).

Disadvantage of this systematic review relates to a small number of studies which are related to local communities and include a small number of respondents. Most of them are retrospective. Also, there are no studies assessing the risk factors associated with traveling to countries that are endemic to these types of infections as well as contact with health facilities therein.

CONCLUSION

The risk of infections caused by carbapenemase-producing Enterobacteria is influenced by individual risk factors such as: length of hospitalization, exposure to invasive procedures and long-term antibiotic therapy, but the local epidemiological situation should not be neglected. Acquiring knowledge of risk factors can be useful in promoting preventive measures and treating infections caused by these microorganisms. Due to the narrow therapeutic possibilities in the fight against the infections caused by these strains, crucial importance is reflected in rational use of antibiotics, timely diagnosis and consistent implementation of measures in preventing hospital infections. Rapid identification of colonized or infected patients, isolation and restriction of carbapenem use is of crucial importance in the spreading control of these multiresistant organisms.

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Analgesic effect of electrostimulation by vaginal probe in neuralgia n. pudendusa

Analgetski efekt elektrostimulacije vaginalnom sondom kod neuralgije n. pudendusa

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ABSTRACT

The pudendal nerve comes out through a large ischiadic foramen. Due to the change in anatomical relationships in a small pelvis, pressure on the nerve and the onset of pain can occur. The clinical picture is characterized by pain located in the zone of inertia n. pudendus, and may be accompanied by sexual dysfunction, disruption of the bowel and bladder discharge. There is no specific treatment for pudendal nerve entrapment (PNE). The treatment includes physical therapy, medication therapy, blockade of the pudendal nerve and surgical decompression. In our case, a 42-year-old woman was referred to our Clinic with the recommendation for urodynamic measurement (UDM). She complained about sharp, intense pain in a small pelvis increasing after emptying the bladder, decreasing in a lying position, and according to Visual Analogue Scale (VAS) it was graded 10/10. She denied any urinary and defecation disorders. Based on the medical documentation and clinical picture, a PNE sy was suspected. The patient was subjected to electrical stimulation of pelvic floor; modulation of pelvic pain. The stimulation resulted in lower pain intensity, and 5 days later the pain according to VAS was estimated to 7/10. The stimulation was extended to a total of 15 treatments which resulted in pain grade 3-4/10, a rare need for bladder emptying, subjective significant improvement in terms of everyday life functionality. A month later, the intensity of pain was at the same level, the patient was subjectively satisfied, and significantly more functional in everyday life.

Key words: neuralgia n. pudendus, electrostimulation, analgesic effect

SAŽETAK

Pudendalni nerv izlazi kroz veliki ishiadički foramen. Zbog promjene u anatomskim odnosima u maloj karlici, može doći do pritiska na nerv i pojave bolova. Kliničku sliku karakteriše bol u zoni inervacije n. pudendusa, a može biti praćena i seksualnom disfunkcijom, poremećajem pražnjenja crijeva i beške. Ne postoji poseban tretman za PNE. Primjenjuje se fizikalna terapija, medikamentna terapija, blokada pudendalnog nerva i hirurška dekompresija. U našem slučaju, 42-godišnja žena došla je na našu Kliniku sa preporukom za provođenje urodinamskog pregleda (UDM). Žali se na oštar, intenzivan bol u maloj karlici koji se povećava nakon pražnjenja beške, a smanjuje u ležećoj poziciji. Prema Vizuelnoj Analognoj Skali (VAS) bol je ocijenjena sa 10/10. Negira poremećaj defekacije i uriniranja. Na osnovu medicinske dokumentacije i kliničke slike, zaključuje se da se može raditi o PNE sy. Započinje se elektrostimulacija mm. pelvičnog dna, modulacija karličnog bola. Tokom stimulacije dolazi do poboljšanja u smislu smanjenja intenziteta boli. Nakon 5 dana bol po VAS-u procenjuje se na 7/10. Stimulacija se produžava na ukupno 15 tretmana nakon čega je VAS 3-4/10, rijetka potreba za pražnjenjem beške, subjektivno značajno poboljšanje funkcionalnosti u svakodnevnom životu. Nakon mjesec dana intenzitet bola na istom nivou, subjektivno zadovoljna, značajno je funkcionalniji u svakodnevnom životu.

Ključne riječi: neuralgija n. pudendusa, elektrostimulacija, analgetski efekt

INTRODUCTION

The pudendal nerve arises from the sacral plexus and includes segments S2, S3 and S4. It is a mixed nerve that transmits sensitivity from genitals such as penis, scrotum, clitoris and lips and innervates the muscles of the perineum and pelvic floor. The nerve comes out through a large ischiadic foramen (Figure 1). Due to the change in anatomical relationships in a small pelvis, pressure on the nerve and the onset of pain can occur (1,2).

The common cause of chronic pain in a small pelvis is the pressure on the pudendal nerve known as Alcock canal sy, pudendal neuralgia

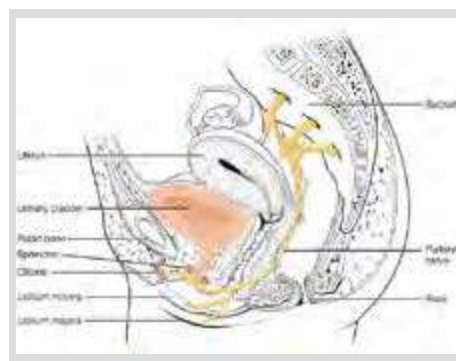


Figure 1
Retrieved from
https://en.wikipedia.org/wiki/Pudendal_nerve

(PN) or, most commonly, pudendal nerve entrapment syndrome (PNE) (2).

The clinical picture is characterized by pains located in the zone of innervation of the pudendal nerve, aggravated in a seated position, and accompanied by sexual dysfunction, disruption of the bowel and bladder discharge (3,4,5,6,7).

Nantes criteria (2) are used to set the PNE diagnosis, which includes: (1) pain in the anatomical area of the pudendal nerves, (2) worsening by sitting, (3) the patient is not awake at night, (4) an objective sensory loss at the clinical examination, and (5) a positive anesthetic block of the pudendal nerve. A detailed anamnesis, examination and appropriate diagnosis is required, which is a case-specific.

There is no specific treatment for PNE. It includes physical therapy, medication therapy (antidepressants, NSAIDs, anticonvulsants, muscle relaxants and opioids), blockade of the pudendal nerve (corticosteroids and/or local anesthetics) and surgical decompression of the nerve. Treatment should begin with less aggressive measures, i.e. conservative therapy which includes physical therapy, medication therapy and seating pads. When conservative therapy does not produce the expected result, a pudendal nerve block may be applied.

If the above techniques do not result in improvement, surgical decompression is usually considered.

CASE REPORT

Based on a neurologist's recommendation a 42-year-old woman was referred to the Clinic of Physiatry and Rehabilitation, Clinical Center University of Sarajevo (CCUS), for UDM. She complained of a sharp, intense pain in the small pelvis increasing after the bladder emptying, decreasing in a lying position, and according to VAS it was graded 10/10. She denied any urinary and defecation disorders. The pain occurred three years ago without any apparent cause. The intensity of pain limited her everyday activities. She was subjected to diagnostic treatment of a gynecologist, urologist, and neurologist. The magnetic resonance (MRI) of the small pelvis showed an enlarged uterus with several myomatous structures, sinisterrized, compressing the urinary bladder. Aerobes, anaerobes, Chlamydia, Ureaplasma, Mycoplasma were negative. Tumor markers were also negative. Gynecological diagnosis was: Cervicitis et adnexitis chr. Antibiotic therapy was administered. Urinoculture x3 negative. Ultrasound (US) of urinary tract was neat. UDM, performed on two occasions, did not show any neurogenic bladder dysfunction (regular sensation, no unwanted contractions of the detrusor, no escape of urine in increasing intra-abdominal pressure, regular capacity and complications). Cystoscopic mucosal biopsy: the findings were in favor of inflammation. Urological therapy was performed with antibiotics. Given that there was no pain regression she was referred to a neurologist due to suspected neuralgia n. pudendosa. MRI L/S spine showed locally discrete ventral osteophytic appositions, at i.v. level. L5/S1 enhanced fat retention directly ventral to the dural bag-epidural lipomatosis. The neurologist concluded that there was a possibility of compressive effect as a cause of pain, recommending the treatment with Neurontin (gabapentin) 300 mg 3x2 tbl, etodolac (Barsa) tbl 2x1. The follow-up examination continued without pain relief, and the treatment included escitalopram (Citram) 1x1, Paracetamol, Propifenazon, Kofein (Kofan) tbl pp. Given that there was no improvement she was referred to the small pelvis MRI,

electromyography (EMG) n. pudendus, UDM review. Repeated UDM did not show a neurogenic bladder dysfunction, but again she failed to empty the bladder after examination. EMG external sphincter showed medium heavy lesion of n. pudendus on both sides, more to the right with signs of spastic, less time compensation.

Somatosensory evoked potentials (SSEP) n. pudendosa - finding indications of mild asymmetric responses that may correspond to a neural lesion. SSEP - n. pudendosa - lesion of the sensory path of the right n. pudendosa. Th: Minipress (Prazosin), Rivotril (Clonazepam), Neurontin (Gabapentin), followed by a checkup. There was no improvement.

Uroflow was done in our Cabinet (voidCys was not repeated given that it was neat when examined on two occasions). Perineum overview: reduced sensation on the dermatome contact of S2, S3, S4, external sphincter tone present, voluntary contraction present. Uroflow: (urinary flow) Qmax 24 cmH₂O, (urinating volume) V 490 ml, (residual urine after urinating) PVR 60 ml.

Based on medical documentation and clinical picture, a PNE syndrome was suspected. Patient was subjected to electrostimulation procedure mm. pelvic floor, modulation of pelvic pain (Work time 2s. Rest time 4s, plate 9 mA) 30 min. on Laborie Medical Technologies. During the stimulation, there was improvement in the sense of lower pain intensity, and after 5 days the pain according to VAS was estimated to 7/10, and less frequent need for bladder discharge. Due to positive effect, the stimulation was extended to a total of 15 treatments which resulted in pain grade 3-4/10, rare need for bladder emptying, subjective significant improvement in terms of everyday functionality. A month later, the intensity of pain was at the same level, the patient was subjectively satisfied and significantly more functional in everyday life.

DISCUSSION

Causes and symptoms of neuralgia n. pudendus are very diverse. The flow of the nerves and the anatomical disturbance in the small pelvis can lead to pain and be accompanied by sexual dysfunction, disruption of the bowel and bladder. It is often difficult to get exact diagnosis and administer adequate therapy.

Jae Wook Lee, et al. (2) had a case of a sixty-year-old man who complained of tingling and pain in the right gluteal region and perineal area. Pain worsened as he sat, rated as 8 on the VAS scale. There was a lower intensity in the lying or standing position. In addition to other diagnostics, MRI was performed which revealed multiple cysts along the sacrospinous ligament at the entrance to the Alcock channel. They assumed that the anatomical site of ganglion cysts and pudendal nerves caused pressure on the nerve. About 4 ml of thick mucoid content was aspirated under ultrasonic guidance. His symptoms decreased after aspiration, and this improvement was maintained during 6-month follow-up.

Seckin, et al. (7) described a case of a 21-year-old man who as a consequence of an injury with a firearm in the small pelvis did not have erection. The finding of the EMG anal sphincter showed a mild and partial axon injury. Preoperative digital subtraction angiography (DSA) of the iliac artery revealed the lack of partial filling on the right distal pudendal artery and poor visualization of the right distal pudendal arteries. An operation to release the Alcock channel to the right was

done. There was a rise in the diameter of the right penile artery during the operation itself. Control pudendal angiography revealed normal flow in the right pudendal artery. The patient was monitored for 2 years and was free of malaise.

Vadim Petrov-Kondratov, et al. (8,9) presented a case of a successful treatment of a 51 year old woman with pudendal neuralgia by pulsed radiofrequency (PRF). She complained of pain in the groin and vulva. The first diagnostic right pudendal nerve block was performed using 3 mL of 0.25% bupivacaine with 6 mg betamethasone using a transglutaneous technique resulting in 8 hours of pain relief by 50%. A MR neurography of the lumbosacral plexus was done where an increased signal in the pudendal channel was obtained. The second pudendal nerve block was made using 3 ml of 0.25% bupivacaine with 40 mg triamcinolone acetonide. This procedure resulted in 8 hours of 100% pain relief. The patient decided to have PRF ablation to the pudendal nerve using a re-transglutaneous approach. After the ablation, the patient reported at least 6 weeks of significant (>50%) pain reduction.

In our case, the etiological diagnosis was not precisely set given that no blocking of n. pudendus was performed which could confirm or exclude suspicion of PNE based on clinical pictures, diagnosis and special EMG findings. Oral therapy did not give satisfactory result. The stimulation of the external sphincter showed a therapeutic effect in terms of pain reduction (from VAS 10/10 to VAS 3-4/10), frequency of urination, and improvement of the patient's quality of life, which lasted for a month. The patient was continuously monitored for eventual repetition of a treatment, recommendation for an attempt to block the nerve, or surgical treatment.

CONCLUSION

Etiology and symptomatology of neuralgia n. pudendus is diverse and requires detailed anamnesis, clinical examination, diagnostic treatment for setting up diagnosis and adequate therapy. Treatment should begin with conservative therapy comprising physical therapy, medication therapy and sitting pads. Antidepressants, non-steroidal anti-inflammatory drugs (NSAIDs), anticonvulsants, muscle relaxants and opioids are usually administered. When conservative therapy fails, a pudendal nerve block with corticosteroids and/or local anesthetics may be applied. If the above techniques do not result in improvement, surgical decompression is usually considered.

Conflict of interest: none declared.

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Therapy adherence and secondary prevention impact on osteoporotic fractures

Uticaj terapijske adherencije i sekundarne prevencije na osteoporotične frakture

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ABSTRACT

Introduction: lack of good education of patient and non-achievement of therapeutic adherence increases the risk of bone fractures in patients with osteoporosis, be it an interruption of the pharmacological or nonpharmacological component of treatment. **History of the disease:** a female patient, aged 83, was DXA diagnosed with osteoporosis 13 years ago (2004) (total T scor for L spine: -3.3 s.d.), and prescribed with bisphosphonate with calcium supplements. The patient was taking therapy for two years, which resulted in bone loss improvement but with lumbar spine finding still significant for osteoporosis (T score: -3.1 s.d). Afterwards the patient interrupted the therapy on her own. Also, she did not conduct the recommended kinesitherapy with osteogenic effects without exceeding the biomechanical competence or other physical procedures having osteogenic effects. She did not attend any checkups, regardless of the spinal pain and „stinging“, until April 2017. In the period from April to August 2017, she suffered three successive attacks of osteoporotic fractures, which were successively treated with vertebroplasty: in June at level L3, in July at levels Th8, Th9 and L2, and in August at level L4. At the time, the patient was not recommended for osteoporosis therapy. After the physiatric examination, the recommended DXA densitometry showed T score for LI: -3.19 s.d. Serum calcium level showed reference value and serum 25 (OH) -D vitamin level showed a value of 23.4 ng / ml (normally 30-100 ng / ml), which was in the domain of insufficiency. The following therapy was prescribed: denosumab subcutaneous injection of 60 mg at 6 months; daily calcium supplement of 1200 mg, daily magnesium supplement of 400 mg daily vitamin D3 supplement of 3200 i.u; physical therapy with an emphasis on kinesitherapy with osteogenic effects without overcoming biomechanical competence. The importance of regular and long-term therapy and regular checkups was explained. Three months later, the first check up revealed improvement of the muscular strength of the paravertebral and lower extremity muscles for one evaluation according to manual muscular test, independence in carrying out daily life activities with adopted protective spine positions, better posture during walking, and improvement of serum 25 (OH) -D vitamin level: 31 ng/ml. The next check up was scheduled in three months, along with the control of 25 (OH) -D vitamin level, and the control DXA densitometry was scheduled in 24 months. **Conclusion:** non-achieved therapeutic adherence is very important risk factor for bone fractures

SAŽETAK

Uvod: izostanak dobre edukacije pacijenta i nepostizanje terapijske adherencije povećava rizik za nastanak koštanih preloma kod pacijenata sa osteoporozom, bilo da se radi o prekidu farmakološke ili nefarmakološke komponente tretmana. Pacijentica staroj 83 godine dijagnosticirana je osteoporoza prije 13 godina (2004.) (ukupni T skor za L kičmu: -3.3 s.d.), te propisani bisfosfonati sa suplementima kalcijuma. Pacijentica je uzimala terapiju dvije godine, nakon čega je došlo do poboljšanja koštane gustoće, s tim da je nalaz na lumbalnoj kičmi i dalje bio u domenu značajne osteoporoze (ukupni T skor: -2.8 s.d.). Nakon toga pacijentica je samoinicijativno prekinula terapiju (2006.). Nije provodila ni proporučenu kineziterapiju sa osteogenim efektom bez prekoračenja biomehaničke kompetencije, niti je provodila druge fizikalne procedure sa osteogenim efektom. Bez obzira što je imala atake bola i „bockanja“ u kičmenom stubu, pacijentica se nije kontrolisala sve do aprila 2017. godine. U periodu od aprila do augusta 2017. godine sukcesivno nastupaju tri atake osteoporotičnih preloma, koji i sukcesivno budu zbrinuti vertebroplastikom: u junu na nivou L3, u julu na nivoima Th8, Th9 i L2, i u augustu na nivou L4. Pacijentici tada nije preporučena terapija za osteoporozu. Nakon fizijatrijskog pregleda uradi se preporučena DXA denzitometrija koja pokaže T skor za LI kralježak -3.19 s.d. Nivo kalcijuma u serumu pokaže referentnu vrijednost, a nivo serumskog 25(OH)-D vitamina pokaže vrijednost od 23.4 ng/ml (normalno od 30-100 ng/ml), što je u domenu insuficijencije. Propisana slijedeća terapija: denosumab subkutane injekcije od 60 mg na 6 mjeseci; dnevni suplement kalcijuma od 1200 mg, dnevni suplement magnezijuma od 400 mg i dnevni suplement D3 vitamina od 3200 i.j; fizikalna terapija sa naglaskom na kineziterapiju sa osteogenim efektom bez prekoračenja biomehaničke kompetencije. Objašnjena važnost redovnog i dugoročnog provođenja terapije, kao i redovnog kontrolisanja. Nakon tri mjeseca obavljen prvi kontroli pregled na kojem se ustanovi: poboljšanje mišićne snage paravertebralne i muskulature donjih ekstremiteta za jednu ocjenu po manuelnom mišićnom testu, neovisnost u izvođenju aktivnosti svakodnevnog života sa usvojenim zaštitnim položajima za kičmeni stub, bolja postura u hodu, te poboljšanje nalaza 25(OH)-D vitamina u serumu: 31 ng/ml. Planiran slijedeći kontrolni pregled za 3 mjeseca sa novim nalazom D vitamina, a kontrolna DEXA denzitometrija planirana za 24 mjeseca. Nepostignuta terapijska adherencija

in patients with osteoporosis. The absence of secondary prevention of osteoporotic fractures is still present in clinical practice. In order to prevent the emergence of further fractures in clinical practice it is necessary to develop a strategic program for measures to be taken.

Key words: osteoporotic fractures, therapeutic adherence, secondary prevention

INTRODUCTION

Depending on the condition of the bone system, etiology or therapy period, treatment of osteoporosis involves the inclusion of a pharmacological and non-pharmacological component, or only non-pharmacological components. This treatment requires good patient education in order to achieve the best therapeutic adherence, as well as long-term and continuous monitoring. The patient should be explained that osteoporosis therapy is a long-term, often lifelong process, and that there are different treatment periods, depending on the therapeutic response of bone tissue. In order to achieve the main goal, which is the primary and secondary prevention of bone fractures, therapeutic adherence in patients with osteoporosis would be achieved by a good doctor-patient relationship, which is primarily based on a good and continuous education of doctors.

Treatment of osteoporosis should be individualized and based on the clinical scenario of each patient, and a discussion about the treatment risks and benefits should take place between the doctor and the patient.

In order to prevent bone fractures, in May 2017 (1) the American Association of Physicians published guidelines for treatment of osteoporosis in men and women with low bone density or osteoporosis which was updated in November of the same year (2). The guidelines contain six recommendations: two strong recommendations, based on strong or moderate evidence, and four weak recommendations, based on poor evidence. Two strong recommendations are as follows:

- Doctors should offer a pharmacological treatment to women with diagnosed osteoporosis with alendronate, risedronate, zoledronic acid, or denosumab, in order to reduce the risk of hip and spinal fracture.
- In postmenopausal women, estrogen therapy or estrogen plus progestogen therapy, or raloxifene, should not be used to treat osteoporosis.

Four weak recommendations are as follows:

- In women with osteoporosis, the pharmacological treatment should last 5 years; Generic medicines should be used whenever possible.
- It is not recommended to monitor bone mineral density (BMD) during treatment for 5 years in women with osteoporosis, as evidence suggests that the risk of fracture formation may be reduced, regardless of changes in BMD.
- For women aged 65 and over who have osteopenia and who

je veoma važan faktor rizika za nastanak koštanih preloma kod pacijenata sa osteoporozom. Izostanak sekundarne prevencije osteoporotičnih preloma i dalje je prisutan u kliničkoj praksi. U cilju prevencije nastanka daljnjih preloma, u kliničkoj praksi je potrebno napraviti strateški program za mjere koje se trebaju poduzeti.

Ključne riječi: osteoporotični prelomi, terapijska adherencija, sekundarna prevencija

have a high risk of fractures, treatment decisions should take into account the patient's tendency, the risk of fracture, the therapeutic benefits, and the cost of the drug.

- In men with clinically recognized osteoporosis, clinicians should offer bisphosphonate therapy in order to reduce the risk of vertebral fractures; evidence of monitoring BMD in males is missing.

In the case of secondary osteoporosis, potentially healing causes should be excluded or treated.

Non-pharmacological treatment of osteoporosis and non-pharmacological preventive measures include physical modalities with osteogenic effect and modification of certain lifestyle factors. Physical procedures which so far have shown the best osteogenic effects are: kinesitherapy, pulsed electromagnetic field (PEMF) and low intensity pulsed ultrasound (LIPUS).

Kinesitherapy is still the most effective non-pharmacological method in the prevention and treatment of osteoporosis. Drugs for osteoporosis have less effect if no kinesitherapy is performed at the same time. The effects of kinesitherapy are reflected through the incorporation of calcium and other minerals into the bone, strengthening muscle strength, improving joint mobility, reducing pain, improving mobility, restoring self-confidence, improving posture and reducing stress on the bone, improving coordination and finally through fall and fracture prevention. It is important to determine the performance and dose of kinesitherapy procedures with osteogenic effect, without exceeding biomechanical competence, especially in patients with rheumatic diseases. These are: body weight exercises, muscle strength exercises, stretching exercises, coordination exercises, and exercises for correct posture. When dosing these exercises it is important not to find individual biomechanical competence. It is also important to ensure optimal calcium and vitamin D intake (3).

The pulsed electromagnetic field (PEMF) is increasingly used for the treatment of osteoporosis and osteopenia, as it has shown a very good osteogenic effect, that is increase of bone cell metabolism and improvement of the bone formation phase (4).

Low intensity pulsed ultrasound (LIPUS) has shown significant effects in bone formation, and its use in the treatment and prevention of osteoporosis is on the rise (5).

Surgical treatment includes vertebroplasty and kyphoplasty, procedures that represent minimal invasive procedures, and are used to treat painful osteoporotic compressive fractures of the vertebral columns. However, after these procedures, there may be an increased risk of developing fractures of adjacent vertebrae (6,7).

A 2008 review of the literature suggests that use of „reminder

plus education focused on doctors and patients" could lead to an increase in BMD testing and increased use of osteoporosis drugs (8). Furthermore, a reminder for the assessment strategy for patient's risk can obviously result in a reduction in fracture incidence and increased osteoporosis therapy. The authors concluded that multi-component tools for doctors and patients can support clinical decision-making in the management of osteoporosis therapy.

In patients with osteoporotic fracture, the first goal of rehabilitation is pain control. Vertebral fractures may be extremely painful and may cause short- and long-term morbidity. Ordinary analgesics can be administered, or analgesic physical modalities, such as transcutaneous electro nerve stimulation, or local thermotherapy can be applied. During this period, it is important to carefully monitor signs of constipation, urinary retention, or respiratory depression, which may occur.

CASE REPORT

An 83 years old female patient was diagnosed with osteoporosis 13 years ago (2004) (total T score for lumbar spine: -3.3 s.d.), and was prescribed bisphosphonates with calcium supplements. The patient was taking the therapy for two years, after which there was an improvement in bone density, with the finding on the lumbar spine still in the domain of significant osteoporosis (total T score: -2.8 s.d.). Afterwards, the patient self-initiatively stopped with her therapy (2006). Also, she did not perform prescribed kinesiotherapy with osteogenic effect without exceeding biomechanical competence, nor did she apply other physical procedures with osteogenic effect. Regardless of having pain attacks and posture deterioration, the patient did not go for check up until April 2017. In the period from April to August three osteoporotic fractures occur, being successively treated with vertebroplasty: in June at level L3, in July at levels Th8, Th9 and L2, and in August at level L4. The patient was not recommended treatment for osteoporosis. Afterwards, she was examined by a physiatrist and was recommended to do DXA densitometry, which showed the T score for the L1 vertebra of -3.19 s.d. Serum calcium levels showed a reference value, and serum 25 (OH) -D vitamin level showed the value of 23.4 ng/ml (normally 30-100 ng / ml), which was in the domain of insufficiency. The following therapy was prescribed: denosumab subcutaneous injection from 60 mg every six months over the period of two years, followed by oral weekly alendronat of 70 mg up to five years; a daily calcium supplement of 1200 mg, a daily magnesium supplement of 400 mg and a daily D3 supplement of 3200 i.u.; and physical therapy with an emphasis on kinesiotherapy with osteogenic effect without exceeding biomechanical competence. The importance of regular and long-term therapy, as well as regular control, was explained. Three months later the first check-up revealed the following: improvement of muscle strength of paravertebral and lower extremity muscles for one grade by manual muscle test, independence in performing daily life activities with adopted protective positions for the spine, better posture during walking, and improvement of 25 (OH)-D vitamin in serum: 31 ng/ml. The next check up was scheduled in 3 months with new D vitamin finding. Control DXA densitometry was scheduled in 24 months.



Figure 1 MRI of vertebral fractures:Th8,Th9 i L3 before vertebroplasty.

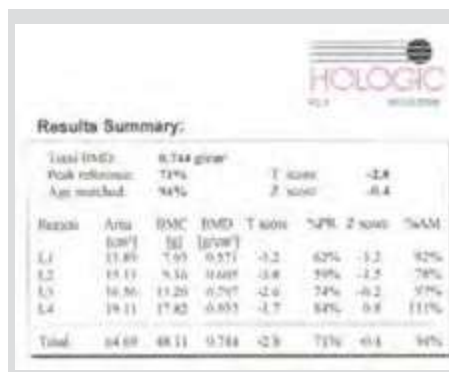


Figure 2 DXA densitometry after the therapy.

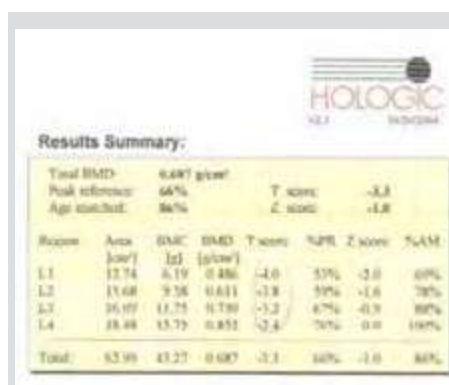


Figure 3 DXA densitometry before the therapy.

DISCUSSION

Poor therapeutic adherence is a well-recognized problem in the treatment of osteoporosis. As with other chronic diseases, poor adherence to the treatment of osteoporosis results in enormous damage to patients and healthcare resources. Most importantly, the low rates of therapeutic adherence consistently result in increased rates of fracture. However, efforts to improve therapeutic adherence in the treatment of osteoporosis seem to be on the rise.

Measures that can contribute to the improvement of therapeutic adherence in the treatment of osteoporosis include: improved patient education, improved doctor-patient relationship, and individual patient preference in assessing the therapeutic choice. Although significant efforts have been made over the past decade to implement these measures, further efforts are needed to be made in the future. Diagnosis, prevention and treatment of osteoporosis should be promoted as well as education of medical staff. It is hoped that these measures, with good pharmacotherapy tolerance, will enable optimum management of patients with osteoporosis, and eradicate this health problem (9).

Osteoporotic fracture is the strongest risk indicator for the onset of future fractures, and at least doubles the likelihood of further fractures. Therefore, the strategy of assessing the treatment of osteoporosis and secondary prevention along with the assessment of the risk of fall is of vital importance in an effort to reduce the risk of further fractures (10). Orthopedic surgeons are usually the first doctors who treat osteoporotic fracture. But their role does not end with the surgical management of fracture. They should play an important role in achieving future preventive and therapeutic measures through coordination with other clinicians (11). The need for a multidisciplinary continuous approach to the treatment of osteoporosis after osteoporotic fracture is well recognized (12).

However, although surgical treatment of an osteoporotic fracture is often the first treatment opportunity and clinical management of osteoporosis, many patients do not receive it. Several studies have shown that osteoporosis examination after fracture is less than optimal, and in patients with low-energy fractures, no assessment was made or received any treatment for osteoporosis (13). Estimation of osteoporosis should be done to all patients because it has been proven that the risk of further fractures in those in the „osteoporotic range“ is reduced by half after the initiation of antiresorptive therapy (14). In women and men with osteoporotic fractures of 75 years of age, it is not necessary to measure bone density and treatment with bisphosphonates is recommended, regardless of bone mineral density (15).

CONCLUSION

Unachieved therapeutic adherence is a very important risk factor for the development of bone fractures in patients with osteoporosis. The absence of secondary prevention of osteoporotic fractures is still present and creates a big problem in clinical practice. In order to prevent the onset of further fractures, it is necessary to develop strategic programs for measures to be taken.

Conflict of interest: none declared.

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Sažetak na našem jeziku, kao i na engleskom - max. 200–250 riječi, s najznačajnijim činjenicama i podacima iz kojih se može dobiti uvid u kompletan rad.

Ključne riječi - Key words, na našem jeziku i na engleskom, ukupno do pet riječi, navode se ispod Sažetka, odnosno Abstracta.

SADRŽAJ

Sadržaj rada mora biti sistematično i strukturno pripremljen i podijeljen u poglavlja i to:

- **UVOD**
- **MATERIJAL I METODE**
- **REZULTATI**
- **DISKUSIJA**
- **ZAKLJUČAK**
- **LITERATURA**

UVOD

Uvod je kratak, koncizan dio rada i u njemu se navodi svrha rada u odnosu na druge objavljene radove sa istom tematikom. Potrebno je navesti glavni problem, cilj istraživanja i/ili glavnu hipotezu koja se provjerava.

MATERIJAL I METODE

Potrebno je da sadrži opis originalnih ili modifikaciju poznatih metoda. Ukoliko se radi o ranije opisanoj metodi dovoljno je dati reference u literaturi. U kliničko-epidemiološkim studijama opisuju se: uzorak, protokol i tip kliničkog istraživanja, mjesto i vrijeme istraživanja. Potrebno je opisati glavne karakteristike istraživanja (npr. randomizacija, dvostruko slijepi pokus, unakrsno testiranje, testiranje s placebom itd.), standardne vrijednosti za testove, vremenski odnos (prospektivna, retrospektivna studija), izbor i broj ispitanika – kriterije za uključivanje i isključivanje u istraživanje.

REZULTATI

Navode se glavni rezultati istraživanja i nivo njihove statističke značajnosti. Rezultati se prikazuju tabelarno, grafički, slikom i direktno se unose u tekst gdje im je mjesto, s rednim brojem i konciznim naslovom. Tabela treba imati najmanje dva stupca s obrazloženjem što prikazuje; slika čista i kontrastna, a grafikon jasan, s vidljivim tekstom i obrazloženjem.

DISKUSIJA

Piše se koncizno i odnosi se prvenstveno na vlastite rezultate, a potom se nastavlja upoređivanje vlastitih rezultata s rezultatima drugih autora, pri čemu se citiranje literature navodi po važećim Vankuverskim pravilima. Diskusija se završava potvrdom zadatog cilja ili hipoteze, odnosno njihovim negiranjem.

ZAKLJUČAK

Treba da bude kratak, da sadrži najbitnije činjenice do kojih se došlo u radu tokom istraživanja i njihovu eventualnu kliničku primjenu, kao i potrebne dodatne studije za potpuniju aplikaciju. Obavezno navesti i afirmativne i negirajuće zaključke.

LITERATURA - Upute za citiranje - pisanje literature

Literatura se obavezno citira po **Vankuverskim pravilima**.

Svaku tvrdnju, saznanje ili misao treba potvrditi referencom. Reference u tekstu treba označiti po redoslijedu unošenja arapskim brojevima u zagradi na kraju rečenice. Ukoliko se kasnije u tekstu pozivamo na istu referencu, navodimo broj koji je referenca dobila prilikom prvog unošenja/pominjanja u tekstu. Literatura se popisuje na kraju rada, rednim brojevima pod kojim su reference unesene u tekst (ulazni broj reference), a naslov časopisa se skraćuje po pravilima koje određuje Index Medicus. Ukoliko je citirani rad napisalo više autora, navodi se prvih šest i doda "et al".

Vrlo je važno ispravno oblikovati reference prema uputama koje se mogu preuzeti na adresama National Library of Medicine Citing Medicine <http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=citmed.TOC&depth=2>, ili International Committee of Medical Journal Editors Uniform Requirements for Manuscripts Submitted to Biomedical Journals:

Sample References http://www.nlm.nih.gov/bsd/uniform_requirements.html.