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ЗА ЦЕЛОКУПНО ЛЕКАРСТВО Осн.1872

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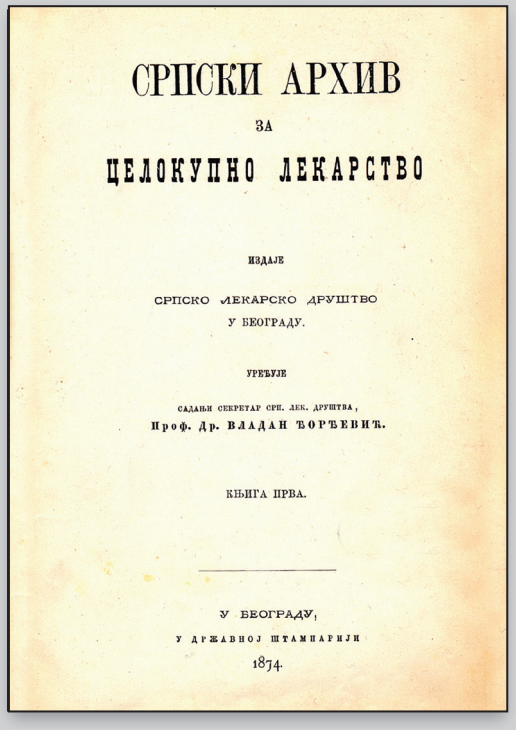

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ЗА

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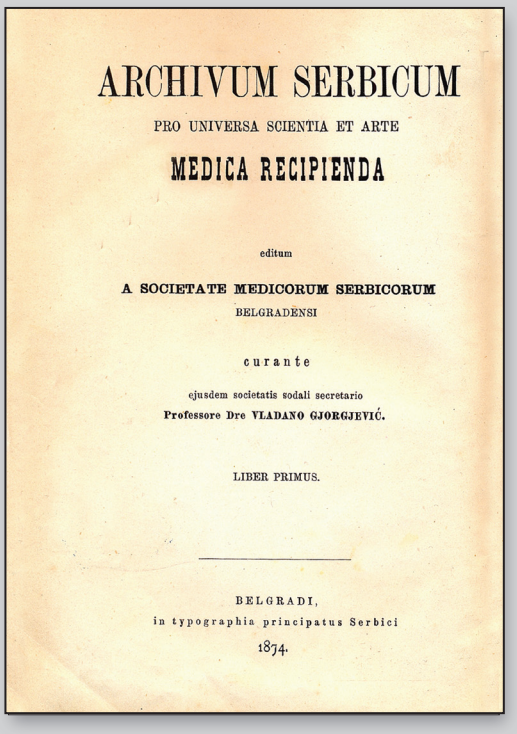

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ORIGINAL ARTICLES • ОРИГИНАЛНИ РАДОВИ

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Facial profile esthetics change of class II malocclusion patients treated with the Herbst appliance as perceived by orthodontists and laypersons

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SUMMARY

Introduction/Objective The recognition of differences in individual assessment of facial attractiveness could be valuable assistance in planning the orthodontic treatment.
The aim of this study was to compare facial profile attractiveness changes of patients treated with the Herbst appliance perceived by orthodontists and laypersons.

Methods The patient sample comprised 33 young Caucasian still-growing patients, aged 14–18 years, with skeletal class II malocclusion treated with the Herbst and multibracket appliances. Facial profile photographs before and after the treatment were shown to 54 orthodontists and 50 laypersons. In the esthetics oriented poll, the evaluators rated the change in facial appearance.

Results The attractiveness scores differed between the two rater groups ($p < 0.001$), with orthodontists being more generous, whereas there was no significant difference between female and male evaluators in both groups ($p > 0.05$). However, scores differed significantly in grading female and male patients ($p < 0.001$), so that female patients got higher scores; younger evaluators graded more critically between different age groups of the evaluators ($p < 0.001$), as well as between the patients with different initial severity of malocclusion ($p < 0.001$).

Conclusion The difference in attractiveness scores differed between two groups, with laypersons being more critical than orthodontists. Higher scores were given to female patients by both groups, as well as by the evaluators in the older age group.

Keywords: esthetics; malocclusion, angle class II; orthodontic appliances

INTRODUCTION

Today's orthodontic patients are mainly concerned about the esthetic outcome of the treatment, given the fact that their facial esthetics is jeopardized by a different type of malocclusion [1, 2]. The class II malocclusion deeply affects facial harmony and changes the patient's appearance. For the majority of class II patients, esthetic issues such as convex profile and retruded chin are the chief complaints when seeking orthodontic treatment and thus of primary importance [3].

Among various treatments of the class II malocclusion, combined orthodontics two-phase treatment which includes the Herbst and multibracket appliances provides significant skeletal changes, especially in adolescents and young adults, and highly improves the soft-tissue profile of these patients [4, 5, 6]. This fixed functional appliance straightens the facial profile due to the sagittal mandibular growth and has high efficiency even after the adolescent period. This appliance can be considered as an alternative to orthognathic surgery in adult patients, especially hesitating ones [7, 8]. Patients with less severe profile convexity problems are reluctant to accept surgical procedure, given the fact that problems like teeth alignment, large overjet, and beauty of the smile and face can be successfully treated with the fixed functional appliance. Therefore, for adult patients whose main concern is not their profile, the Herbst appliance can be considered a reliable alternative to orthognathic surgery [6].

Facial attractiveness is a complex issue, especially among adolescents, who tend to be strongly concerned about their facial appearance [9]. The recognition of differences in individual assessment of facial attractiveness could be valuable assistance in planning the orthodontic treatment.

Several studies have shown that the perception of facial attractiveness differs between patients, peers, and dental professionals [10, 11, 12].

Some studies showed orthodontists being more generous than laypersons [13–17], assumingly based on their clinical experience, while others reported agreement between evaluators of different professions [18, 19]. Nevertheless, prior knowledge of the treat-

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Figure 1. A patient's profile photograph before the treatment

Figure 2. A patient's profile photograph after the treatment

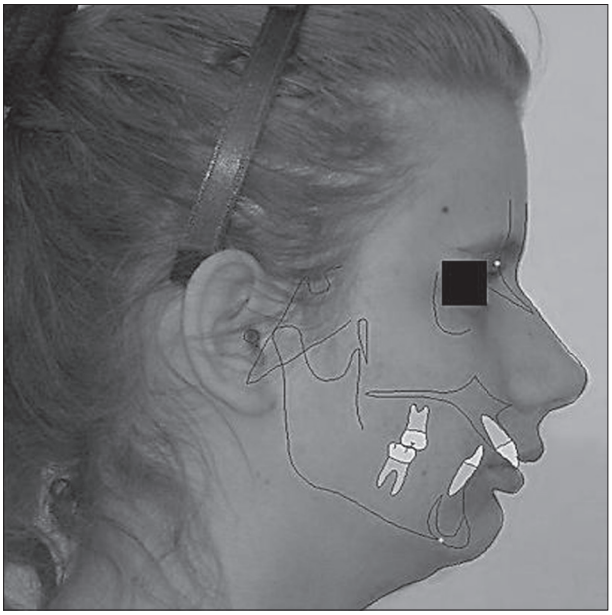

Figure 3. Profile cephalometric drawing superimposed on a patient's profile photo before the treatment

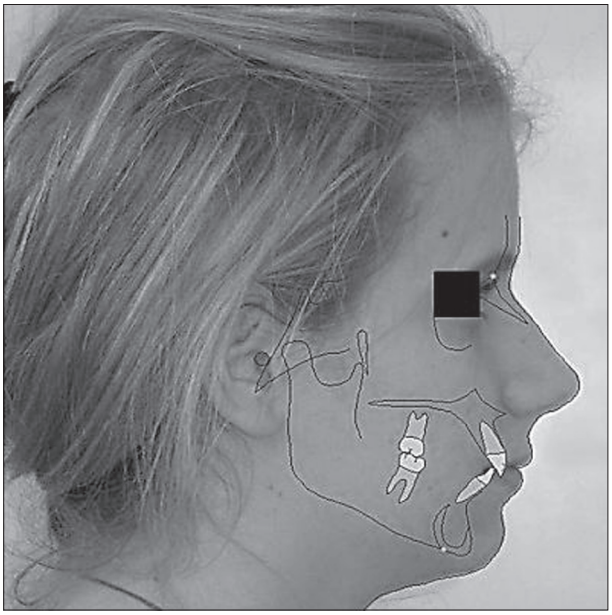

Figure 4. Profile cephalometric drawing superimposed on a patient's profile photo after the treatment

ment procedure usually causes biased view of treatment outcome and facial improvement evaluation in the group of orthodontists [1].

The purpose of this study was to analyze the difference in esthetic evaluations by orthodontists and laypersons of profile photographs of skeletal class II patients who had finished the treatment with the Herbst and multibracket appliances.

METHODS

The patient sample comprised 33 still-growing patients, aged 14–18 years, skeletal class II, division 1, and Caucasian ethnicity. All the patients were successfully treated with a combined two-phase therapy, which included the cast splint Herbst appliance followed by multibracket appliances treatment. The treatment duration was on average 20 months, respectively. After the combined two-phase treatment, all the patients achieved the Class I occlusion.

The patients' pre- and posttreatment profile photographs were used. The right-side profile photographs were taken in a standing position, in central occlusion. Before every recording, the operator ensured that the subject's forehead, neck, and ear were clearly visible (Figures 1 and 2).

All cephalometric points and measurements were carried out by one author (JM) and repeated after six months. The cephalometric analysis had indicated that all the patients had a skeletal class II pattern. (Figures 3 and 4). Key cephalometric parameters are shown in Table 1.

Table 1. Key cephalometric parameters

Parameter	Definitions	Before treatment Mean ± SD	After treatment Mean ± SD	p
SNA (°)	Upper jaw position in sagital plane	81.0 ± 2.8	80.1 ± 2.9	p < 0.001
SNB (°)	Lower jaw position in sagital plane	74.7 ± 3.3	76.6 ± 3.2	p < 0.001
ANB (°)	Sagital upper–lower jaw relationship	6.1 ± 1.8	3.9 ± 0.9	p < 0.001
SN/SpP (°)	Upper jaw position in vertical plane	9.6 ± 3.2	9.8 ± 3.5	p = ns
SN/MP (°)	Lower jaw position in vertical plane	32.4 ± 5.3	32.2 ± 5.2	p = ns
SpP/MP (°)	Vertical upper–lower jaw relationship	22.8 ± 4.9	22.4 ± 4.7	p = ns
Co-Gn (mm)	Condile–gnathion length	110.6 ± 5.0	113.0 ± 4.7	p < 0.001
I/SpP (°)	Upper incisor protrusion	121.5 ± 7.0	112.4 ± 4.9	p < 0.001
i/MP (°)	Lower incisor protrusion	95.3 ± 5.3	102.1 ± 7.9	p < 0.001
I/i (°)	Interincisal angle	120.8 ± 7.8	123.3 ± 9.8	p = ns
CmSnLs (°)	Nasolabial angle	113.6 ± 8.8	116.6 ± 8.6	p = ns
Si-LiPg (mm)	Mentolabial sulcus depth	-7.6 ± 1.7	-5.1 ± 1.1	p < 0.001
Ls-SnPg (mm)	Upper lip protrusion	5.1 ± 1.7	3.3 ± 1.7	p < 0.001
Li-SnPg (mm)	Lower lip protrusion	-4.4 ± 2.3	-3.9 ± 2.6	p < 0.001
G-Sn-Pg (°)	Facial convexity angle	159.6 ± 5.5	163.4 ± 7.1	p < 0.001

Eleven patients had a more pronounced malocclusion before the treatment (which was determined by the prominence of ANB (A point–nasion–B point) angle with the value of greater than or equal to 7°, with overjet larger than 10 mm, and the mentolabial sulcus depth of over 8 mm). In order to avoid bias and evaluators' presumption about patients with higher malocclusion severity, these patients were randomly infiltrated into the sample.

A group of 104 evaluators participated in the survey in order to judge the profiles of each patient. The evaluators were divided into two groups:

1) Orthodontist group comprised 54 specialists in orthodontics, 22 females and 32 males, mean age of 37.5 years;
2) Lay group comprised 50 people with no dental knowledge, classified as lay in the area; in this group, 34 were male and 16 were female and the mean age was 22.4 years.

Before grading, each examinee was asked to fill out a short questionnaire concerning gender, age (groups were divided according to 20–34- and 35–50-year ranges) and profession. All examinees were introduced with the grading procedure and it was explained to them that they were expected to evaluate only the change in facial attractiveness, comparing 'before' and 'after' photographs, not the level of attractiveness in general for each subject.

The experimental procedure used in this study can be defined as follows:

(a) The examinee is shown two black-and-white cropped photos of the patient, one before and one after the treatment, and is asked to grade the esthetics change, on a modified Likert scale, from '0 – no change' to '5 – excellent'.
(b) The photo pair is kept on the screen until the examinee clicks on one of the choices (0 to 5). This kind of electronic questionnaire provides privacy for each examinee and enables time and concentration during the grading of patients.

Ethical approval for this research was obtained from the Ethical Committee, Faculty of Dentistry, University of Belgrade. All the patients undergoing orthodontic treatment at the Clinic of Orthodontics signed informed consent prior to the treatment. The patients in this study have consented to their clinical information, including radiographs and photographs, to be used for any research or presentations associated with the Faculty of Dentistry, University of Belgrade.

Statistical analysis

SPSS ver. 18 software (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis of data. Descriptive statistics for the assessment of changes in the esthetic appearance were performed with the measure of central tendency (mean and median) and the measure of dispersion (standard deviation, min-max). Influence of each investigated parameter (variables concerning a patient's related factors, as well as an evaluator's related factors) on facial improvement scores, were analyzed using Mann–Whitney or Kruskal–Wallis tests, depending on the number of categories. Univariate and multivariate linear regression analyses were used to evaluate the relationship between facial improvement scores (dependent variable) and potential determinants. Statistical significance was defined as $p < 0.05$. Linear regression model was used to determine predictors of facial improvement scores. Differences were considered significant when the p-value was < 0.05.

RESULTS

Statistical significance was found for patient- and evaluator-related factors. In Table 2 one can observe higher mean improvement for female patients (mean = 2.5 ± 1.4), with the difference between patients' gender being statistically significant ($p < 0.001$). The mean improvement scores were also higher for patients with more pro-

Table 2. Patients' related factors

Parameters		Facial improvement scores				p
		Mean	SD	Median	Min-max	
Patients' gender	Male	2.4	1.4	2	0–5	p < 0.001
	Female	2.5	1.4	3	0–5	
Malocclusion prominence	Lower	2.4	1.4	3	0–5	p < 0.001
	Higher	2.6	1.4	3	0–5	

Table 3. Evaluators' related factors

Parameters		Facial improvement scores				p
		Mean	SD	Median	Min-max	
Evaluators' gender	Male	2.3	1.5	2	0-5	p = 0.060
	Female	2.8	1.4	3	0-5	
Evaluators' age	20–34 years	2.5	1.4	3	0-5	p < 0.001
	35–50 years	3.1	1.1	2	0-5	
Evaluators' profession	Orthodontist	3.1	1.2	3	0-5	p < 0.001
	Layperson	1.9	1.4	2	0-5	

Table 4. Linear and multiple regression analysis

Parameters	Univariate regression model		Multivariate regression model	
	B* (95% CI)	Significance	B* (95% CI)	p
Patients' gender	0.165 (0.065–0.265)	p < 0.001	0.200 (0.107–0.292)	p < 0.001
Malocclusion prominence	0.176 (0.078–0.275)	p < 0.001	0.209 (0.118–0.299)	p < 0.001
Evaluators' gender	0.590 (0.490–0.689)	p < 0.001	0.055 (-0.048–0.159)	p = 0.294
Evaluators' age	0.605 (0.254–0.956)	p < 0.001	0.556 (0.235–0.877)	p < 0.001
Evaluators' profession	-1.197 (-1.285–(1.108))	p < 0.001	-1.170 (-1.269–(1.071))	p < 0.001

*Unstandardized coefficient B

nounced malocclusion (mean = 2.6 ± 1.4), with statistically significant difference between these two groups of patients (p < 0.001).

Table 3 shows the difference between the evaluators' related factors, such as gender, age, and profession. The facial improvement scores were influenced by the evaluators' age (p < 0.001), as well as profession (p < 0.001). In the group of orthodontists, the mean improvement score was 3.1 ± 1.2, while in the layperson group this value was 1.9 ± 1.4.

Four out of five predictors in the regression model in Table 4 show to be significant: patient's gender (p < 0.001), prominence of malocclusion (p < 0.001), evaluator's age (p < 0.005), and evaluator's profession (p < 0.001).

Predictors of facial improvement scores

Multiple linear regression analysis was used to determine predictors that might have an effect on the attractiveness improvement scores. When univariate predictor showed significance for overall model, the multivariate regression model was introduced. In Table 4, one can observe that patients' gender, prominence of malocclusion, and evaluators' age and profession appeared as independent predictors of facial attractiveness improvement scores.

DISCUSSION

In this study, the patients were still-growing orthodontic patients whose primary concerns were the looks of profile, frontal view of their teeth, and smile. All the patients were in the post-puberty period of growth, which is adequate timing for using the Herbst appliance, given the fact that in this age clinicians could expect long-term stability with less probability of relapse, compared to class II treatment with removable functional appliances in growing adolescents (patients in the puberty period of growth). According to Pancherz and Ruf [4], this kind of treatment has a positive effect on facial attractiveness, due to anterior movement of the chin and thus straightening the profile. Moreover, this appliance displayed an effect on skeletal and dental features, and consequently on soft tissue structures which cover them [5]. It is worth mentioning that patients with a more severe class II skeletal problem (which highly affects profile appearance) are advised to seek surgical, rather than orthodontic treatment [6].

Dunlevy et al. [13] emphasized that opinions and grading of an esthetical change among orthodontists and layperson often differ. Therefore, it is of high importance for orthodontists to know what the public will consider as most relevant for improving a patient's face.

Many authors tried to determine whether and to what extent perception of facial appearance differs between professionals and laypersons. Some authors have reported general agreement between clinicians and the public [13–18], as opposed to some studies which showed differences between these two groups [11, 12]. This study conflicts with a couple of studies that have found that laypersons tended to be more generous in evaluating profiles than orthodontists [18, 19].

The difference in scoring facial change was found in both groups of evaluators, depending on the age of evaluators. The group of evaluators aged 35–50 graded facial change with higher scores than the younger group, aged 20–34. This result coincides with the study conducted by Naini et al. [20] who implied that older judges are more generous scorers than younger examiners.

This difference could also be explained by the fact that elderly people appreciate beauty and youth more than younger ones, who tend to be more critical [21]. Attractiveness scores were influenced by the gender of patients. This was the case with both raters group, and confirms data from the literature which suggest that attractiveness ratings of female patients are influenced by multiple factors [22, 23].

Nevertheless, in order to eliminate all extrinsic and intrinsic distracting factors for female patients (such as hairstyle, make-up, jewelry, and skin complexion) black-and-white cropped photographs were used (revealing only forehead, nose, chin, chin–throat length, up to the ear). Moreover, female patients got higher scores, given the fact that slightly convex profile is considered more attractive in females than males. This was in the line with the findings of von Bremen et al. [6].

However, the grading was not affected by the gender of evaluators in either group. This result is consistent with other studies, which also showed that there was no significant difference in attractiveness scores between female and male evaluators [24, 25, 26].

There are limitations to using a questionnaire to measure a subjective phenomenon, such as facial attractiveness. In the present study, biases results after presenting pre- and posttreatment photographs, especially in the group of orthodontists, are expected and could explain higher rankings than in the layperson group, which coincides with results from a previous study [1].

The results of this study showed that layperson group graded changes of facial attractiveness more critically. One can only assume that the lower grades in the layperson group present their unawareness of seriousness of this orthodontic problem and the complexity of the treatment procedure. However, an objective opinion could be one of the guidelines for orthodontists in their pursuit of patient's satisfaction with the treatment outcome.

The degree of patient's facial esthetics change after the treatment viewed from a layperson's perspective could provide valuable information for orthodontists. In order to avoid patient dissatisfaction after finished treatment, it is crucial for clinicians not to overestimate the treatment outcome as their perception of facial esthetics improvement is usually biased. Therefore, this type of study might help in coinciding the patient's wishes and the orthodontist's predictions on the outcome of the treatment.

One of the important aspects of the present study is the finding that in cases with more pronounced malocclusion the impact of the esthetical improvement is higher; this is contrary to what many practitioners typically expect and has been shown in other studies (it is often assumed that high initial deficiency would rarely result in something that represents a considerable improvement) [27, 28]. The bigger the change was, the bigger the impact on the difference in the average grade before and after the treatment. However, if the initial problem is relatively small, other distracting factors may have a dominant impact on the overall esthetics.

CONCLUSION

The results of this study showed an improvement of facial esthetics in class II malocclusion patients after the orthodontic treatment with Herbst and multibracket appliances. This research indicates a significant difference between orthodontists and laypersons judging the change in facial improvement after treatment, with orthodontists being more generous. Higher scores were given to female patients by both groups, as well as by the evaluators in the older age group. The severity of pre-treatment status noticeably influences the outcome of esthetic evaluations; thus, patients with a more pronounced malocclusion got higher scores in both rater groups.

ACKNOWLEDGMENT

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Процена промене фацијалне естетике код болесника са II класом малоклузије лечених Хербстовим апаратом

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САЖЕТАК

Увод/Циљ Препознавање разлика у индивидуалној процени фацијалне атрактивности може бити од велике помоћи у планирању ортодонтске терапије.

Циљ рада је био да се упореди промена атрактивности профила лица болесника третираних Хербстовим апаратом од стране ортодоната и лаика.

Методе Узорак се састојао се од 33 испитаника узраста 14–18 година, који су имали малоклузију II класе и лечени Хербстовим апаратом у комбинацији са горњим и доњим фиксним апаратом. Њихове профилне фотографије пре и после терапије прегледали су 54 ортодонта и 50 лаика. Они су оцењивали, у виду анкете, промену фацијалне атрактивности.

Резултати Оцене фацијалне привлачности разликовале су се између две групе ($p < 0,001$), тако што су ортодонти давали веће оцене, док није било статистички значајне разлике између полова у обе групе ($p > 0,05$). Међутим, резултати су се значајно разликовали при оцењивању мушких и женских болесника ($p < 0,001$), те су болеснице добиле веће оцене. Такође, резултати су се значајно разликовали између различитих старосних група оцењивача ($p < 0,001$), тако да је млађа група давала ниже оцене, те била критичнија у процени. Разлика је била значајна и код оцењивања болесника са различитим степеном изражености аномалије ($p < 0,001$).

Закључак Разлика у оценама фацијалне привлачности постојала је између две групе, те су лаици били критичнији у оцењивању у односу на ортодонте. Веће оцене добиле су болеснице од стране обе групе, а старија узрасна група оцењивала је већим оценама све болеснике.

Кључне речи: естетика; малоклузија II класе; ортодонтски апарати

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Stress and strain analyses of removable partial denture abutment tooth in relation to the position of the minor connector

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SUMMARY

Introduction/Objective For optimum loading distribution, the angle formed by the occlusal rest and the vertical minor connector from which it originates should be less than 90°.
The objective of the article was to visualize the optimum angle between the occlusal rest and the minor connector in terms of intensity and distribution of occlusal loads using finite element analysis. It was the intention, concerning biomechanical behavior, to document that the optimum angle between the occlusal rest and the minor connector should be less than 90°.
Methods Three different virtual models of partial edentulous Kennedy III class were created using the CATIA design computer program with different angles between the occlusal rest and the minor connector. Stress distribution after simulated occlusal loading was analyzed using the finite element method.
Results Comparing the results obtained for three models, the highest stress values were seen in model 3 (the angle between the occlusal rest and the small connector is greater than 90°) whether the load is applied in the middle or at the end of the saddle.
Conclusion Within limitations and on the basis of the study results, the minimum compressive stress was seen in model 1, where the angle between the occlusal rest and the minor connector was less than 90° whether the load is applied in the middle or at the end of the saddle. It is recommended that obtuse angle between the rest and the minor connector should be avoided due to potential hazardous stress concentration on abutment teeth.
Keywords: minor connector; occlusal rest; finite element analysis; stress and strain

INTRODUCTION

A tooth as a part of the orofacial system is subjected to great occlusal loads during normal function. As a result of occlusal loading, reactionary stresses are generated and distributed throughout the entire whole tooth structure. The same is factual for a tooth acting as an abutment tooth of a removable partial denture (RPD), where most occlusal forces are distributed from the occlusal rest to the abutment. Teeth and their supporting tissues are best suited for resisting axially directed forces [1]. When not loaded parallel to the long axis, such forces may generate stresses and strains in the tooth and the periodontal ligament, causing various problems, such as extreme tooth movement, non-carious cervical lesions formation, and cervical alveolar bone loss [2, 3]. Occlusal loads exerted on an RPD are transmitted to abutment teeth and oral mucosa. Therefore, when planning an RPD, one faces two different biological tissues and the need for even distribution of the occlusal and other forces on the periodontal tissue of the remaining teeth and in the mucoperiosteum on the edentulous alveolar ridge [4]. To employ the stated, the design of the RPD requires biomechanical considerations in order to minimize potential hazardous loading on supporting tissues. Therefore, each element in the RPD design should fulfill requirements concerning function and esthetics, but also enables patient comfort and preserves supporting tissue health and well-being.

A minor connector is the connecting link between the major connector of an RPD and the other units such as clasps, indirect retainers, and occlusal rests [5]. From the biomechanical perspective, it possesses a very important role to connect the aforementioned elements of the RPD to the major connector. In such a way it enables the RPD to act as a single unit rather than elements acting separately and individually. In this way, forces applied to one part of the RPD are transmitted to the other parts and are dissipated by all teeth and supporting tissues. For optimum loading distribution, angle formed by the occlusal rest and the vertical minor connector from which it originates should be less than 90° [6, 7]. Only in this way can the occlusal forces be directed along the long axis of the abutment tooth and slippage of the

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RPD away from the abutment can be prevented [7]. So far, there have been no biomechanical studies supporting the aforementioned statements.

The objective of this study was to visualize the optimum angle between the occlusal rest and the minor connector in terms of intensity and distribution of occlusal loads using finite element analysis. With this aim, the intention was to document in terms of biomechanical behavior that the optimum angle between the occlusal rest and the minor connector should be less than 90°.

METHODS

Three different virtual models of Kennedy III class partial edentulous were created using the CATIA design computer program (Dassault Systèmes, Vélizy-Villacoublay, France). The surface geometry of all three models was obtained based on digital data obtained by scanning denture, teeth, and jaw models. Three denture models were set up using lower jaw models of Class III partial edentulism with a tooth-borne removable partial denture. Morphologic details and dimensions were used to define a series of planes at different levels. The basic morphology outlines were reconstructed, with detailed morphological characteristics obtained from the literature [8, 9]. The teeth surfaces were reconstructed in the finite element models by fitting polynomial surfaces through geometric records. The geometric characteristics of occlusal rest seat were taken from the literature. Each rest seat was spoon-shaped, 1.5 mm deep, occupied one third of the mesiodistal length of the tooth, and was approximately one half the buccolingual width of the tooth, measured from cusp tip to cusp tip [10]. The occlusal rests that were fully fitted to the corresponding rest seats were separately produced as hemisphere shapes. The following three 3D models were created for this study:

1. The first model was that of a tooth-bounded saddle where the angle between the occlusal rest and the minor connector is less than 90°;
2. The second model was that of a tooth-bounded saddle where the angle between the occlusal rest and the minor connector is 90°;
3. The third model was that of a tooth-bounded saddle where the angle between the occlusal rest and the minor connector is greater than 90°.

The geometric characteristics of the tooth-bounded saddle were obtained by measurements of dimensions and shapes of saddles in a large number of master casts. From the basic geometry created, the elastic properties of various materials were attributed using approximate values found in the literature [11, 12] (Table 1). It was assumed that the mechanical behavior of the teeth, rests and minor connectors was linear elastic, homogeneous, and isotropic.

Table 1. Mechanical properties of the materials

Material	Young's modulus (MPa)	Poisson's ratio
Enamel	4.1×10^4	0.30
Dentin	1.9×10^4	0.31
Periodontal ligament	0.00689×10^4	0.45
Co-Cr alloy	23×10^4	0.33

According to literature data, the intensity of the occlusal force is within the range from 50 N in edentulous patients to 1000 N in extreme cases of a full dental arch [12, 13]. The values in the 25–300 N range are considered physiological for denture wearers. The occlusal force intensity of 250 N in RPD wearers was found by Witter et al. [14].

For this reason, a vertical load of 250 N was applied according to two simulated situations:

1. In the first simulation, the load was applied in the middle of the tooth-bounded saddle in all three models;
2. In the second simulation, the load was distributed at the end of the tooth-bounded saddle in all three models.

Each model was meshed structurally with solid elements defined in tetrahedral bodies. The final models had a total number of 42,176 elements and 63,572 nodes for model 1, 53,141 elements and 77,213 nodes for model 2, and 60,119 elements and 80,123 nodes for model 3.

Described virtual three-dimensional finite element models of the tooth-bounded saddle with a different angle between the occlusal rest and the minor connector were analyzed using the ANSYS 6.1 (ANSYS, Inc., Canonsburg, PA, USA) FEA program.

RESULTS

The results of the study are presented graphically as maps of stress distribution within the saddle and the occlusal rest minor connector junction.

When a vertical load was applied over the middle of the tooth-bounded saddle, the highest maximum compressive stress was found in the saddle area at the site of applied load in the model where the angle between the occlusal rest and the minor connector was modeled as less than 90° (Figures 1 and 2). Under the same condition of loading,

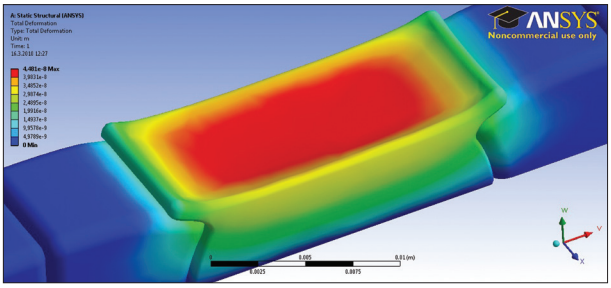

Figure 1. Stress distribution in model 1 when the load was applied in the middle of the tooth-bounded saddle

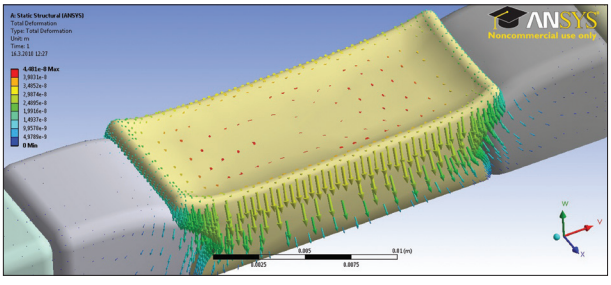

Figure 2. Schematic diagram of stress distribution in model 1 when the load was applied in the middle of the tooth-bounded saddle

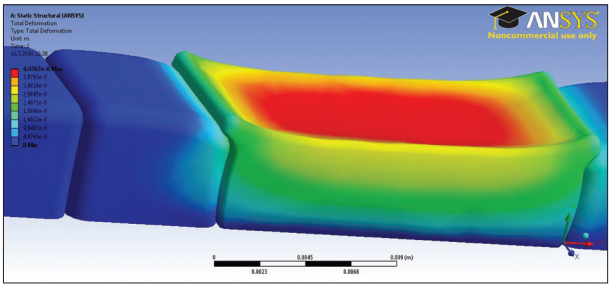

Figure 3. Stress distribution in model 2 when the load was applied in the middle of the tooth-bounded saddle

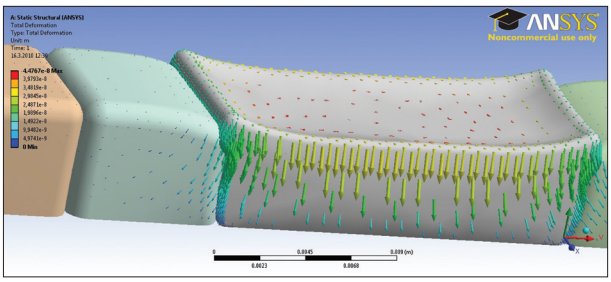

Figure 4. Schematic diagram of stress distribution in model 2 when the load was applied in the middle of the tooth-bounded saddle

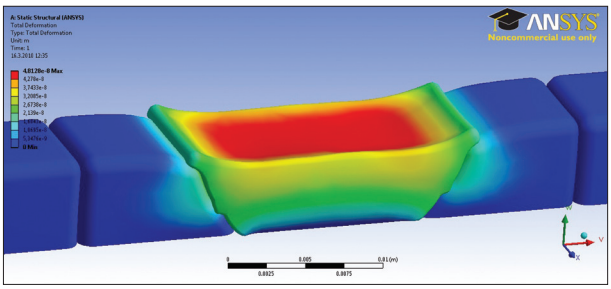

Figure 5. Stress distribution in model 3 when the load was applied in the middle of the tooth-bounded saddle

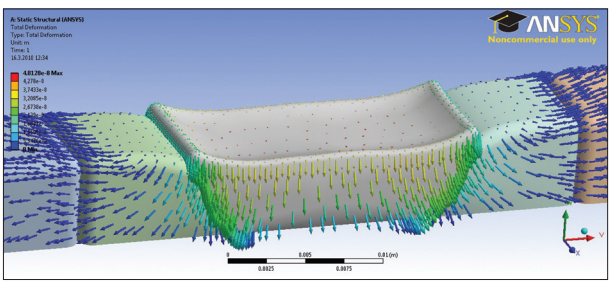

Figure 6. Schematic diagram of stress distribution in model 3 when the load was applied in the middle of the tooth-bounded saddle

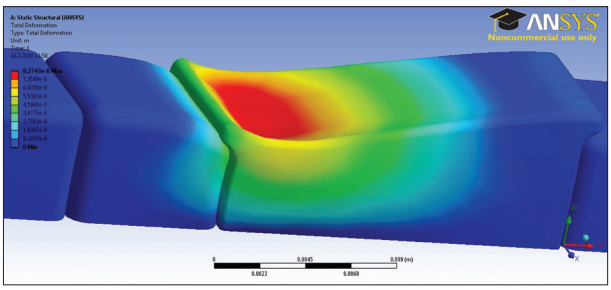

Figure 7. Stress distribution in model 1 when the load was applied at the end of the tooth-bounded saddle

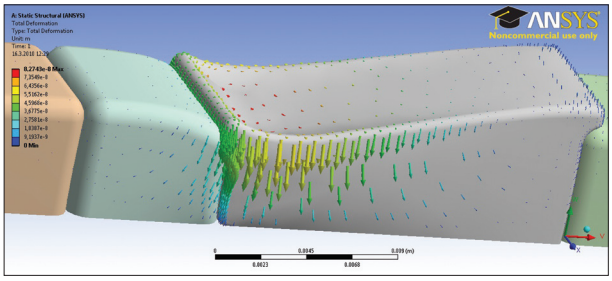

Figure 8. Schematic diagram of stress distribution in model 1 when the load was applied at the end of the tooth-bounded saddle

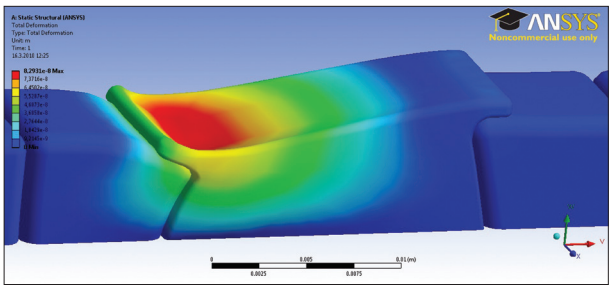

Figure 9. Stress distribution in model 2 when the load was applied at the end of the tooth-bounded saddle

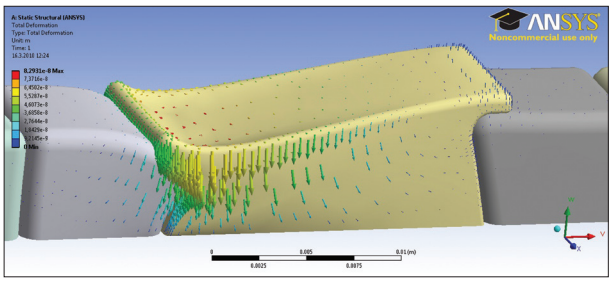

Figure 10. Schematic diagram of stress distribution in model 2 when the load was applied at the end of the tooth-bounded saddle

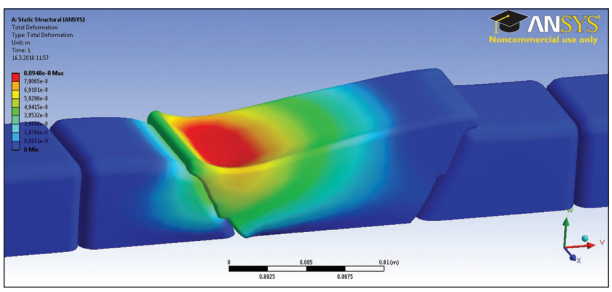

Figure 11. Stress distribution in model 3 when the load was applied at the end of the tooth-bounded saddle

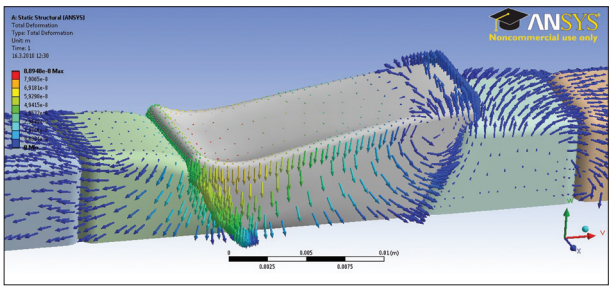

Figure 12. Schematic diagram of stress distribution in model 2 when the load was applied at the end of the tooth-bounded saddle

Table 2. Maximum and mean stress values in all models when the occlusal load was applied in the middle and the end of the tooth-bounded saddle

Models	Load applied in the middle of the saddle	Load applied at the end of the saddle
Model 1	σ_{max} = 2.6 MPa	σ_{max} = 5.4 MPa
	σ_{mean} = 1.2 MPa	σ_{mean} = 2.9 MPa
Model 2	σ_{max} = 2.5 MPa	σ_{max} = 6.4 MPa
	σ_{mean} = 1.2 MPa	σ_{mean} = 2.4 MPa
Model 3	σ_{max} = 4.5 MPa	σ_{max} = 7.7 MPa
	σ_{mean} = 2.0 MPa	σ_{mean} = 3.5 MPa

the stress intensity decreased increasing the distance from the loading site with a uniform distribution throughout the whole saddle. The pattern of stress distribution is the same in model 2 – the angle equals 90° as seen in Figures 3 and 4), whereas the stress intensity increases. Concerning the third model with an obtuse angle between the occlusal rest and the minor connector, the stress was also the highest at the loading point and gradually distributed to the supporting tissues (Figures 5 and 6). Accordingly, as seen in Table 2, the highest stress values after loading the middle of the saddle are obtained in the third model, where the occlusal rest minor connector angle is modeled as greater than 90° (Table 2).

When vertical load was applied at the end of the tooth-bounded saddle, the pattern of stress distribution was different to that seen in the simulated situation of loading in the middle of the saddle. The loading on one side of the saddle promotes unequal stress distribution with the dominant concentration of stresses at the loading point, seen in all three models (Figures 7, 9, and 11). The schematic view of stress distribution in all three models under vertical loads with the point of attack at the end of the tooth-bounded saddle is shown in Figures 8, 10, and 12. It is evident that there is a stress concentration in the saddle structure as well as in the abutment tooth on the side of the applied load. By comparing the results obtained for three models, the highest stress values were obtained in model 3 (Table 2).

DISCUSSION

Since the intention of the study was to visualize and document stress and strain distribution in the junction between the occlusal rest and the minor connector, the computer simulations were simplified. Creating the virtual models was done without intense morphological details, especially when anatomy details of the abutment tooth are concerned. Accepting the simplifications involved in the study, the values of stresses encountered during occlusal loading simulation were considered more qualitatively than quantitatively. Another limitation of this study concerns the intensity of occlusal force applied to the saddle and the rest afterward. The phenomenon of any horizontal movement of the rest was neglected and the RPD was assumed stable, as it is obliged to be when designed properly. Moreover, the assumed isotropic, homogeneous, and elastic characteristics of the materials may present the limiting factor in the study. However, despite the materials' intrinsic anisotropic nature, there is still no competent literature data concerning inhomogeneity and anisotropy. Since results were not considered quantitatively, one may speculate that such limiting factors did not have a contributing effect on the obtained data.

When simulating loading in the middle of the saddle, uniform distribution of stresses on abutment teeth and surrounding tissues is visible. On the other hand, loading applied on one side of the saddle exerted higher stresses on the abutment on that side. Accepting the aforementioned, it may be speculated that from the clinical perspective one is obliged to create uniform occlusal contacts in harmony with the natural dentition. Premature contacts on one side of the saddle will cause the potentially unstable leverage effects and might overload the abutment with consecutive side effects.

After evaluating the obtained results it is evident that the angle formed by the occlusal rest and the vertical minor connector from which it originates should be less than 90°. The angle greater than 90° fails to transmit occlusal forces along the supporting vertical axis of the abutment tooth with generated higher stresses. Also, the results of this study showed that the highest maximum compressive stress was found in the saddle area at the site of applied loads in all models. The minimum stress values were seen in model 1, where the angle between the occlusal rest and the minor connector is less than 90° whether the load is applied in the middle or at the end of the saddle. The findings that horizontal axis of the occlusal rest should be inclined toward the abutment to prevent slippage of the prosthesis away from the abutment are in agreement with previous researches [9]. The opposite was found by Sato et al. [15], who stated that such inclination may cause a high-stress concentration. According to them, a standard-shape rest with a zero degree horizontal axis produced less stress and may prevent slippage. Despite the statement that the inner line angle of an occlusal rest should be rounded [16], results of the study by Sato et al. [15] showed that over-roundness was associated with the high-stress concentration and decreased yield strength. The authors explained that such results may be attributed to the fact that the loaded point moved to the thinnest portion (the most protruded point of occlusal rest base). Although minority of scientific studies are dealing with the occlusal rest biomechanical behavior, it may be, however, stated that stress distribution on the residual ridge beneath the RPD base is dependent on the occlusal rest design [17].

CONCLUSION

Despite the defects in the model geometry and the implemented assumptions, the results still can provide some mechanical insight of the influence of the angle between the occlusal rest and the minor connector on stress distribution on supporting tissues. Within limitations and on the basis of the study results, the minimum compressive stress was seen in model 1, where the angle between the

occlusal rest and the minor connector was less than 90° whether the load was applied in the middle or the end of the saddle. Therefore, it may be confirmed that, from the biomechanical aspect, the optimum angle between the occlusal rest and the minor connector should be less than 90°. It is recommended that an obtuse angle between the rest and the minor connector is avoided due to potential hazardous stress concentration on abutment teeth.

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Анализа напона и деформација унутар ретенционог зуба парцијалне скелетиране протезе у зависности од угла са малом спојницом

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САЖЕТАК

Увод/Циљ Оклузални наслон и мала спојница треба да заклапају међусобни угао мањи од 90 степени, како би се обезбедило најповољније преношење оптерећења.
Циљ рада је био да се методом коначних елемената прикаже угао између оклузалног наслона и мале спојнице који је најповољнији за преношење оклузалног оптерећења. Намера је била да се, посматрано са биомеханичког аспекта, документује да је угао мањи од 90 степени између оклузалног наслона и мале спојнице најповољнији.

Метод Израђена су три различита виртуелна модела крезубе вилице класе крезубости Кенеди III у програму *CATIA* са моделованим различитим угловима између оклузалног наслона и мале спојнице. Анализа дистрибуције напона и деформација након симулираног оклузалног оптерећења извршене су методом коначних елемената.

Резултати После симулираног оклузалног оптерећења сва три модела највећи напон је уочен код модела 3 (угао између оклузалног наслона и мале спојнице већи од 90 степени), без обзира на то да ли је оптерећење апликовано на средини или на крају седла.

Закључак У оквиру ограничења у истраживању, најмањи компресиони напон уочен је у моделу 1 (угао између оклузалног наслона и мале спојнице мањи од 90 степени) без обзира на то да ли је оптерећење апликовано на средини или на крају седла. Препоручује се да се туп угао између оклузалног наслона и мале спојнице избегава због могућих штетних концентрација напона на ретенционом зубу.

Кључне речи: мала спојница; оклузални наслон; метода коначних елемената; напон и деформације

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Incidence and morphological features of thyroid papillary microcarcinoma in Graves' disease

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SUMMARY

Introduction/Objective Association of Graves' disease (GD) and thyroid cancer is reported in a wide range from 0% to 33.7%. Papillary thyroid carcinoma (PTC) is the most commonly diagnosed malignancy in GD, namely its variant – papillary thyroid microcarcinoma (PTMC). The increasingly frequent PTMC disclose favorable biological behavior with low mortality and recurrence rates.
The aim of this work is to report our experience on the frequency and morphological features of PTMC in surgically treated patients with GD.

Methods Over a period of three years, total or near-total thyroidectomy was performed in 129 patients with GD.

Results Incidental PTMC was diagnosed in 24 (18.7%) patients with GD. The mean tumor diameter was 3.03 ± 2.17 mm. The average age of patients in the GD with PTMC group was 48.50 ± 13.07 years, while in the GD without PTMC group it was 41 ± 13.12 years, and it proved to be statistically significant (p = 0.045). Most of the PTMC were unifocal (83%), and the most common morphological features of PTMC were intraparenchymal localization (62.5%), follicular morphology (66.7%), and infiltrative growth pattern (62.5%). Extrathyroidal extension, lymphatic invasion and multifocality of PTMC were more commonly related with subcapsular localized PTMC. The presence of at least one nodule in the GD with PTMC group was 58.3%, while in the GD without PTMC group it was 26.7%, and it was statistically significant (p = 0.003).

Conclusion Our results showed a high incidence of PTMC (18.7%) in patients with GD. Clinically, the most important morphological characteristics of PTMC were related with its subcapsular localization.

Keywords: Grave's disease; thyroid papillary microcarcinoma; morphology

INTRODUCTION

Graves' disease (GD) is an organ-specific autoimmune disease of the thyroid gland that occurs in the presence of autoantibodies to TSH receptors, leading to gland hyperfunction, hyperproduction of hormones (thyroxine, triiodothyronine), and the development of a specific clinical presentation [1]. Macroscopically, the thyroid gland is usually diffusely enlarged, and the histological picture is characterized by follicular hyperplasia with intraluminal/follicular infolding, occasionally in the form of papillary proliferation. Thyroid gland lobularity and vascularisation are increased and it is possible to detect a patchy lymphoid infiltration (LI) in the stroma. In long-standing medically treated Grave's disease, a nodular transformation of the adenomatous type can be detected, as well as development of different degrees of fibrosis, cellular atypia and oncocytic cell transformation [2, 3]. Association of GD and thyroid carcinoma is well documented with frequencies ranging from 0% to 33.7% [4–8]. The most common malignancy in reported studies of GD is papillary carcinoma (PTC), namely its variant papillary microcarcinoma (PTMC) – defined as incidentally discovered PTC with size less than or equal to 10 mm [9]. The increasingly frequent PTMC disclose favorable biological behavior with low mortality and recurrence rates [10, 11, 12].

The malignant potential of well-differentiated thyroid carcinomas of follicular origin in GD is still contradictory. Some studies suggest that immunological basis of GD, which is characterized by permanent autoantibody stimulation of gland epithelial and tumor cells, as well as the presence of antiapoptotic Il-4 and Il-10, could affect the growth, survival, and biological behavior of thyroid carcinomas [6, 13, 14, 15].

The aim of this work is to report our experience on the frequency and morphological features of PTMC in surgically treated patients with GD.

METHODS

From January 2013 to December 2015 in the Clinic for Endocrine and General Surgery at the Military Medical Academy in Belgrade,

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Serbia, total or near-total thyroidectomy was performed in 129 patients. General epidemic and clinical data (gender, age, type of surgery) were obtained from the medical history of patients. Indication for surgery in 125 patients was medically uncontrolled thyroid hyperfunction, compressive symptoms, nodular presence or esthetic reason. In four patients, indication for thyroidectomy was clinical suspicion for PTC, after fine-needle aspiration biopsy was performed. Macroscopic processing of surgical specimens was done according to guidelines for handling surgical specimens from Rosai and Ackerman's Surgical Pathology [2]. The scar lesion – fibrous and/or calcified foci – was fully processed. The diagnosis of PTMC was reached according to the classification of the World Health Organisation [9]. The following morphological features of PTMC were analyzed: size, multifocality, localization, histomorphology (classical, follicular, tall cell), growth pattern (infiltrative vs. circumscribed), extrathyroidal extension, lymphovascular invasion and lymph node metastasis. According to the localization, PTMC were divided into those localized in the peripheral or subcapsular/superficial zone according to the criteria applied by Niemeier et al. [16], and those localized deep in the thyroid parenchyma. The study of remaining non-neoplastic thyroid tissue included the search for nodular transformation and abundance and frequency of LI. We defined nodular transformation as the presence of at least one nodule in the gland (adenomatoid, colloid, oncocytic). The abundance and frequency of LI are graded by the 0–4 scale according to Williams and Doniach [17]. In cases where we incidentally discovered lymph nodes in peri isthmic or peri thyroid tissue, they were fully processed and examined for the presence of metastasis.

Review of all cases was done by two pathologists (SC, BK). Cases where there was a different opinion in the diagnosis of PTMC and four cases of PTC with preoperative suspicion for malignancy were excluded from the series.

The data are presented as mean ± standard deviation or count (percentage), depending on the data type. Significant differences between groups were assessed using the t-test, Mann–Whitney U-test, and χ^2 test, depending on the data type and distribution. Data were analyzed using SPSS 20.0 (IBM corp.) statistical software. All p-values less than 0.05 were considered significant.

RESULTS

In the analyzed period, a total of 125 patients with GD without previous suspicion of malignancy were surgically treated. After histopathological examination the diagnosis of PTMC was made in 24 (19.2%) patients, with the mean tumor size of 3.03 ± 2.17 mm (0.45–7 mm). The mean weight of the gland in the GD with PTMC group was 37 ± 40.90 g, and in the GD without PTMC group it was 54.94 ± 43.64 g. Statistical significance was not determined according to the weight of the gland (Z = -0.940, p = 0.347). One hundred and one (80.8%) patients were female, while 24 (19.2%) patients were male. Eighteen of the patients who had PTMC were female, whereas six were male, to which no statistically significant difference can be attributed (χ^2 = 0.644, p = 0.564). The average age of patients in the GD with PTMC group at the time of surgery was 48.50 ± 13.07 years, while in the GD without PTMC group it was 41 ± 13.12 years, and it proved to be statistically significant (t = 2.023, p = 0.045). Clinical and pathological characteristics of the patients are shown in Table 1.

Table 1. Clinical and pathological characteristics of patients

Variable	TOTAL	GD without PTMC	GD with PTMC	p-value
Number of patients	125	101	24	/
SEX				
Female	101 (80.8%)	83 (82.2%)	18 (17.8%)	0.564[a]
Male	24 (19.2%)	18 (75%)	6 (25%)	/
Age (years)	44.27 ± 13.28	43.09 ± 13.12	49.13 ± 13.07	0.045[b]
Thyroid weight (g)	53.95 ± 43.02	54.94 ± 43.64	49.80 ± 40.90	0.347[c]
NODULAR PRESENCE				
Without nodular transformation	84 (67.2%)	74 (73.3%)	10 (41.7%)	/
With nodular transformation	41 (32.8%)	27 (26.7%)	14 (58.3%)	0.003[a]
LYMPHOID INFILTRATION				
Grade 0	35 (28%)	30 (29.7%)	5 (20.8%)	/
Grade I	80 (64%)	65 (64.36%)	15 (62.5%)	/
Grade II	10 (8%)	6 (5.94%)	4 (16.7%)	0.129[d]
Grade III	0 (0%)	0 (0%)	0 (0%)	/
Grade IV	0 (0%)	0 (0%)	0 (0%)	/

[a]χ^2 test;
[b]t-test;
[c]Mann–Whitney U-test;
[d]χ^2 test for trend

PTMC characteristics

Most of the PTMC were unifocal (n = 20; 83%), and multifocality was detected in only four cases (16.2%). The most common localization of PTMC was intraparenchymal (n = 15; 62.5%), two were located in the isthmic region, while the subcapsular localization was detected in nine cases (37.5%). Follicular morphology of the tumor was the most common (n = 16; 66.7%), followed the classical (n = 5; 20%) and tall-cell morphology (n = 3; 12.5%). Infiltrative growth pattern was found in 15 cases (62.5%), compared to nine circumscribed cases (37.5%). Lymphatic invasion was present in four cases (n = 4; 16.7%), and vascular invasion was not found in any of the cases. Extrathyroidal microscopic extension was detected in three of 24 cases (12.5%), and it was related to subcapsular localization of PTMC. Subcapsular PTMC were also more commonly related with morphological features such as multifocality and lymphatic invasion. Three of four cases with lymphatic invasion and all cases with multifocal distribution were subcapsular PTMC. In 12 cases of the GD with PTMC group, between one and five lymph nodes were found. In none of these cases lymph node metastases were found. The pathomorphological characteristics of PTMC of all patients are shown in Table 2. Figures 1A–D show several histomorphological findings.

Table 2. Pathomorphological characteristics of PTMC for all patients

Case No.	Age (years)	Sex	Size (mm)	TNM	Localization	Morphology	GP	Multifocality	LV
1	35	M	0.9	T1aNx	IP	Fol.	Circ.	No	L0V0
2	73	M	0.9	T1aNx	SC	Fol.	Inf.	No	L0V0
3	46	F	4	T1aN0 (0/2)	IP	Fol.	Inf.	No	L0V0
4	56	F	4	T1aNx	IP	Fol.	Inf.	No	L0V0
5	48	F	3.9	T1aN0 (0/4)	IP	Fol.	Inf.	No	L1V0
6	58	M	1.8	T1aNx	IP	Fol.	Circ.	No	L0V0
7	63	F	1.35	T1aNx	IP	Clas.	Inf.	No	L0V0
8	63	F	2.4	T1aNx	IP	Fol.	Inf.	No	L0V0
9	33	F	2.4	T1aN0 (0/4)	IP	Clas.	Inf.	No	L0V0
10	47	F	7	T3Nx	SC	Tall	Inf.	No	L1V0
11	49	F	5	T1aN0 (0/2)	IP	Fol.	Circ.	No	L0V0
12	46	M	3.3	T1aN0 (0/3)	SC	Fol.	Circ.	Yes	L0V0
13	25	F	7	T3Nx	SC	Clas.	Inf.	No	L1V0
14	60	F	1.95	T1aNx	IP	Clas.	Inf.	No	L0V0
15	53	F	6	T1aNx	IP	Clas.	Circ.	No	L0V0
16	36	F	2	T1aNx	SC	Fol.	Inf.	No	L0V0
17	58	F	6	T1aNx	IP	Fol.	Inf.	No	L0V0
18	64	M	1.2	T1aN0 (0/1)	SC	Fol.	Circ.	No	L0V0
19	47	F	1.5	T1aN0 (0/2)	SC	Tall	Inf.	Yes	L0V0
20	41	M	0.9	T1aN0 (0/2)	IP (Ist.)	Fol.	Circ.	No	L0V0
21	24	F	1.35	T1aN0 (0/3)	IP	Fol.	Circ.	No	L0V0
22	65	F	2	T1aNx	SC	Fol.	Inf.	Yes	L0V0
23	35	F	0.45	T1aN0 (0/1)	IP (Ist.)	Fol.	Circ.	No	L0V0
24	54	F	7	T3N0 (0/5)	SC	Tall	Inf.	Yes	L1V0

GP – growth pattern; LV – lymphovascular invasion; IP – intraparenchymal; SC – subcapsular; Ist.– isthmic; Fol. – follicular morphology; Clas. – classical morphology; Tall – tall cell morphology; Inf. – infiltrativ growth; Circ. – circumscribed

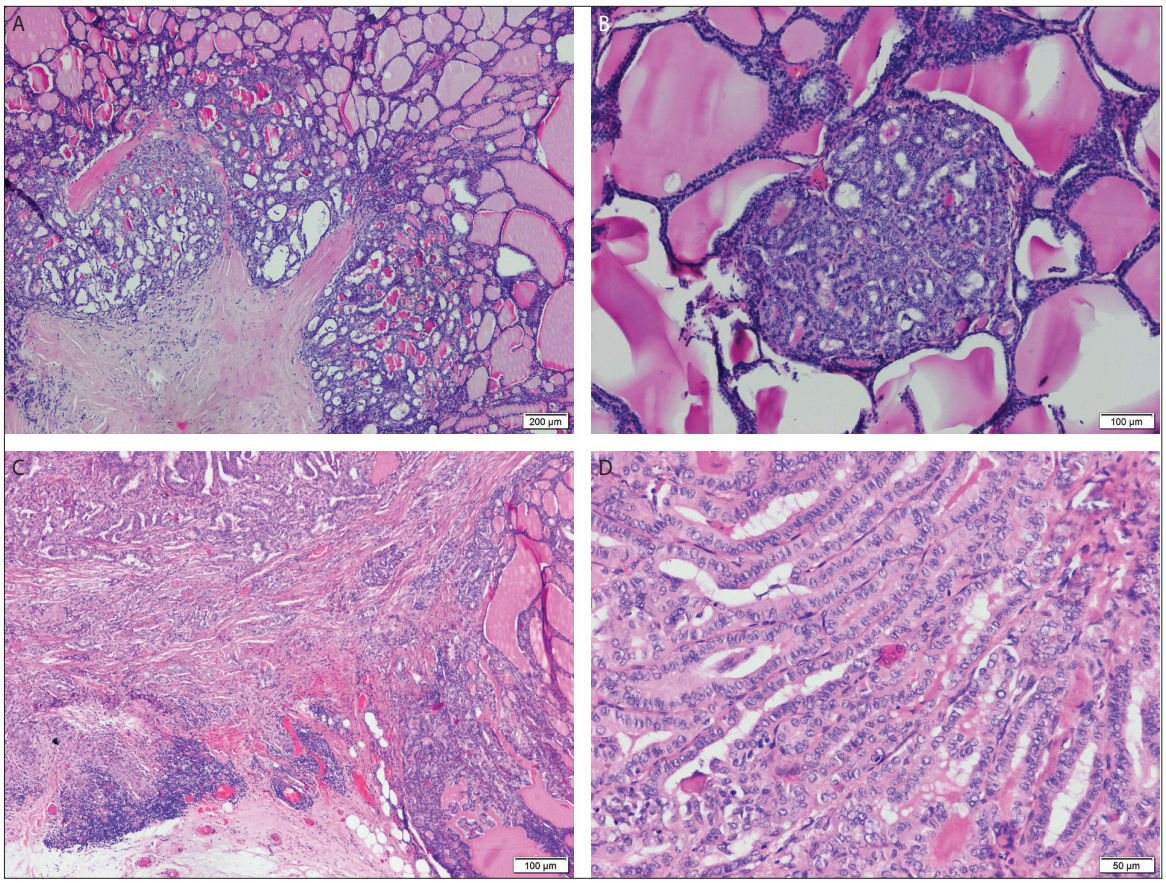

Figure 1. A – follicular PTMC with infiltrative growth pattern (H&E, ×40); B – submillimeter size circumscribed PTMC (H&E, ×100); C – subcapsular PTMC with extrathyroidal extension (H&E, ×40); D – tall cell PTMC (H&E, ×200)

Additional findings in GD in relation to PTMC

Nodular presence

The nodular presence was detected in 41 of 125 (32.8%) cases. In five cases (12%) the nodules were solitary, with diameter ranging from 7 mm to 25 mm. Three of them were of the adenomatous type and one was of colloid type. In 36 cases (82%) the nodules were multiple with the diameter ranging from 2 mm to 30 mm. The morphology of these nodules was a mix of hyperplastic/adenomatous type and/or colloidal type. Oncocytic nodules were detected in three cases. The presence of nodules in the GD with diagnosed PTMC group was found in 14 of 24 cases, or in 58.3%, while in the GD without PTMC group the presence of nodules was found in 27 of 101, or in 26.7%, and it proved to be statistically significant ($\chi^2 = 8.786$; $p = 0.003$).

Presence of lymphoid infiltration

In the group of analyzed patients the most prevalent presence of lymphoid infiltration was within grade I. Grade I of LI was detected in 84 out of 125 cases, or in 64%, followed by grade 0 and grade II with 28% and 8% of the cases, respectively. In the GD with PTMC group, the results were very similar: grade 0 of LI was present in five out of 24 cases, or in 20.8%, grade I of LI was present in 15 out of 24 cases, or in 62.5%, followed by grade II in four of 24 cases or 16.7%. Grades III and IV of LI, which would correspond to lymphocytic and Hashimoto's thyroiditis, respectively, according to the applied criteria, were not detected in any of the cases. PTMC was most commonly detected within Grade I, but we did not prove it to be statistically significant ($p = 0.129$).

DISCUSSION

Reported presence of malignancy in GD is very different, but in two recent studies its frequency is very high, with the rate of 32% and 33.7%, respectively [8, 18]. The increase of cancer incidence in GD is well-presented in the study reported by Phitayakorn et al. [19]. This study involves a time interval of 25 years divided into three periods. In the first period (1985–1993), the frequency of carcinoma was 0%, while in the third period (2003–2010), the frequency of carcinoma was 16.4%. In the cohort study reported by Chen et al. [20], patients with GD, particularly in older age, are at a greater risk of developing thyroid carcinoma compared to general population.

The most common malignancy in GD is PTC, with the participation of 23–88% of its variant PTMC [5, 6, 18, 21, 22]. Our results show a high incidence of malignancy in surgically treated patients with GD (28/129; 21.7%) with high participation of PTMC (24/28; 85.7%). The incidence of PTMC in patients with GD is 18.7% (24/129). The frequency of PTMC in GD was statistically significantly higher ($p = 0.045$) in older patients of our series, similar by to the results of other studies [19, 21, 22]. These results are in accordance with the general trend of worldwide increasing incidence of PTMC, most often as early clinical detection or as incidental pathohistological findings in patients undergoing thyroid surgery for benign thyroid lesions [10–12].

Thyroid nodules in GD are a common finding and its prevalence is different depending on the detection method: thyroid palpation, ultrasonography, or pathohistological examination. Thyroid nodules are found in 28.5–53% of patients with GD using thyroid ultrasonography as the most sensitive method [18, 23, 24]. Relation of thyroid nodules and carcinoma in GD is already established in a number of studies and increases the risk of developing thyroid carcinoma [5, 18, 21–24]. Carcinoma can be localized within nodules or into thyroid parenchyma outside nodules, most often as an incidental PTMC. In our work, the presence of thyroid nodules was detected in 32.8%, which is similar to 33.6% reported by Tam et al. [22] and 39% reported by Ergin et al. [21]. The frequency of PTMC was statistically significantly higher in thyroid glands with present nodules as opposed to the gland without present nodules, which is consistent with results of previous studies [5, 18, 21–24]. This result is also opposed to the study by Wei et al. [18], who reported a higher incidence of PTMC in GD without nodules. Localization of PTMC in our work was outside of detected nodules except in two cases. This could be a result of a larger number of analyzed slides in case of nodular presence, usually in order to assess its invasive growth.

The presence of lymphoid infiltrate in the thyroid glands of GD is usually small, most often in the form of patchy and small groups of lymphocytes, usually in the interfollicular stroma, and sometimes with germinal center formation. Foci of LI were accompanied by secondary changes in thyrocyte, usually in the form of its degeneration and rarely oncocytic transformation [1, 2, 3, 25]. According to the medical records in our work, the clinical significance of a moderate amount of LI was associated with medically uncontrolled thyroid hyperfunction. This could be an expected finding, because intrathyroid lymphocytes are one of the main sources of autoantibodies [1]. Also, the presence of LI can lead to the follicular destruction and increased hormone release. The abundance and frequency of LI in our work were not statistically significantly associated with the presence of PTMC. The interpretation of secondary changes related to the presence of LI, development of fibrosis, and cellular atypia is problematic since it could be associated with therapy-induced changes, especially in the long-standing and medically treated disease, which was not a subject of this analysis [2, 3].

In addition to the differences in the reporting cancer frequency in GD, opinions and results about its malignant potential are also disparate. A study by Pellegriti et al. [13] shows that well-differentiated thyroid cancers in GD have a more aggressive biological behavior, which is, according to Ozaki et al. [26], also applicable to tumors with diameter under 10 mm. Other studies, however, show that there are no differences in the biological behavior of cancer in GD according to other pathological conditions, and the prognosis of PTMC is excellent [6, 13, 27, 28].

Usually, clinical behavior of PTMC is favorable, with excellent outcome. In rare cases, PTMC can show aggressive behavior presented by local lymph node metastases, extrathyroid invasion, or local recurrence, while a distant metastasis and fatal outcome are extremely rare [29]. A potentially different biological behavior of PTMC can be related to patient's age, specifically in children and younger adolescents up to 19 years old. Clinical presentations and behavior of PTMC are mostly related to its morphological features such as tumor size, its multifocality, infiltrative growth, lymphovascular invasion, histological type, and its localization [10, 30]. According to Niemeier et al. [16], the most specific and sensitive assessment of aggressiveness of PTMC is obtained by applying the combined molecular-pathological score.

Clinical impact of morphological characteristics of PTMC in our work was related to their peripheral/subcapsular localization. Only PTMC within this localization is able to infiltrate thyroid capsule and can show extrathyroidal extension which was in our work present in 12.5% of cases. Other features (multifocality, lymphatic invasion), related to potentially more aggressive biological behavior of PTMC, were also more commonly associated with subcapsular PTMC. Another important morphological characteristic of PTMC in our results was the high frequency of PTMC with tall cell morphology, detected in 12.5% of cases. Tall cell variant is a clinically more aggressive form of PTC with reported incidence of 4–12% [30]. In recent studies, Boutzios et al. [8] presented higher incidence of tall cell variant of PTC (18%) in patients with GD, and Wei et al. [18] reported incidence of PTC with tall cell morphology in 16% (7% were tall cell variant, and 9% of PTC showed tall cell features). These results indicate that tall cell morphology as a pure PTC variant or as a part of PTC with tall cell features could be a more common finding in patients with GD than in euthyroid patients. It is also interesting that all cases of PTMC with tall cell morphology in our work were of subcapsular localization.

More precise results could be expected in larger series, which is the main flaw and limitation of our present work.

Pathohistological diagnosis of PTMC is rarely problematic, but from a practical standpoint, it is important to emphasize that differentially diagnostic lesions can be mostly seen in GD. Foci of papillary proliferations can be problematic, especially the ones localized in the vicinity of the fibrotic area with the picture of pseudoinvasion. Small hypercellular and often pseudo-encapsulated nodule with nucleomegaly and some degree of hypochromasia could be a diagnostic challenge. The most significant differential diagnostic issue represents stellate fibrotic foci as solitary or multifocal findings. In these cases, the definite diagnosis usually requires a serial section examination in order to assess invasive growth, and/or the detection of more typical PTC nuclear features or psammoma body.

CONCLUSION

Thyroid carcinomas in GD are not rare, and in our results, most of them represent an incidental PTMC. Clinical impact of PTMC is mostly related to its morphological features and tumor localization. Reporting of these features and long-term follow-up could help a better understanding of true biological nature of PTMC in GD.

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Учесталост и морфолошке карактеристике папиларног микрокарцинома штитасте жлезде у Грејвсовој болести

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САЖЕТАК

Увод/Циљ Удруженост Грејвсове болести (ГБ) и карцинома штитасте жлезде пријављује се у широком распону од 0% до 33,7%. Папиларни карцином (ПК) штитасте жлезде је најчешћи малигнитет у ГБ, односно његова варијанта – папиларни микрокарцином (ПМК). Упркос сталном порасту учесталости ПМК, његова стопа рецидива и смртности је константна и ниска.

Циљ рада је да се одреде учесталост и морфолошке карактеристике ПМК код болесника са ГБ и тиреоидектомијама.

Методе У периоду од јануара 2013. године до децембра 2015. године анализирани су општи клинички и морфолошки параметри код 129 болесника са ГБ и учињеном тоталном или скоро тоталном тиреоидектомијом.

Резултати Код 24 (18,7%) болесника са ГБ дијагностикован је ПМК. Пречник тумора износио је 3,03 ± 2,17 *mm* (0,45–7 *mm*). Старост болесника у групи са ГБ и ПМК износила је 48,50 ± 13,07 година, а у групи без ПМК 41 ± 13,12 година и била је статистички значајна ($p = 0,045$). Микроскопски, најзаступљенији параметри били су: само један фокус ПМК (83,3%), фоликуларни подтип ПМК (66,7%), ифилтративна форма раста (62,5%), интрапаренхимска локализација (54,2%). Присуство најмање једног чвора у штитастој жлезди детектовано је код 26,7% болесника са ГБ без ПМК, док их је у групи са ПМК било више (58,3%), статистички високо значајних ($p = 0,003$).

Закључак Учесталост карцинома штитасте жлезде код болесника са ГБ је висока и износи 18,7%. Његове клинички најзначајније морфолошке карактеристике везане су за супкапсуларну локацију тумора.

Кључне речи Грејвсова болест; штитаста жлезда; папиларни микрокарцином; морфологија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The importance of compression elastography in the evaluation of thyroid nodule malignancy

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SUMMARY

Introduction/Objective Compression, also called strain elastography imaging techniques, represent new echotomographic modality, which is a promising method for the differentiation of benign from malignant lesions, not only in the thyroid gland but also in other organs.
The objective of this study is to evaluate the importance of compression elastography in the differentiation of benign and malignant thyroid nodules.

Methods We performed echotomographic examinations in B mode, and examinations using compression elastography in a total of 186 persons (152 females and 34 males, with the average age of 45.3 ± 13.5 years), with 264 nodules in the thyroid gland. Elastography was done in two steps: the first one through scoring elastographic figures, and the second one through the determination of the resistance index (strain ratio – SR).

Results Using elastography scores by Fukunari, 44 of 60 malignant nodules had a score of 3–4, while 152 of the 204 benign nodules had a score of 1–2. Using the receiver operating characteristic (ROC) analysis, the best cut-off point obtained using elastography scores was 2, with a sensitivity of 73.3% and specificity of 74.5%. Using the software-calculated SR we found that out of 89 nodules with SR ≥ 2.5, 52 were malignant nodules, while out of 175 nodules with SR < 2.5, 167 were benign nodules. Using the ROC analysis, the best cut-off point obtained using SR was > 2.5, with a sensitivity of 86.7%, and specificity of 81.9%.

Conclusion As a follow-up of standard echotomographic examination in B mode, compression elastography is a newly developed and promising technique in the differentiation of benign from malignant lesions.
Keywords: compression elastography; nodule; thyroid gland; malignancy

INTRODUCTION

Thyroid nodules are very common in the general population, especially in iodine-deficient areas. It is estimated that nodules are seen in 35–50% of people living in areas with deficiencies in iodine. However, a normal thyroid gland has some thyrocytes (follicular cells) with the tendency of autonomous growth. All of them can occur in nodules regardless of the amount of iodine, with increased frequency in the population [1]. Thyroid ultrasonography is one of the most practical, easily manageable, accessible, and non-invasive methods which can detect nodules. One of the most important assessments is to determine the nature of the nodules, or to differentiate benign from malignant thyroid nodules. The classical technique of echotomographic examination in B mode cannot differentiate benign from malignant nodules with great certainty. Hypoechoic changes, the presence of microcalcifications, variable peripheral edge, intranodal hypervascularization, and pathologically suspected lymph nodes in the neck are some of the uncertain indicators of malignant nodules, insufficient to evaluate the nature of thyroid nodules. Today, thanks to different software modes and modern ultrasonography devices, it is possible to additionally assess the nature of various nodules of the thyroid gland [2, 3].

Elastography is a new, non-invasive method, which estimates the elasticity of tissue by measuring different degrees of distortion during the application of an external force. The basic principle of ultrasound elastography is that tested tissue compression shows resistance. As well as palpation, elastography can measure tissue deformation or strain caused by the external compression [4]. Nowadays, these assessment methods are showing promise in the differentiation of benign from malignant lesions, and not just in the thyroid gland but also in the breasts, liver, spleen, and prostate. Malignant lesions are characterized by lower elasticity compared to the structure of normal tissue, partly due to the uncontrolled proliferation of malignant cells, increased vascularization, and, somewhat less frequently, due to the presence of fibrotic changes in them.

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The aim of this study is to evaluate the importance of strain ultrasound elastography in the differentiation of benign from malignant thyroid nodules, especially when combined with standard echotomography examination in B mode.

METHODS

This cross-sectional study included 186 patients with 264 thyroid nodules (152 females and 34 males, with the mean age of 45.3 ± 13.5 years). All the patients had solid lesions (nodules) and were referred to the Department of Radiological Diagnostics of the KBC "Dr. Dragiša Mišović – Centar" in Belgrade, Serbia, from March 2014 to June 2016. Patients with nodules of over 3.5 cm in size, completely cystic, anechogenic without solid components, and nodules in close contact with the carotid artery, were excluded from the study in order to increase the reliability of the findings. Echotomographic thyroid examinations were performed using the Aplio XG (Toshiba, Tokyo, Japan) ultrasound device, with a 10 MHz linear transducer.

All the patients were examined by using three ultrasonic methods. The first step was a standard echotomographic examination in B mode, the second was to test the resistance of the tissue through elastography scores, and the third step was to measure the resistance index (strain ratio – SR) as an indicator of the semi-quantitative elastography method for tissue resistance.

To avoid potential differences in operation between different researchers, all examinations were performed by a single researcher with a long experience in dealing with different modalities of ultrasound equipment. Also, in this way we standardized and equalized the compression on the tissue, during the elastography performance. Strain elastography was performed by repeated compressions (up to seven), with the same volume, and in the same time intervals (about 0.5 seconds). All compressions were based on the centrally positioned nodules in a region of interest, in the longitudinal view of the surrounding normal thyroid tissue for elasticity comparison.

Based on the classification by Fukunari [5], each nodule was scored by the elastographic figure. A score of 1 means the majority of the nodule, fully stained in green. A score of 2 signifies that only the nodule center is stained green, and the periphery is blue. A score of 3 denotes a predominantly blue nodule with green parts, and the score of 4 means that the entire nodule is blue. Elastography scores represent different degrees of elasticity of the lesion, from the highest (score 1) to the lowest elasticity (score 4). Scores of 1 and 2 represent indicators of benign nodules, while scores of 3 and 4 represent indicators of malignant ones. After the scoring, we recorded the SR, which is the software-calculated ratio of elasticity between two regions of interest, in our case between the nodule and the rest of the normal thyroid tissue. We evaluated each lesion three times, using a variety of static images, and the average value was recorded as the final result.

After the echotomographic examinations, all the patients were sent to perform the ultrasound-guided fine-needle aspiration biopsy. The results were processed by an experienced pathologist. Fifty-two patients (52 nodules) with cytological findings suspicious for malignancy, and 36 patients (48 nodules) with benign cytological findings, were operated on and histopathologic findings confirmed the earlier diagnoses. We compared the cytological and histopathological results with elastographic images, and evaluated the sensitivity, specificity, negative and positive predictive value, and accuracy of the techniques or methods. Quantitative data are presented in mean ± standard deviation, and qualitative as frequencies. Receiver operating characteristic (ROC) curve was used to determine optimal cut-off values to differentiate between benign and malignant nodules. The value of $p < 0.05$ was adopted as statistically significant. The data were statistically analyzed by MedCalc v.11.4.2 (MedCalc Software, Ostend, Belgium) statistical software.

RESULTS

Among 186 patients who were included in the study (152 female and 34 male, with the mean age of 45.3 ± 13.5 years), 264 nodules were obtained. Using classical echotomographic examination of the thyroid gland in B mode, out of 264 discovered nodules, 180 (68%) had homogeneous echostructure, while 84 nodules (32%) had heterogeneous echostructure – partially cystic. Most of the nodules (120; 45%) were isoechogenic, 104 (40%) were hipoechogenic, while the minority were hyperechogenic nodules (40; 15%). A total of 192 nodules (73%) had proper edges, while the edges were irregular in 72 nodules (27%). Visible calcifications were present in 56 nodules (21%), while calcifications were not seen in 208 nodules (79%) (Table 1). Using elastography scores by Fukunari [5], out of 204 benign nodules, 152 nodules (74%) had a score of score 1–2 (60 nodules had a score of 1, and 92 nodules a score of 2), and 52 nodules (26%) had a score of 3–4 (46 nodules had a score of 3, and 6 nodules a score of 4). Out of 60 malignant nodules, 44 nodules (73%) had a score of 3–4 (14 nodules had a score of 3, and 30 nodules a score of 4), while 16 malignant nodules (27%) had a score of 1–2 (all with a score of 2), as shown in Figures 1–5. The sensitivity of elastography scores for getting positive results in malignant nodules was 73.3%. the specificity of elastography scores for negative results in benign nodules was 74.5%. The positive predictive value was 45.8%, and the negative predictive value was 90.4%. The accuracy in the differentiation between benign and malignant nodules was 74.2% (Figure 6). Using the ROC analysis, the best cut-off point obtained using elastography scores to differentiate benign and malignant thyroid nodules was 2, with a sensitivity of 73.3% and a specificity of 74.5% (area under the ROC curve = 0.83, 95% confidence interval: 0.78–0.87, $p < 0.0001$) (Figure 7). Using the ROC analysis, the best cut-off point obtained using SR to differentiate between benign and malignant thyroid nodule was found to be > 2.5, with sensitivity of 86.7% and specificity of 81.9% (area under the ROC curve = 0.88, 95% confidence

Table 1. Number of nodules in each score by Fukunari [5]

Elastographic score	Malignant nodules	Benign nodules	TOTAL
3–4	44	52	96
1–2	16	152	168
Total	60	204	264

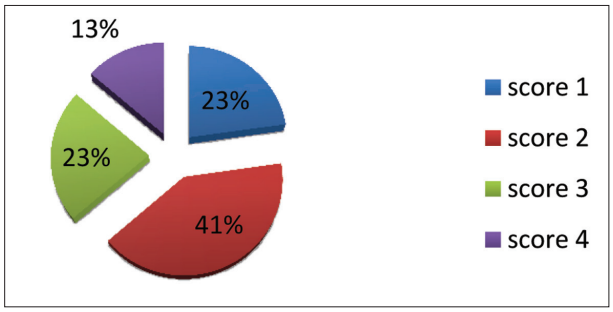

Figure 1. Total percentage of nodules in each score category

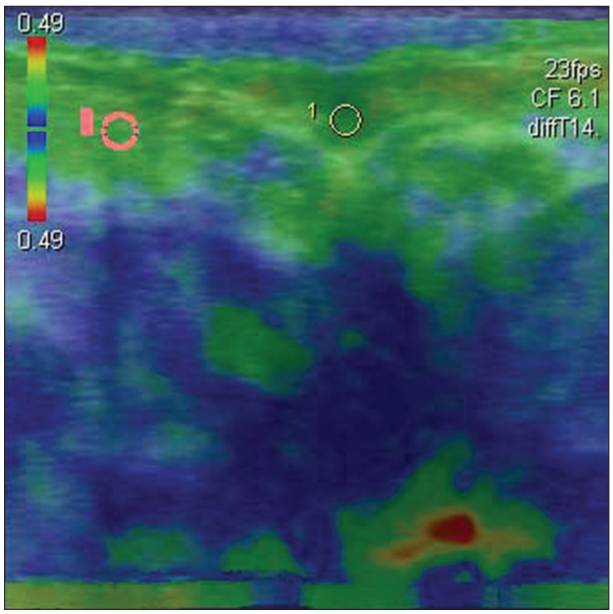

Figure 2. Score 1 by Fukunari [5] – findings obtained by biopsy proving benign nodule

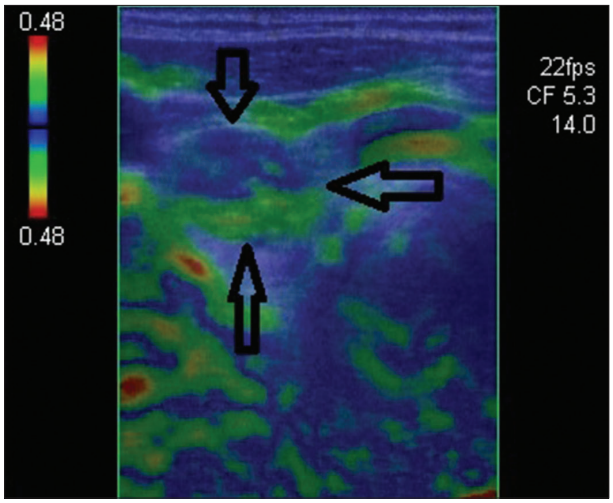

Figure 3. Score 2 by Fukunari [5] – findings obtained by biopsy proving benign nodule

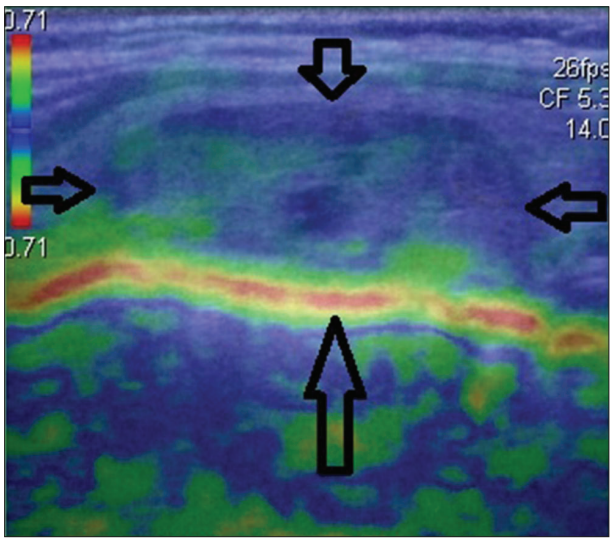

Figure 4. Score 3 by Fukunari [5] – findings obtained by biopsy proving papillary carcinoma

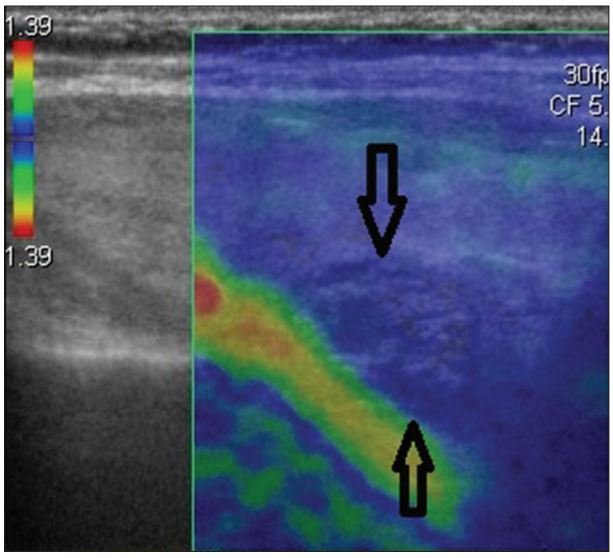

Figure 5. Score 4 by Fukunari [5] – findings obtained by biopsy proving papillary carcinoma

interval: 0.83–0.91, $p < 0.0001$), as shown in Table 2 and Figures 8 and 9. Using the software-calculated SR, while performing elastography, we took the criterion that SR ≥ 2.5 was an indicator of malignancy. Out of 89 nodules with SR ≥ 2.5, 52 were malignant. Out of 175 nodules with SR < 2.5, as much as 167 were benign. The SR for obtaining positive results in malignant nodules was 86.7%. The specificity of SR for obtaining negative results in benign nodules was 81.9%. The positive predictive value was 58.4%, and the negative predictive value was 95.4%. The accuracy of SR in differentiating benign from malignant nodules was 82.9% (Table 3). The results obtained by fine-needle aspiration biopsy showed that 204 nodules were benign (77%), while 60 nodules (23%) were malignant. Fifty-two patients (52 nodules) with malignant cells that are detected by fine-needle aspiration biopsy, and 36 patients (48 nodules) with benign cells, were operated on. Histopathologic findings for 52 patients (52 nodules) with malignant nodules were presented in Table 3.

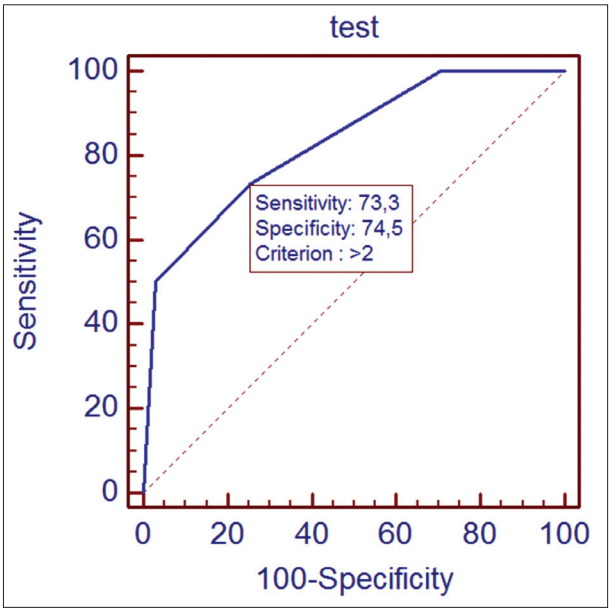

Figure 6. ROC analysis by using elastography scores

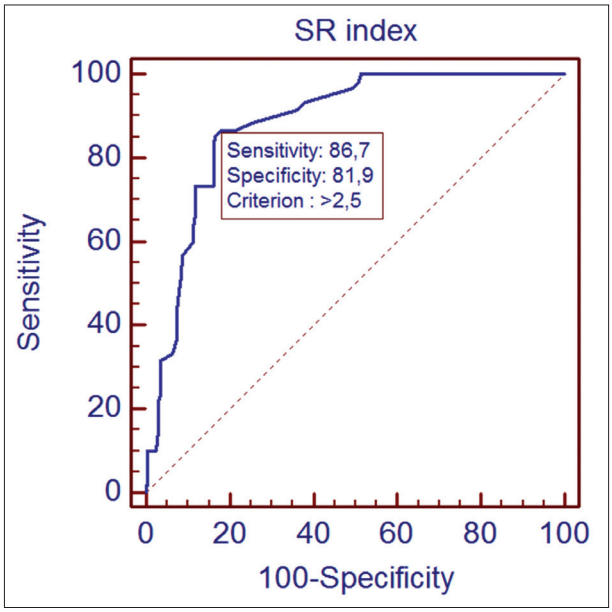

Figure 7. ROC analysis by using strain ratio

Table 2. Number of nodules in each category by strain ratio

Strain ratio	Malignant nodules	Benign nodules	Total
SR ≥ 2.5	52	37	89
SR < 2.5	8	167	175
Total	60	204	264

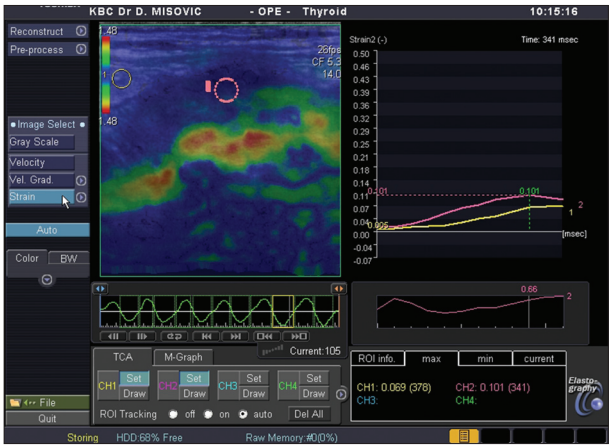

Figure 8. A nodule with elastography score of 2, SR 0.66 – biopsy-proven benign characteristics of a thyroid adenoma

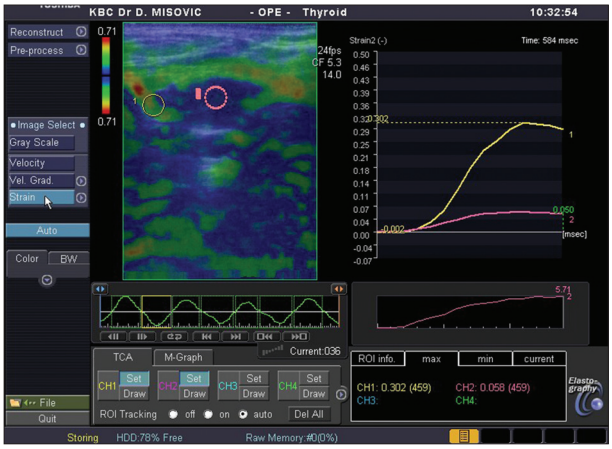

Figure 9. A nodule with an elastography score of 3–4, SR 5.71, biopsy-proven follicular carcinoma

Table 3. Histopathologic types of malignant nodules in each elastographic score and strain ratio category

Type	Elastographic score 3–4 (%)	Elastographic score 1–2 (%)	SR ≥ 2.5 (%)	SR < 2.5 (%)	TOTAL (%)
Papillary carcinoma	33 (63.5)	7(13.5)	38 (73.1)	2 (3.8)	40 (76.9)
Follicular carcinoma	3 (5.8)	4 (7.7)	4 (7.7)	3 (5.8)	7 (13.5)
Anaplastic carcinoma	2 (3.8)	2 (3.8)	2 (3.8)	2 (3.8)	4 (7.7)
Primary thyroid lymphoma	0 (0)	1 (1.9)	1 (1.9)	0 (0)	1 (1.9)
Total	38 (73.1)	14 (26.9)	45 (86.5)	7 (13.5)	52 (100)

DISCUSSION

One of the primary methods of clinical examination of the thyroid gland includes the palpation. Palpation gives us information about the shape, size, as well as the hardness and elasticity of the thyroid gland. However, palpation is a subjective method of examination. Measurements of the elasticity and stiffness of soft tissue assessment are useful in the differentiation of tumor, inflammation, and normal thyroid tissue. It is generally accepted that benign lesions show less stiffness and greater elasticity than malignant lesions, and higher stiffness and lower elasticity than normal tissue [4–7].

Classical echotomographic examination of the thyroid gland can detect characteristics of nodules that indicate malignancy. In addition to their appearance, ranging from

hypoechogenic to almost anechogenic, malignant nodules are echotomographically different, and the difference is mostly exhibited through the absent or incomplete (rarely closed) peripheral vascular edge. Microcalcifications are often present in the nodules, and intense central vascularization is almost always present. However, even at about 50% of benign nodules, intranodal vascularization may be present. Approximately, the most accurate echotomographic diagnosis of thyroid cancer is only possible when all the above symptoms are simultaneously present in thyroid nodules, with high specificity but low sensitivity [8, 9]. Therefore, unified and comprehensive information obtained by the classic echotomographic examination in B mode and color Doppler are the most reliable results predictive for malignancy.

Fine-needle aspiration biopsy under ultrasound control is still the most precise method for diagnosing cancer with high sensitivity and specificity, with a very small number of false-positive (2.3%) and false-negative (0.2%) results. The accuracy of differentiating benign from malignant lesions of the thyroid gland is more than 95% [10, 11].

We examined a newly-developed diagnostic method (ultrasound elastography), which estimates the degree of distortion of tissues when applying an external force. In the diagnostic algorithm, this method is placed between classic echotomographic examination in B mode, and fine-needle aspiration biopsy, and has been introduced in order to further increase ultrasound accuracy. It is based on the principle that softer tissue and tissue parts are more easily deformed, and have greater elasticity than harder tissues. Some of the main benefits of elastography are its simple feasibility, non-invasivity, and its convenience during a routine echotomography performance. In addition, this technique enables the dynamic visualization of lesions in the region of interest during the compression. Elastography is performed in two steps: determining scoring nodules on the elastography figure, and calculating the SR using the software. Scoring is a subjective estimate of the lesion of interest (nodule), classified into one of the scores based on the color distribution in B mode. Different colors (green, red, blue) on the echotomographic figure represent different elasticity areas of the tissue in the region of interest [12]. In our study, we used the scoring by Fukunara [5], ranging from the most elastic (score of 1; mostly benign nodules) to the least elastic (score of 4; mostly malignant nodules). Using these elastography scores, we discovered malignant nodules with the sensitivity of 73.3%. The same score system was used by Wang et al. [12], while the score of the Ueno classification was used by Ciledag et al. [4] and Itoh et al. [13]. Many studies indicate that this part of elastography is sufficient for the assessment of different tissue elasticity and for the differentiation between benign and malignant nodes [14, 15]. Malignant thyroid nodules have less elasticity (score 3–4), while benign nodules exhibit higher elasticity (score 1–2), as shown by our results of sensitivity (73.3%) and specificity (74.5%); ($p < 0.05$) [14–18]. However, in determining the SR, we get even higher values of sensitivity (86.7%) and specificity (81.9%), and the results pointed to increased reliability and accuracy of the test (82.9%). We demonstrated that malignant nodules had a significantly higher SR than benign nodules ($p < 0.001$). Determination of SR represents a software-calculated quantitative measure of elasticity, which may provide more reliable information. In our study, all SR values ≥ 2.5 represent a predictor of malignant nodules, in accordance with the values that we obtained by the ROC analysis. Lyshchik et al. [19] suggested that SR > 4 is a strong predictor of malignant nodules, with a sensitivity of 82% and a specificity of 96%. However, Kagoya et al. [7] used the SR > 1.5 as an indicator of malignant nodules, with sensitivity of 90%, and specificity of 50%.

While performing this technique, the depth of tissue should be taken into consideration when the comparison between the nodules and the normal tissue is made, and it should be the same or similar when calculating the SR. The estimation should be performed in the longitudinal mode of the thyroid gland examination because it shows a sufficiently large portion of normal tissues that are used to compare and calculate the SR [12, 20]. Rago et al. [14] showed that the size of nodules does not affect the value of the SR and elastography predictability. However, other researchers have indicated that the size of nodules may affect the SR, so in some studies all nodules up to 3 cm in size are included [12], while some studies include all nodules up to 4 cm in size [4]. In our study, we included all nodules up to 3 cm. Nodules larger than 3 cm were not compressed with the same intensity throughout, and the results of the SR index values were inadequate. Presently, there is no reliable information which indicates that minimal nodule size might be involved in this method. Researchers in some studies advise that, during the elastography procedure, nodules near the carotid artery must be treated with care due to the fact that pulsations of the carotid arteries can disrupt proper interpretation of the elastography figure [4, 12]. Consequently, our study avoided patients whose nodules were near the carotid artery, and the method was performed using the external compression. However, the study of Dighe et al. [21] showed that the pulsation of the carotid artery can be used, instead of external compression by researchers, for the evaluation of elastography figures. Elastography analysis results obtained in this way were similar to ours ($p < 0.05$). In our study, a small number of false-negative results inform us that elastography can reduce the number of patients referred to surgeons for the suspicion of malignancy and therefore delay the eventual surgical intervention due to malignancy.

One of the biggest disadvantages of this method is subjectivity. Strain elastography in all its forms remains an examiner-dependent method and requires a trained and experienced operator to perform valid free-hand cyclic compressions that can yield reliable and reproducible readings. The free-hand probe pressure is difficult to standardize among different operators and strain variations due to changes in the amplitude and velocity of compression that cannot be avoided. Non-uniform compressions produce intra- and interobserver variability [20]. For this reason, a newer elastography technique, called shear wave elastography (SWE) has been developed. This method is designed

to provide quantitative, more objective information on elasticity in real time. SWE uses acoustic pressure from the probe for the standardization of compression. The tissue compression force does not depend on the skill of the person performing the examination, ensuring high reproducibility and objectivity of the results. SWE can produce quantitative and more precise results than strain elastography. Although SWE requires a more complex system to generate the shear waves, it allows visualization of smaller displacements compared to strain elastography [22].

Also, histological features of nodules may lead to pitfalls. Fibrosis within a nodule, calcification, partially cystic or colloid components, isthmus location, nodule size, and the multinodular nearness appearance are correlated to increased levels of stiffness [22, 23]. Follicular carcinomas may lead to false negative results in strain elastography, as they may be soft, and therefore may be missed [24].

CONCLUSION

Elastography is a newly-developed and a very promising technique in the differentiation between benign and malignant lesions, especially when combined with standard echotomography examination in B mode. However, it is important that all of these newly-developed techniques are performed properly and with great attention because of their influence on the possible cancellation or reduction of unnecessary surgical procedures.

ACKNOWLEDGEMENT

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Значај компресионе еластографије у процени малигнитета чворова штитасте жлезде

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САЖЕТАК

Увод/Циљ Компресиона еластографија је нов начин ултразвучног прегледа за који се очекује да са већом поузданошћу разликује доброћудне од злоћудних промена не само у штитастој жлезди него и другим органима.

Циљ овог истраживања је да проценимо значај ултразвучне еластографије у разликовању бенигних и малигних чворова штитасте жлезде.

Методе Ултразвучне прегледе у Б моду и компресиону еластографију обавили смо код 186 особа (152 женског пола и 34 мушког пола, са 45,3 ± 13,5 година живота), са 264 чвора у штитастој жлезди. Еластографију смо радили у два корака – најпре смо одредили степен растегљивости (еластичности) ткива, приказаног као колорна мапа, а потом, упоређујући са околни ткивом, одредили индекс еластичности (ИЕ).

Резултати Користећи скорове еластографије према Фукунари, 44 од 60 малигних чворова имало је скор 3–4, док је 152 од 204 бенигна чвора имало скор 1–2. Анализом пријемне карактеристике, најбоља гранична тачка добијена коришћењем скорова еластографије је 2, са сензитивношћу 73,3% и специфичношћу 74,5%. Користећи софтверски израчунат ИЕ, од 89 чворова са ИЕ ≥ 2,5 било је 52 малигних нодуса, док је од 175 нодуса са ИЕ < 2,5 било чак 167 бенигних чворова. Користећи пријемну анализу, најбоља гранична тачка добијена коришћењем индекса отпора је > 2,5, са сензитивношћу од 86,7% и специфичношћу 81,9%.

Закључак Придружена стандардном ултразвучном прегледу, компресиона еластографија пружа нову могућност која обећава поузданије разликовање бенигних од малигних промена.

Кључне речи: компресиона еластографија; чвор; штитаста жлезда; малигност

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Cardiac surgery in patients with chronic renal failure

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SUMMARY
Introduction/Objective Patients with chronic renal failure (CRF) undergoing cardiac surgery are believed to have more postoperative complications and significantly higher mortality rate.
The aim of the paper was to determine preoperative predictors of exacerbation of CRF and the outcome in patients with CRF submitted to cardiac surgery.
Methods A retrospective study included 169 patients hospitalized from 2012 to 2015 (age 67.71 ± 8.46 years, 72.3% male). The analysis included numerous perioperative characteristics.
Results Preoperative stage I CRF was present in 62 (37%), stage II in 77 (46%), and stage III–V in 30 (17%) patients. Exacerbation of CRF was registered in 37 (21.9%), and the lethal outcome in 16 (9.5%) patients. Stage II of CRF (odds ratio [OR] 4.76; 95% confidence interval [CI] 1.31–17.28; p = 0.018) and stage III–V of CRF (OR 11.39; 95% CI 2.87–45.14; p = 0.001) were designated as predictors for exacerbation of CRF following cardiac surgery. In patients with CRF stage I and II, multivariate analysis designated previous cerebrovascular insult (OR 3.36; 95% CI 1.04–10.93; p = 0.044) and ejection fraction ≤ 35% (OR 5.35; 95% CI 1.83–15.64; p = 0.02) as predictors for the exacerbation of CRF. The only predictor of postoperative dialysis requirement was higher stage of CRF (OR 5.81; 95% CI 1.22–27.81; p = 0.028). CRF stage III–V was a predictor of lethal outcome (OR 7.64; 95% CI 1.49–39.27; p = 0.015).
Conclusion Higher stage of CRF in patients submitted to cardiac surgery is a predictor of exacerbation of renal failure and the lethal outcome.
Keywords: chronic renal failure; cardiac surgeries; morbidity; mortality

INTRODUCTION

There is a significant increase in the number of patients with chronic renal failure (CRF) who require cardiac surgery. Heart diseases are more frequent in this specific population compared to the general population [1]. Terminal renal failure is recognized as a significant risk factor for the outcome of cardiac surgery. The prognosis of patients diagnosed with cardiovascular disease and CRF is much worse than in patients without this associated morbidity. In patients with impaired renal function, cardiac surgery may cause aggravation of pre-existing renal failure or irreversible renal damage [1]. The problems these patients have are further complicated during open heart surgery when extracorporeal circulation is used.

In order to reduce perioperative risk, an optimal perioperative strategy is required. Most published studies emphasize the importance that preoperative clinical status has on postoperative mortality and morbidity in this category of patients, but little is known about the predictive factors for long-term survival. In patients with mild to moderate reduction of glomerular filtration rate, there is quite consistent evidence that surgical revascularization is a better therapeutic option compared to percutaneous coronary intervention (PCI) [2]. This especially applies to patients with CRF caused by diabetes mellitus, who are recommended "off pump" surgery [3, 4]. In patients with the terminal phase of the renal disease, there is no such clear evidence in favor of surgical approach. Better long-term results in these patients are being achieved with the surgical approach, but with the higher rate of intra-hospital events and complications, whereas vice versa is true for PCI comparing to coronary artery bypass grafting (CABG).

Only 15 years ago, patients with CRF were believed to have unacceptably high operative risk and only rarely underwent cardiac surgery [5, 6]. Perioperative treatment strategy of these patients in intensive care units is constantly improving. Based on the research results and improving clinical practice results, the attitude towards candidates for cardiac surgery with CRF has been dramatically changed.

In our study, we sought to determine the following:

1) Present preoperative clinical CRF patient profile;
2) Examine occurrence of postoperative morbidity and mortality in patients with CRF who underwent cardiac surgery.

METHODS

This retrospective analysis included 169 patients with CRF who underwent cardiac sur-

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gery (coronary, valvular, and combined) during the 2012–2015 period, at the Vojvodina Institute of Cardiovascular Diseases. Patients with CRF were divided into three groups according to the creatinine clearance (CC) values [1]. The first group comprised patients with CC ≥ 90 mm/min./1.73 m^2, the second group – patients with CC 60–89 mm/min./1.73 m^2, and the third group – patients with CC 15–59 mm/min./1.73 m^2. The third group included all the patients with CRF stage III, IV, and V, considering the small number of patients in each stage separately. The following comorbidities and patient characteristics were assessed: high blood pressure (> 140/90 mmHg), diabetes, blood lipid levels, chronic obstructive pulmonary disease, peripheral arterial occlusive disease, cerebrovascular insult, myocardial infarction, heart failure (HF) class using New York Heart Association (NYHA) III/IV classification, left ventricle ejection fraction (EF) ≤ 35%. The following postoperative complications were assessed: new onset of myocardial infarction, heart rhythm disturbances, cerebrovascular insult, sepsis, sternal infection, return to the intensive care unit, respiratory insufficiency, pericardial effusion, exacerbation of renal failure, and lethal outcome.

Heart surgeries were performed using extracorporeal circulation, in moderate hypothermia and perfusion pressure > 50 mmHg. Heart was stopped using the cold antegrade St Thomas' Hospital cardioplegia (with 40 mmol/l K^+). Intraoperative and postoperative monitoring included electrocardiography, transesophageal echocardiography, oxygen saturation (pulse oximetry), diuresis, continual measurement of arterial, central venous, and pulmonary arterial pressure.

Criteria for the diagnosis of HF were defined as the need to ensure hemodynamic support with the inotropes – adrenaline, dobutamine (> 5 mg/kg), and/or high doses of dopamine for longer than 24 hours, and the value of cardiac index < 2.0 l/min./m^2 [2].

Postoperative myocardial infarction was defined as > 10-fold increase of cardiac enzymes (troponin and CK-MB) following the surgery compared to reference levels [5]. Heart rhythm disturbances were defined as the new onset of arrhythmia – atrial fibrillation or ventricular tachycardia. Cerebrovascular event was defined as an isolated neurological deficit after the surgery. Respiratory failure was defined as a requirement for mechanical ventilation for longer than 24 hours. Septic attack was diagnosed on the basis of sepsis criteria (white blood cell count, body temperature, respiratory rate, inflammatory mediators: CRP, fibrinogen, procalcitonin, and positive blood cultures). Pericardial effusion was defined as the presence of abnormal amount of fluid in pericardial cavity – more than 100 ml. Exacerbation of renal failure was defined as significant increase of nitrogenous substances in blood comparing to preoperative values [4]. Hospital mortality was defined as any death that occurred during index hospitalization.

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 19.0 (IBM Corp., Armonk, NY, USA). A p-value of < 0.05 was considered statistically significant. The Kolmogorov–Smirnov test was used for the determination of quantitative data distribution. Differences of mean values were tested by the independent samples t-test or Mann–Whitney U-test and the results are presented as mean (standard deviation). The relations between categorical variables were tested using the χ^2 test and the results are presented as frequencies and percentages. Univariate and multivariate binary logistic regression were performed to determine the effects of all the factors on the dependent variable. Only variables designated as significant by a univariate analysis were entered into multivariate regression analysis.

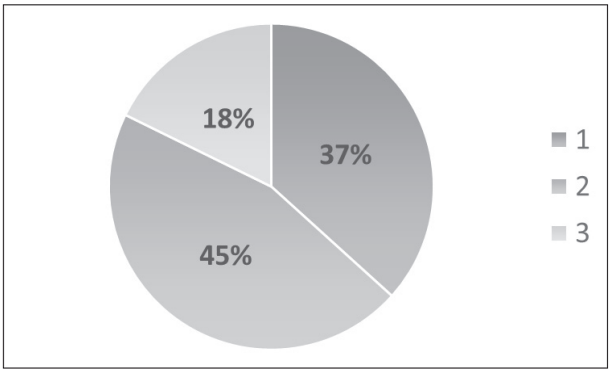

Figure 1. Representation of patients with chronic renal failure based on creatinine clearance; 1 – patients with creatinine clearance ≥ 90 mm/min./1.73 m^2; 2 – patients with creatinine clearance of 60–89 mm/min./1.73 m^2; 3 – patients with creatinine clearance of 15–59 mm/min./1.73 m^2

RESULTS

The study was conducted on 169 CRF patients, mean age 67.71 ± 8.46 years, of whom 125 patients (73.9%) were males. The number and percentage of patients according to the CRF stage are presented in Figure 1. The majority of patients (45%) were classified as CRF stage II.

High blood pressure was detected in 166 patients (98.2%). There was no statistically significant difference between the number of patients with high blood pressure within different stages of renal failure (p > 0.05). Diabetes mellitus was present in 64 (37.8%) patients. There was no statistically significant difference in the incidence of diabetes mellitus throughout renal failure stages (p > 0.05).

Significant difference was noted in the incidence of patients with HF (p = 0.018), NYHA III/IV stage (p = 0.032), and EF ≤ 35% according to the renal failure stage. Comparing patients with stage II CRF to those with stage I, there was statistically significant difference in the incidence of patients with NYHA III/IV stage (p = 0.017) and EF ≤ 35% (p = 0.013). There was statistically significant difference in the incidence of patients with HF (p = 0.005), NYHA III/IV (p = 0.022), and EF ≤ 35% (p = 0.026) when comparing patients with stage III–IV CRF to those with stage I.

Previous myocardial infarction (MI) was present in 83 (49.1%) patients. There was no significant difference in the incidence of MI throughout the stages of renal failure (p > 0.05) (Table 1).

Table 1. Preoperative characteristics of patients with CRF

Variable	Renal failure stage			p
	1	2	3	
	n (%)	n (%)	n (%)	
SEX (male)	49 (79%)	51 (66.2%)	25 (73.5%)	0.242
HBP	60 (96.8%)	76 (98.7%)	34 (100%)	0.473
DM	19 (30.6%)	32 (41.6%)	13 (38.2%)	0.410
HLP	45 (72.6%)	56 (72.7%)	25 (73.5%)	0.995
COPD	12(19.4%)	11 (14.3%)	6 (18.2)	0.712
PAOD	4 (6.5%)	3 (3.9%)	1 (2.9%)	0.677
CVI	7 (11.3%)	16 (20.8%)	5 (14.7%)	0.309
MI	28 (45.2%)	36 (46.8%)	19 (55.9%)	0.579
HF	19 (30.6%)	32 (41.6%)	20 (60.6)	0.018
NYHA III/IV	8 (12.9%)	23 (29.9%)	11 (32.4%)	0.032
EF ≤ 35	7 (11.3%)	22 (28.6%)	10 (29.4%)	0.030

HBP – high blood pressure; DM – diabetes mellitus; HLP – hyperlipoproteinemia; COPD – chronic obstructive pulmonary disease; PAOD – peripheral arterial occlusive disease; CVI – cerebrovascular insult; MI – myocardial infarction; HF – heart failure; NYHA – New York Heart Association; EF – ejection fraction

Table 2. Postoperative complication in patients with CRF

Postoperative complications	Renal failure stage			p
	1	2	3	
	n (%)	n (%)	n (%)	
NOMI	0 (0%)	2 (2.6%)	2 (5.9%)	0.185
NOHA	24 (38.7%)	35 (45.5%)	15 (44.1%)	0.716
NOCVI	2 (3.2%)	4 (5.2%)	0 (0%)	0.383
SEPSIS	2 (3.2%)	3 (3.9%)	1 (2.9%)	0.690
STERINF	2 (3.2%)	4 (5.2%)	1 (2.9%)	0.788
RET to ICU	6 (9.7%)	10 (13%)	7 (20.6%)	0.320
RESP FAIL	4 (6.5%)	9 (11.7%)	8 (23.5%)	0.049
PER EFF	5 (8.1%)	12 (15.6%)	5 (14.7%)	0.387
AC RF	3(4.8%)	15 (19.5%)	14 (41.2%)	0.000
Mortality	2 (3.3%)	7 (9.1%)	7 (20.6%)	0.021

NOMI – new onset of myocardial infarction; NOHA – new onset of heart arrhythmias; NOCVI – new onset of cerebrovascular insult; STERNINF – sternal infection; RET to ICU – return to the intensive care unit; RESP FAIL – respiratory failure; PER EFF – pericardial effusion; AC RF – acute exacerbation of renal failure

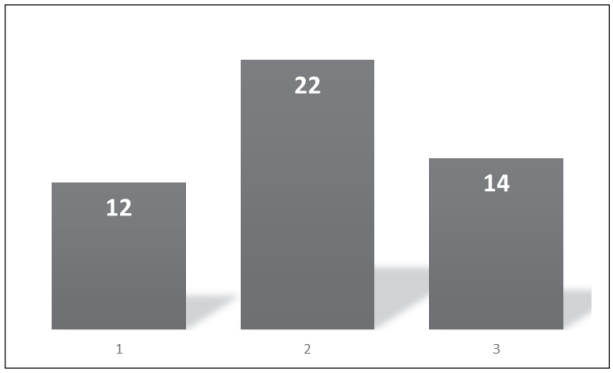

Figure 2. Representation of patients with postoperative heart failure according to chronic renal failure stage; 1 – patients with creatinine clearance ≥ 90 mm/min./1.73 m^2; 2 – patients with creatinine clearance of 60–89 mm/min./1.73 m^2; 3 – patients with creatinine clearance of 15–59 mm/min./1.73 m^2

The number of patients who underwent coronary surgery was 90 (53.2%), valvular surgery 36 (21.3%), and combined surgery 43 (25.5%). Mean duration of extracorporeal circulation was 88.23 ± 35.52 min.

There was no significant difference in the incidence of patients with the new onset of MI in regard to the CRF stage ($p > 0.05$). A new onset of MI was a rare complication after surgery in patients with CRF. There was no significant difference in the incidence of patients with arrhythmias, CVI and sepsis rate in regard to the CRF stage ($p > 0.05$).

Significant difference in the incidence of respiratory failure with regard to the CRF stage ($p = 0.049$) was noted, and it was most notable between CRF stage I and stages III–IV ($p = 0.016$). Postoperative occurrence of HF – defined as the need to ensure hemodynamic support with the inotropes (adrenaline, dobutamine > 5 mg/kg, and/or high doses of dopamine for periods longer than 24 hours), and the value of cardiac index < 2.0 l/min./m^2 – is presented in Figure 2 ($p < 0.0001$).

A highly significant difference was present in the incidence of acute exacerbation of renal failure with regard to the CRF stage ($p < 0.01$) – between the CRF stages I and II ($p = 0.011$), II and III–V ($p = 0.016$), and between the CRF stages I and III–V ($p < 0.01$). Also, there was a significant difference in the mortality rate in regard to the CRF stage ($p = 0.021$) – between the CRF stages I and III–V ($p = 0.006$) (Table 2).

A total of 16 deaths were registered. Thereof, seven patients underwent coronary surgery, six underwent combined surgery and three patients underwent valve surgery. Seven patients were in CRF stage III–V, seven patients were in CRF stage II, and two patients were in CRF stage I.

Univariate analysis designated CRF stage as a predictor of the occurrence of respiratory failure after surgery (OR = 2.1; 95% CI 1.12–4.07; $p = 0.021$) and CRF stage III–V (OR = 4.46; 95% CI 1.23–16.15; $p = 0.023$). CRF stage was also the predictor of in-hospital mortality (OR = 2.72; 95% CI 1.27–5.08; $p = 0.009$) and CRF stage III-V (OR = 7.64; 95% CI 1.49–39.27; $p = 0.015$). Preoperative CRF stage was the predictor of acute exacerbation of renal failure following surgery (OR = 3.14; 95% CI 1.69–5.79; $p < 0.01$), stage II (OR = 4.76; 95% CI 1.31–17.28; $p = 0.018$), and CRF stage III–V (OR = 11.39; 95% CI 2.87–45.14; $p = 0.001$).

In patients with CRF stage I and II, the univariate predictors of acute exacerbation of CRF were preoperative CVI (OR = 3.06; 95% CI 1.01–9.25; $p = 0.048$), NYHA III/IV (OR = 4.5; 95% CI 1.60–12.64; $p = 0.004$), EF ≤ 35% (OR = 5.05; 95% CI 1.78–14.30; $p = 0.002$), and CRF stage (OR = 4.76; 95% CI 1.31–17.28; $p = 0.018$). In patients with CRF stage I and II, the multivariate predictors of acute exacerbation of CRF were previous CVI (OR = 3.36; 95% CI 1.04–10.93; $p = 0.044$) and EF ≤ 35% (OR = 5.35; 95% CI 1.83–15.64; $p = 0.02$).

DISCUSSION

The global number of cardiac surgery procedures performed in patients with CRF is constantly increasing [2, 3]. According to the available data, adverse cardiovascular events are the most important cause of death in patients with CRF. The development of exacerbation of CRF is correlated with substantial short- and long-term morbidity

and mortality. The pathogenesis is multifactorial. Hemodynamic, inflammatory, metabolic and nephrotoxic factors are involved and overlap each other, which leads to further aggravation of renal failure. Preoperative risk factors include advanced age, impaired left ventricular function or congestive HF, diabetes, chronic obstructive pulmonary disease, and the urgency of the operation [7, 8].

In large studies that have addressed this issue, the most commonly described postoperative morbidity included: respiratory failure, arrhythmias, acute exacerbation of renal insufficiency, pericardial effusion. Most of these complications might be explained, to an extent, through hypervolemic state developed early after the surgery (inadequate fluid management). Contrary to our findings, the need for dialysis was over 15% [9].

The high frequency of postoperative arrhythmias can be compared with the results of other studies as a result of electrolyte imbalance in the perioperative period [10–15]. The tendency to infection is a common feature for these patients, due to reduced chemotaxis, lymphopenia, decreased cell-mediated immunity, and reduction functions of monocytes. In our study, patients underwent antibiotic protection so no significant occurrence of infection was recorded compared to other studies where the presence of sternal infection was around 8% [16–19].

A retrospective analysis of patients undergoing CABG at the Cleveland Clinic found that the level of creatinine over 168 mmol/l results in a higher perioperative morbidity (2.8%) and mortality (3.7%). Hospital mortality in patients with CRF submitted to cardiac surgery goes up to 36.7% as reported in large multicenter studies [20–23]. Our results are in line with the reports of cardiovascular centers around the world, and also with the largest multicenter study performed so far, in which the mortality was 12.5% [24]. Our analyses designated the following factors as predictors of postoperative mortality of CRF patients: COPD and diabetes. Isolated coronary surgery is associated with the lowest incidence of acute exacerbation of CRF, followed by valvular and combined operations as is the case with our group of patients [25].

In terms of prevention of acute exacerbation of renal failure after surgery, a proper preoperative patient management is required, especially for those on chronic dialysis, patients with pulmonary diseases and metabolic disorders (diabetes). As our understanding of the pathogenesis of renal injury following cardiac surgery grows, better preventive and therapeutic strategies will arise. Current approaches include deferring elective surgery, until there is adequate recovery following pre-existing renal injury, careful pre-operative risk stratification of patients, and consideration of less invasive procedures in those at greatest risk [26].

CONCLUSION

Preoperative chronic renal failure leads to an increased morbidity and mortality in patients submitted to cardiac surgery. Therefore, a careful preoperative evaluation is warranted as well as optimal perioperative management and treatment strategy for the purpose of risk reduction.

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Кардиохирургија код болесника са хроничном бубрежном инсуфицијенцијом

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САЖЕТАК

Увод/Циљ Сматра се да болесници са хроничном бубрежном инсуфицијенцијом (ХБИ) после кардиохируршких операција имају више оперативних компликација и знатно повећану смртност.

Циљ рада је био да се одреде преоперативни показатељи погоршања ХБИ и смртног исхода код болесника са ХБИ подвргнутих кардиохируршким операцијама.

Методе Ретроспективно су анализиране периоперативне карактеристике код 169 болесника (старости 67,71 ± 8,46 година, 72,3% мушкараца) лечених од 2012. до 2015.

Резултати Преоперативно, у I стадијуму ХБИ било је 62 (37%), у II – 77 (46%), а III–V стадијуму 30 (17%) болесника. Погоршање ХБИ регистровано је код 37 (21,9%), а смртни исход код 16 болесника (9,5%). II стадијум ХБИ (*OR* 4,76; 95% *CI* 1,31–17,28; *p* = 0,018) и III–V стадијум ХБИ (*OR* 11,39; 95% *CI* 2,87–45,14; *p* = 0,001) били су предсказатељи погоршања ХБИ. Код болесника са I и II стадијумом ХБИ, мултиваријантни предсказатељи погоршања ХБИ били су претходни мождани удар (*OR* 3,36; 95% *CI* 1,04–10,93; *p* = 0,044) и ејекциона фракција ≤ 35% (*OR* 5,35; 95% *CI* 1,83–15,64; *p* = 0,02). Једини предсказатељ постоперативне дијализе је виши стадијум ХБИ (*OR* 5,81; 95% *CI* 1,22–27,81; *p* = 0,028). III–V стадијум ХБИ био је предсказатељ смртног исхода (*OR* 7,64; 95% *CI* 1,49–39,27; *p* = 0,015).

Закључак Тежи стадијум ХБИ код болесника подвргнутих кардиохируршким операцијама јесте предсказатељ погоршања бубрежне инсуфицијенције и смртног исхода.

Кључне речи: хронична бубрежна инсуфицијенција; кардиохирургија; морбидитет; морталитет

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Radiofrequency ablation for hepatocellular carcinoma – analysis of the clinical outcome

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SUMMARY

Introduction/Objective Radiofrequency ablation (RFA) is a minimally invasive treatment modality for primary and metastatic liver tumors. It can be performed percutaneously or as a laparoscopic or open surgical procedure under ultrasound or computerized tomography guidance.
The objective of the study was to evaluate the clinical outcome of the initial 16 patients with hepatocellular carcinoma (HCC) managed by percutaneous RFA at a tertiary institution and to assess the efficacy of this procedure in the management of selected patients with HCC.

Method From June 2011 until December 2013, 16 patients with early-stage HCC were managed by percutaneous radiofrequency ablation at the Clinic for Digestive Surgery, Clinical Center of Serbia, Belgrade. All the patients were treated by the same team composed of an interventional radiologist and a liver surgeon. We analyzed the clinical outcome and the biologic effect of this treatment by comparing the pre- and post-treatment levels of alpha-fetoprotein (AFP).

Results Post-treatment values of liver transaminase levels returned to the pre-treatment values from Day 3. Post-treatment hospital stay was two days. Post-procedural complications included mild pain in all patients, skin necrosis at the site of the electrode puncture in five patients, and transient hepatic decompensation in one patient. In all the patients the AFP level correlated with the findings of liver imaging (ultrasound and/or magnetic resonance imaging with liver-specific contrast agent) indicating viability of the treated tumor.

Conclusion RFA is a feasible and effective procedure providing favorable clinical outcome in patients with early-stage HCC.

Keywords: radiofrequency ablation; hepatocellular carcinoma; biologic effect; percutaneous approach

INTRODUCTION

Radiofrequency ablation (RFA) is a minimally invasive treatment modality for primary and metastatic liver tumors. It can be performed as percutaneous or as laparoscopic or open surgical procedure under ultrasound (US) or computerized tomography (CT) guidance. When performed percutaneously, patients can be managed using local analgosedation avoiding general anesthesia. Radiofrequency ablation is an alternative treatment option to hepatic resection for patients with small, primary liver tumors [1, 2, 3]. It is a safe procedure achieving survival as long as 10 years in the latest reports and it is considered a curative treatment modality [4]. For selected patients with early-stage hepatocellular carcinoma (HCC), RFA can be used as a first-line treatment option. However, the results of the treatment are dependent on operator experience [5].

The advantages of RF ablation are the following: a) it is an effective treatment for primary and metastatic liver tumors in selected patients who are unsuitable for surgical resection; b) treatment-related serious complications are infrequent and discomfort is minimal; c) the procedure may be used repeatedly to treat recurrent liver tumors; d) the percutaneous approach is minimally invasive, produces few complications, and may be applied in ambulatory settings; e) it is a relatively quick procedure associated with quick recovery so chemotherapy may be resumed almost immediately if required; f) it is less expensive than other treatment options; g) no surgical incision is needed.

The clinical outcome of patients treated with RFA can be assessed using laboratory analysis and imaging modalities. Several studies confirmed that alpha-fetoprotein (AFP) measurements indicate the necrotic effect of locoregional thermal ablation [6, 7, 8], while other studies confirmed the biologic role of AFP in a neoplastic growth [9]. AFP is a fetal-specific glycoprotein normally produced primarily by the fetal liver. Normally, AFP levels decline rapidly after birth, reaching undetectable levels (< 10 ng/ml) within several months after birth [6, 7, 8]. Increased AFP levels indicate the presence of cancer, most commonly liver cancer, ovarian cancer, or germ cell tumor of the testicles. However, not every liver, ovarian, or testicular cancer will produce significant quantities of AFP [8, 9].

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In the present study we analyzed the clinical outcome of the first 16 patients with early-stage HCC treated by RFA at a tertiary institution and we assessed the efficacy of this procedure using post-procedural imaging and measuring pre- and post-treatment levels of AFP.

The study objective was to evaluate the clinical outcome of the first 16 patients with an early-stage HCC managed by a percutaneous radiofrequency ablation and to assess the efficacy of this procedure using post-procedural imaging and serum levels of AFP.

METHODS

From June 2011 until December 2013, 16 patients with early-stage HCC were managed by percutaneous radiofrequency ablation at the Clinic for Digestive Surgery, Clinical Center of Serbia, Belgrade. During the same period, five additional patients were managed by the same procedure for a solitary metastatic tumor in the liver as they were not eligible for open surgery due to their co-morbidities. All the patients were managed by the same team composed of an interventional radiologist and a liver surgeon. According to revised version of the Barcelona Clinic Liver Cancer system endorsed by the American and European Association for Study of the Liver, the patients diagnosed at an early stage are defined as follows [10]: "very early" with a single node less than 2 cm in size, in Child–Pugh A class, with no symptoms and no change in performance status; and "early", when single node, smaller than 5 cm, or up to 3 nodes < 3 cm each, in Child–Pugh A-B class, with no symptoms and no change in performance status.

All the patients included in this study met the following criteria: (a) no extrahepatic spread of a disease; (b) liver tumor less than 5 cm in diameter; (c) adequate functional liver parenchyma; (d) no obstructive jaundice; and (e) no simultaneous operative procedures on other organs.

The preoperative workup of the patients included: (a) accurate liver imaging utilizing multi-detector CT or magnetic resonance imaging (MRI); (b) the assessment of hepatic functional reserve; (c) AFP measurement.

In all the patients, biochemical liver function tests were monitored before and on the first day after the RFA. Static biochemical liver function tests were used to assess hepatic functional reserve since all the patients had liver cirrhosis. Liver abscess, subphrenic abscess, bile leakage, sepsis, chest involvement, and post-procedural bleeding were considered procedure-related complications. Follow-up protocol included laboratory analysis, AFP measurement, and US examination one, three, and nine months after the RFA and laboratory analysis, AFP measurement and MRI with a liver-specific contrast agent (Primovist®, Bayer AG, Leverkusen, Germany), six and 12 months following RFA. After the first year, the patients were followed on six months bases using abdominal MRI and AFP level. The follow-up period for patients included in the study ranged 24–42 months.

In all the patients, RFA was performed using a Cool-tip® (Valleylab, Tyco) water-cooled, single, RF tumor ablation electrode, with a 30 mm non-insulated tip, connected to a 480 kHz 200 watt generator (Valleylab Cool-tip® RF System). RF energy was applied by gradually increasing the output to maximum power, achieving thermal ablation of tumor tissue. Under US guidance, the electrode was repositioned until the complete tumor ablation was performed.

Table 1. Patient and tumor characteristics

Variable	RFA, n = 16 pts
Sex (M/F)	11/5
Age	60.5 (47–79)
Cirrhosis etiology	
Hepatitis B	5
Hepatitis C	9
Co-infection hepatitis B and C	1
Hepatitis B and ethylic	1
Liver function status	
Child–Pugh A	13
Child–Pugh B	3
Tumor number	
1	15
2	1
Tumor size (mm)	25.5 (21–40)
Tumor localization	
Segment II/III	2
Segment IVB	1
Segment V	4
Segment VI	5
Segment VII	3
Segment VIII	2

RFA – radiofrequency ablation

Four patients were treated under local analgosedation and 12 under general anesthesia. Other patients' data are presented in Table 1.

All the patients had liver cirrhosis of viral origin with Child status A or B. Child B patients had either lower albumin level (30–35 g/l), moderate ascites responsive to diuretics treatment and/or increased bilirubin level (Child–Pugh B8). However, only Child B patients with sufficient remnant liver volume and compensated liver function were considered for RFA. All patients managed by percutaneous RFA in this study had compensated liver function. All the patients included in this study were not candidates for liver resection due to their co-morbidites, liver function, or due to tumor localization that would require major liver resection with insufficient liver remnant.

The patients were diagnosed either by liver biopsy (histological confirmation of HCC and cirrhosis during the previous surgery / liver resection or by ultrasound-guided biopsy performed in another institution) or by typical radiological findings of HCC (arterial enhancement and wash-out on portal phase) on CT or MRI imaging in combination with an increased level of AFP (normal range up to 10 ng/ml).

Informed consent was obtained from all the patients before RFA.

All data were prospectively collected and entered into a computerized data base. All the data are expressed as median with the range.

RFA procedure

All RFA treatments were performed under a standard protocol using the Cool-tip® RF needle with a 3 cm exposed tip. The ablation was performed with a curative intent, aiming to achieve a margin of 1 cm. After completing the procedure a safe margin of 1cm was assessed by measuring the ablated zone. In all the patients the ablated zone was at least 1cm larger than the size of the tumor. The patients were reviewed by a multidisciplinary team before the approach of RFA was decided (intercostal in 10 and subcostal in six patients). The percutaneous ablation was performed by ultrasound guidance under local analgosedation (four patients) or under general anesthesia (12 patients). The ablation was performed using the manual mode with continuous monitoring of the impedance, RF current, and the temperature (Figures 1–3). The non-insulated tip of the electrode was continuously perfused with cold saline via internal channels inside the needle throughout the ablation to maintain the tip temperature below 15°C, preventing charring around the electrode tip.

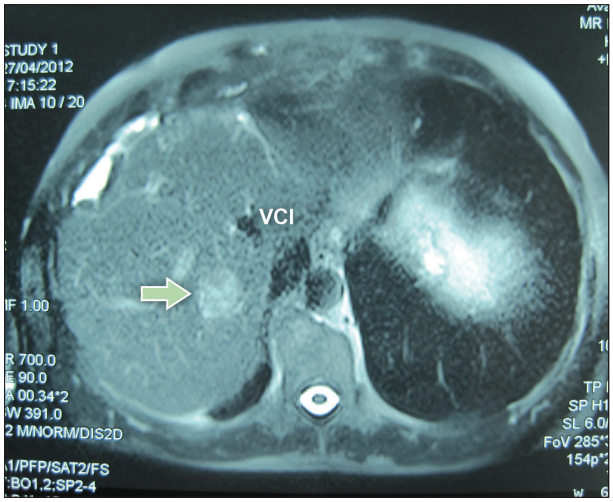

Figure 1. Pretreatment abdominal MRI demonstrating a tumor in liver segment VIII

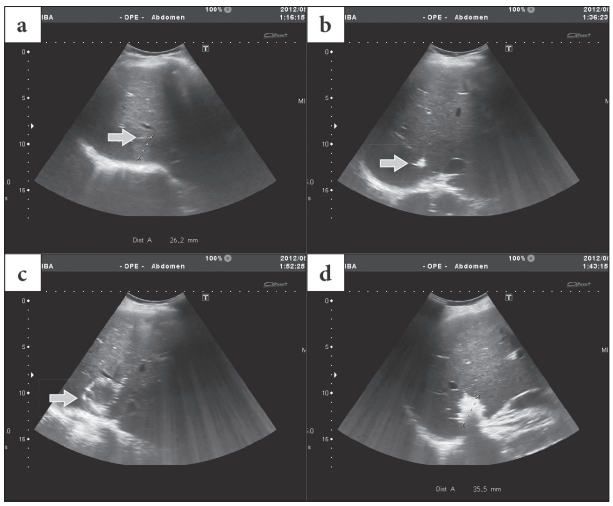

Figure 2. US-guided radiofrequency ablation procedure for hepatocellular carcinoma in liver segment VIII; a) US tumor measurement, localization and positioning of the needle; b) start of ablation; c) progress of ablation; d) end of procedure, ablation zone

RESULTS

Sixteen patients with early-stage HCC in cirrhotic livers underwent curative-intent RFA. Peri-procedural transfusion was not required in any patient.

The pretreatment and post-treatment profile of liver enzymes (bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and prothrombin time (PT)) and hemoglobin and platelets is presented in Table 2. The post-treatment values of AST and ALT returned to pretreatment values from Day 3.

The post-treatment hospital stay was two days [2–8]. Post-procedural complications included mild pain in 14 patients, skin necrosis at the site of the needle puncture in five patients, and transit hepatic decompensation in one patient (Table 3).

During the follow-up period, two patients were managed by transarterial chemoembolization (using a mixture of Lipiodol and cisplatin) as the tumor size exceeded 3 cm (42 mm) or due to tumor recurrence. Mortality was not recorded during the follow-up period.

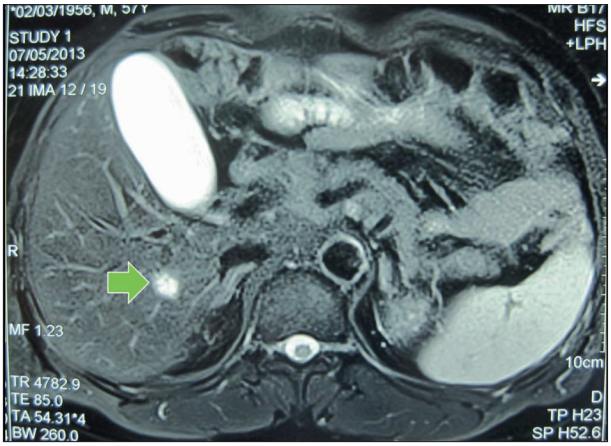

Figure 3. Post-treatment abdominal MRI using a liver-specific contrast agent demonstrating complete tumor ablation

Table 2. Pre- and post-treatment profile of liver enzymes and hemoglobin/platelets

Variable	Before RFA	After RFA
Bilirubin (μmol/L)	24.1 (8.6–92.3)	28.1 (18.2–80.4)
AST (U/L)	56.5 (34–227)	226.5 (87–529)
ALT (U/L)	46.5 (22–137)	147.5 (33–411)
Prothrombin time	147.5 (33–411)	14 (11.3–24.3)
Hemoglobin (g/L)	129 (107–158)	116.5 (97–156)
Platelets (10^9/L)	113 (40–178)	89.25 (40–162)

RFA – radiofrequency ablation; AST – aspartate aminotransferase; ALT – alanine aminotransferase

Table 3. Post-procedural complications

Variable	n (%)
Pain (mild)	14 (87)
Nausea	0
Vomiting	0
Fever	0
Skin necrosis	5 (31)
Transit liver decompensation	1 (6)

Table 4. Pre- and post-procedural measurement of AFP; correlation with liver imaging

Patient	Pre- AFP	AFP 1M	AFP 6M	MR/Primovist
1	21.9	6.5	5.9	Complete ablation
2	26	10.6	7.7	Complete ablation
3	1,703	24.1	11.1	Complete ablation
4	147.2	5.7	7.2	Complete ablation
5	145.1	366.6	11.8	Complete ablation
6	4,848.8	22.9	14.3	Complete ablation
7	1,216.7	55.1	11.4	Complete ablation
8	13.4	8.7	8.8	Complete ablation
9	1,746	33.1	21,748	Incomplete/recurrence
10	30.1	4.7	5.2	Complete ablation
11	12.5	5.5	4.9	Complete ablation
12	14,964	8,232	/	/
13	13.2	15.6	9.9	Complete ablation
14	40.5	12.8	10.1	Complete ablation
15	13.8	10.6	11.2	Complete ablation
16	409.4	55.1	14.8	Complete ablation

AFP – alfa-fetoprotein; 1M – one month after the treatment; 6M – six months after the treatment

In one patient, one month following RFA, alpha-fetoprotein level had increased from pretreatment levels of 145.1 ng/ml to 366.6 ng/ml. An abdominal US examination confirmed incomplete tumor ablation and the patient was re-treated using the same procedure. At the six-month follow-up AFP measured 11.8 ng/ml. This result correlated with the finding of complete ablation verified by the abdominal MRI using liver-specific contrast agent.

In one patient at the six-month follow-up, AFP level significantly increased to more than 20,000 ng/ml. This result correlated with the finding of recurrent tumor adjacent to the ablation zone verified on abdominal MRI using a liver-specific contrast agent. The tumor recurrence was managed by liver resection and intraoperative exploration has demonstrated new encapsulated tumor adjacent to the ablation zone. At the time of surgery, the patient liver function improved to Child A status.

In the two presented patients with recurrent tumor, the AFP level correlated with the findings of liver imaging (abdominal US and/or MRI with liver-specific contrast agent) indicating viability of the treated tumor (Table 4).

DISCUSSION

Radiofrequency ablation for small HCC demonstrated a survival benefit comparing to percutaneous ethanol injection as indicated by randomized controlled trials and meta-analyses and systemic reviews [11, 12]. For many years, these results established RFA as a standardized local thermal ablation technique for treating small liver tumors.

Novel ablation thermal and non-thermal techniques, including microwave ablation and irreversible electroporation, have some potential to overcome the limitations of RFA but further clinical investigations are required [13].

The complete ablation rate of RFA for liver tumors was found to vary from 50% to 95% in different reports [14]. In the present study, a complete ablation was achieved in 14 out of 16 patients (87%) after the initial RFA and in 94% of patients after the repeated procedure in one patient having a recurrent tumor. Only one patient required surgical treatment due to recurrent tumor adjacent to the ablation zone.

Significantly different results of RFA are usually attributed to the differences in the electrode design, generators, and application techniques. The importance of operator experience was reported by Poon et al. [5]. They report that complete ablation was achieved in 84% of the first 50 patients managed by RFA, while in the following 50 patients 100% ablation rate was achieved. In the present study, the success rate for initial 16 patients with small HCC managed by RFA was 87%. In the same study by Poon at al., close collaboration between the surgeons and radiologists was suggested in order to shorten the learning curve and plan the best strategy for ablation. In the present study, pretreatment analysis, deciding on the RFA approach, RFA procedure, and post-treatment follow-up were performed in a multidisciplinary team approach by an interventional radiologist and a liver surgeon.

Complication rates following RFA of liver tumors range 0–27% [15, 16]. Reported RFA treatment-related complications include pneumothorax; symptomatic pleural effusion; bleeding from the needle track or into the treated tumor; biliary fistula; biliary stricture; biloma; abscess in the treated tumor; skin burn; cholecystitis; thermal injury to adjacent structures including the diaphragm, stomach, duodenum, and transverse colon; liver failure; segmental hepatic infarction; paralysis of the hemidiaphragm; arterial-portal venous fistula; systemic hemolysis; tumor lysis syndrome; myoglobinemia or myoglobinuria; transient acute renal failure; and prolonged post treatment pain for lesions near the hepatic capsule [15]. According to Livraghi et al. [17] and Mulier et al. [18], the complication rates are higher after open or laparoscopic RFA compared to the percutaneous approach. A possible explanation is that more difficult cases are treated by open or laparoscopic approach and due to surgery-related complications.

In the present study, during the follow-up period, early and late post procedure complications did not occur. This is mainly due to proper patient selection and due to collaboration between the interventional radiologist and liver surgeon, which is rarely reported in literature [5].

In the present study, two out of the first 16 patients had HCC in seg. VIII (and one more patient with metastatic liver tumor not included in this series) not eligible for open surgery. In the reviewed literature this tumor localization is considered difficult for percutaneous approach or associated with serious complications [19, 20]. The two patients had uneventful post-procedural course and demonstrated no complications in the follow-up period.

The most common complication in the present study was skin burns at the site of the electrode puncture detected in the first five patients treated by RFA. In the reviewed literature, the majority of reported skin burns after percutaneous radiofrequency ablation occur along the edge of the grounding pads or during the tract ablation phase [14, 21, 22]. In the present study, skin burns resulted

from an excessive tract ablation to prevent tract seeding; however, in the following 11 patients, this complication did not occur.

The most important result of the study is related to the comparison of alpha-fetoprotein measurements and liver imaging indicating the biologic effect of the applied treatment.

There is a consensus in the literature that AFP regulates neoplastic growth through the presence of an alpha-fetoprotein cell surface receptor that undergoes internalization to the cell interior growth [9].

In HCC there is a strong correlation between AFP values, tumor dimensions, and microvascular invasion, as predictors of HCC recurrence [23]. According to reports from the literature, AFP is a surrogate of tumoral activity and vascular invasiveness. AFP-mRNA concentration is used as a marker of HCC cell dissemination into the circulation, which is an additional proof of this correlation [24, 25].

In the present study, the AFP level increased from pretreatment level of 145.1 ng/ml to 366.6 ng/ml one month after the RFA in one out of 16 patients. Abdominal US examination confirmed incomplete tumor ablation and the patient was re-treated using the same procedure. At the six-month follow-up, AFP measured 11.8 ng/ml, correlating with the finding of complete ablation on abdominal MRI using liver-specific contrast agent.

In another patient at the six-month follow-up, the AFP level significantly increased to more than 20,000 ng/ml, correlating with the finding of recurrent tumor adjacent to the ablation zone on abdominal MRI using liver-specific contrast agent. The tumor recurrence was managed by liver resection. The pathology finding demonstrated a new encapsulated tumor adjacent to the ablation site.

In other patients, normal AFP values correlated with complete tumor ablation verified by liver imaging techniques. Therefore, in all the patients the AFP level correlated with the findings of liver imaging (abdominal US and/or MRI using liver-specific contrast agent) indicating viability of the treated tumor.

New tumor markers are continuously discovered and investigated, but they are still far from the routine clinical practice.

The present study confirmed the need for the development of a new predictive model combining radiological and biological features based on biological markers as already indicated by Giovanni B et al. [26].

CONCLUSION

Percutaneous RFA is a first-line treatment option for carefully selected patients with small-sized/early-stage HCC in a cirrhotic liver when performed at a tertiary institution by a multidisciplinary team. The procedure is associated with a minimal morbidity offering curative treatment for this difficult category of patients.

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Радиофреквентна аблација хепатоцелуларног карцинома – анализа клиничког исхода

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САЖЕТАК

Увод/Циљ Радиофреквентна аблација (РФА) минимално је инвазиван начин лечења примарних и метастатских тумора јетре. Може се извести перкутано, лапароскопски или отворено хируршки, под контролом ултразвука или компјутеризоване томографије.

Циљ овог рада је да прикаже клинички исход лечења првих 16 болесника са хепатоцелуларним карциномом (ХЦК), третираних перкутаном РФА у терцијалној установи и да процени ефикасност ове процедуре у лечењу селектованих болесника са ХЦК.

Метод У периоду од јуна 2011. до децембра 2013. године 16 болесника са раним ХЦК третирани су перкутаном РФА. Анализирали смо клинички исход и биолошки ефекат лечења поредећи ниво алфа-фетопротеина (АФП) пре и после третмана.

Резултати Ниво трансаминаза после интервенције враћао се на вредности пре третмана од трећег дана. Боравак у болници је трајао два дана (2–8). Постпроцедуралне компликације су биле: благи бол код свих болесника, некроза коже на месту пункције електродом код пет болесника и пролазна декомпензација јетре код једног. Код свих болесника ниво АФП је корелирао са налазом на јетри (ултразвук и/или магнетна резонанца са специфичним контрастом за јетру), указујући на вијабилност третираног тумора.

Закључак РФА је изводљива и ефикасна процедура која обезбеђује повољан клинички исход код болесника са раним ХЦК.

Кључне речи: радиофреквентна аблација; хепатоцелуларни карцином; биолошки ефекат; перкутани приступ

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Subscale correlations between MSSS-88 and PRISM scales in evaluation of spasticity for patients with multiple sclerosis

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SUMMARY

Introduction/Objective Patient-reported outcomes have been recognized as an important way of assessing health and well-being of patients with multiple sclerosis (MS).
The aim of the study is to determine the correlation between different subscales of Patient-Reported Impact of Spasticity Measure (PRISM) and Multiple Sclerosis Spasticity Scale (MSSS-88) scales in the estimation of spasticity influence on different domains

Methods The study is a cross-sectional observational study. MSSS-88 and PRISM scales were analyzed in five domains (body-function domain, activity domain, participation domain, personal factors/wellbeing domain, and hypothesis). For statistical interpretation of the correlation we performed the Spearman's ρ-test, concurrent validity, divergent validity, and the linear regression model.

Results We found a significant correlation between subscales of evaluated MSSS-88 and PRISM scales for body domains; the highest correlation was between the need for assistance/positioning (NA/P) and walking (W). Spasticity has the weakest correlation with the need for intervention (NI). The presence of pain has a negative impact and significant positive correlation between pain discomfort and NI. In the domain of body function for males, there was a non-significant correlation between muscle spasms and NI. The same applies for social functioning and social embarrassment domains, as well as for emotional health and psychological agitation for personal factors / wellbeing domain. The differences between genders of MS patients persist in different domains; muscle spasms are strong predictors for NI, and body movement is a strong predictor versus W for NA/P.

Conclusion MSSS-88 and PRISM scales can be considered reliable in measuring different domains of disability for MS patients with spasticity. Because it is shorter, quicker, and simple to use, it is concluded that the PRISM scale can successfully compete with and replace the MSSS-88 scale in certain domains.
Keywords: multiple sclerosis; spasticity; scales; patient-oriented scales

INTRODUCTION

Multiple sclerosis (MS) presents a chronic autoimmune disorder with particular influence on the central nervous system, characterized by inflammation, demyelination, and axonal degeneration, and is the most common cause of neurologic disability in young adults [1, 2]. The epidemiology assessment incidence and prevalence can demonstrate the existence of spatial, temporal, and demographic variations of disease risks which are important for identifying genetic and environmental factors that act together to cause the disease [3].

The important group of clinical manifestations refers to the functional disability with various degrees of neurological affection and therefore reduction of functional capacity. Although the symptoms individually vary, the majority of persons with MS present with some degree of spasticity. The reported prevalence of spasticity in MS is up to 65% in Europe and 85% in the USA [4, 5]. Spasticity is often disabling and may affect the physical, psychological, and social well-being of patients with MS [6, 7].

Outcome measurement is important for assessing disability, and selecting an appropriate scale of measurement is one of the most important steps in clinical research. Many of the available disability outcome measures used in clinical trials of MS are insensitive to change over time, inadequately validated, or insensitive to patient-perceived health status or the quality of life [8].

To be appropriate to the task, a scale must be valid, accurate, precise, efficient, and easy to use, sensitive to changes in the disease without being sensitive to symptom fluctuations, and it needs to cover the whole range of the disease [9]. Outcome measures are difficult to choose because of the diversity and the progressive and fluctuating nature of disease.

Patient-reported outcomes have been increasingly recognized as an important way for

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assessing health and well-being from a personal perspective. For this purpose, the Multiple Sclerosis Spasticity Scale (MSSS-88) has been developed to address how spasticity affects daily life of people with MS [10]. Previously, we have validated MSSS-88 in MS patients with spasticity and also provided findings on the correlation among different functional scales [11]. We hypothesized that correlations in different domains in MSSS-88 scale is expected with different domains of daily activities for patients with MS. Since Patient-Reported Impact of Spasticity Measure (PRISM) was originally developed and validated in the spinal cord injury population, we have previously validated PRISMSR (PRISM in the Serbian language) in persons with MS [12].

The PRISMSR shows adequate validity and reliability for assessing the impact of spasticity on the quality of life in persons with MS, provides a unique personal experience of spasticity, and may complement other clinical outcome measures [13]. We tried to demonstrate whether these two scales correlate completely, or in certain domains.

Therefore, the aim of our study was to assess the correlation among different subscales of PRISM and MSSS-88 scales in the estimation of spasticity influence on different domains of daily activities for patients with MS.

METHODS

The cross sectional observational study included 58 patients with diagnosed MS that we recruited at the "Dr Miroslav Zotović" Clinic for Rehabilitation. This type of study was used since our participants differed in the variable of interest, while they shared variables such as educational background, socioeconomic status, and ethnicity; thus, the study environment wasn't manipulated. Patients were evaluated separately regarding gender [males (n = 17) and females (n = 41)].

Prior to the inclusion in the study, the patients were informed about the study protocol and informed consent was obtained. The study was approved by the Institutional Review Board for Human Research of the Clinic for Rehabilitation in Belgrade.

The criteria for inclusion in the study were as follows: age above 18 years; duration of MS for more than a year, from the diagnosis established by magnetic resonance imaging and oligoclonal band; remission of the disease longer than three months and the presence of spasticity either subjectively reported or documented on clinical examination.

MSSS-88 and PRISM scales were analyzed in five domains (body function domain, activity domain, participation domain, personal factors / well-being domain, and hypothesis domain). Body domain included MSSS-88 subscales [muscle stiffness (MSS), muscle spasms, pain and discomfort (PD), body movement (BM), and walking (W)] and PRISM subscales [need for intervention (NI) and need for assistance/positioning (NA/P)]. Activity domain included MSS-88 subscale activities of daily life (ADL) and PRISM subscale daily activities (DA). Participation domain included MSSS-88 subscale social functioning (SF) and PRISM subscales social embarrassment (SE) and social avoidance/anxiety (SAA). Personal factors / well-being domain included MSSS-88 emotional health (EH) subscale and PRISM SAA and psychological agitation (PA) subscales. Hypothesis domain included MSSS-88 (PD, W, ADL, SF, and EH subscales, and PRISM positive impact (PI) subscale.

MSSS-88 scale contains a total of 88 questions divided into eight subscales: MSS – 12 items, PD – nine items, MS – 14 items, ADL – 11 items, W – 10 items, BM – 11 items, EH – 13 items, SF – eight items. Each item is ranked on a four-point Likert scale: 1 (not bothered at all), 2 (a little bothered), 3 (moderately bothered), and 4 (extremely bothered).

PRISM scale consists of 44 items grouped into seven subscales. SAA – 11 items, PA – five items, DA – six items, NA/P – five items, PI – four items, NI – five items, and SE – five items. The participants answered to which extent each statement is true for their situation using a five-point Likert-type scale (0 – "never", 1 – "rarely", 2 – "sometimes", 3 – "often," and 4 – "very often"). The reported score for PI is reversed (0 – "very often", 4 – "never"); thus, the higher the score, the lower the positive impact of spasticity.

Statistical analysis

Data were presented as whole numbers (n) and as percentage (%). The χ^2 test was used for statistical interpretation of categories distribution for different parameters in Table 1.

For statistical interpretation of correlation strength and significance among different subscales of evaluated scales (MSSS-88 and PRISM), we performed Spearman's ρ-test, where ρ was indicated as the measure of strength, while p-value represented statistical significance. Statistical significance was set at $p < 0.05$. Body function, activity and participation domains, and personal factors / well-being domains were analyzed through concurrent validity, while hypothesis was analyzed by divergent validity. We used the linear regression model for predictor subscales of MSSS-88 and on subscale values of PRISM.

Table 1. Demographic and multiple sclerosis-related characteristics of the sample (n = 58)

Parameters	Categories	n (%)	p
Gender	male	17 (31%)	< 0.001
	female	41 (69%)	
Education	high school	42 (72%)	< 0.001
	college/university	16 (28%)	
Employment	unemployed	7 (12%)	< 0.001
	employed	19 (33%)	
	retired	32 (55%)	
Marital status	single	11 (19%)	< 0.001
	married	35 (60%)	
	divorced/widowed	12 (21%)	
Type of MS	primary progressive MS	32 (55%)	< 0.001
	relapse-remitting MS	8 (14%)	
	secondary progressive MS	18 (31%)	

RESULTS

The mean age of the studied participants was 45 ± 10 years. Females, individuals with high school education, those who were retired as well as married were significantly more frequent than others ($p < 0.001$) (Table 1). The significantly predominant type of MS was the primary progressive (55%), followed by the secondary progressive (31%), and relapsing-remitting (8%) ($p < 0.001$) (Table 1).

There is a significant positive correlation between every tested subscale, with the highest positive correlation for the NA/P subscale of the PRISM, and the BM subscale ($\rho = 0.727$) and for the W subscale of the MSSS-88 scale ($\rho = 0.730$) (Table 2). The weakest positive correlation was obtained between the PI subscale of PRISM and the SF subscale of the MSSS-88 scale ($\rho = 0.339$) (Table 2).

There is a significant positive correlation between every tested subscale except for the PI subscale of the PRISM with the SF subscale ($\rho = 0.259$; $p = 0.101$) and with the EH subscale of the MSSS-88 ($\rho = 0.289$; $p = 0.066$) (Table 3). There is the highest positive correlation for the NA/P subscale of the PRISM and the BM subscale of the MSSS-88 ($\rho = 0.752$) and for the W subscale of the MSSS-88 scale ($\rho = 0.761$) (Table 3). The weakest positive correlation was obtained between the PI subscale of the PRISM and the SF subscale of the MSSS-88 scale ($\rho = 0.259$) (Table 3).

There is a significant positive correlation between every tested subscale except for the NI subscale of the PRISM and muscle spasms subscale of the MSSS-88 ($\rho = 0.471$; $p = 0.056$), for the SE subscale of the PRISM and the SF subscale of the MSSS-88 ($\rho = 0.288$; $p = 0.260$), for the PA subscale of the PRISM and the EH subscale of the MSSS-88 ($\rho = 0.455$; $p = 0.066$), and the PI subscale of the PRISM with the PD subscale of the MSSS-88 ($\rho = 0.443$; $p = 0.074$) (Table 4). There is the highest positive correlation for the PI subscale of the PRISM and the EH subscale of the MSSS-88

Table 2. Correlations between subscales of the MSSS-88 and PRISM scales

MSSS-88 subscales	PRISM subscales	ρ	p
CONCURRENT VALIDITY: Body function domain			
MSS	NI	0.568	0.000
MS		0.652	0.000
PD		0.607	0.000
BM	NA/P	0.727	0.000
W		0.730	0.000
CONCURRENT VALIDITY: Activity domain			
ADL	DA	0.671	0.000
CONCURRENT VALIDITY: Participation domain			
SF	SE	0.384	0.003
	SAA	0.619	0.000
CONCURRENT VALIDITY: Personal factors / well-being domain			
EH	SAA	0.593	0.000
	PA	0.553	0.000
DIVERGENT VALIDITY: Hypothesis domain			
PD	PI	0.418	0.001
W		0.625	0.000
ADL		0.530	0.000
SF		0.339	0.009
EH		0.417	0.001

MSS – muscle stiffness; MS – muscle spasms; PD – pain and discomfort; BM – body movement; W – walking; AD – activities of daily life; SF – social functioning; EH – emotional health; NI – need for intervention; NA/P – need for assistance/positioning; DA – daily activities; SE – social embarrassment; SAA – social avoidance/anxiety; PA – psychological agitation; PI – positive impact; ρ – correlation factor

Table 3. Correlations between subscales of the MSSS-88 and PRISM scales in female subjects

MSSS-88 subscale	PRISM subscales	ρ	P
CONCURRENT VALIDITY: Body function domain			
MSS	NI	0.616	0.000
MS		0.702	0.000
P D		0.615	0.000
BM	NA/P	0.752	0.000
W		0.761	0.000
CONCURRENT VALIDITY: Activity domain			
ADL	DA	0.668	0.000
CONCURRENT VALIDITY: Participation domain			
SF	SE	0.450	0.003
	SAA	0.620	0.000
CONCURRENT VALIDITY: Personal factors / Well-being domain			
EH	SAA	0.561	0.000
	PA	0.643	0.000
DIVERGENT VALIDITY: Hypothesis domain			
PD	PI	0.430	0.004
W		0.600	0.000
ADL		0.503	0.001
SF		0.259	0.101
EH		0.289	0.066

MSS – muscle stiffness; MS – muscle spasms; PD – pain and discomfort; BM – body movement; W – walking; AD – activities of daily life; SF – social functioning; EH – emotional health; NI – need for intervention; NA/P – need for assistance/positioning; DA – daily activities; SE – social embarrassment; SAA – social avoidance/anxiety; PA – psychological agitation; PI – positive impact

Table 4. Correlations between subscales of MSSS-88 and PRISM scales in male subjects

MSSS-88 subscale	PRISM subscales	ρ	P
CONCURRENT VALIDITY: Body function domain			
Muscle stiffness	NI	0.438	0.007
Muscle spasms		0.471	0.056
P D		0.537	0.026
BM	NA/P	0.630	0.006
W		0.667	0.003
CONCURRENT VALIDITY: Activity domain			
ADL	DA	0.691	0.002
CONCURRENT VALIDITY: Participation domain			
SF	SE	0.288	0.260
	SAA	0.640	0.005
CONCURRENT VALIDITY: Personal factor s/ well-being domain			
EH	SAA	0.682	0.002
	PA	0.455	0.066
DIVERGENT VALIDITY: Hypothesis domain			
PD	PI	0.443	0.074
W		0.688	0.002
ADL		0.615	0.008
SF		0.607	0.009
EH		0.809	0.000

MSS – muscle stiffness; MS – muscle spasms; PD – pain and discomfort; BM – body movement; W – walking; AD – activities of daily life; SF – social functioning; EH – emotional health; NI – need for intervention; NA/P – need for assistance/positioning; DA – daily activities; SE – social embarrassment; SAA – social avoidance/anxiety; PA – psychological agitation; PI – positive impact

Table 5. Predictor parameters of the MSSS-88 for the subscales of the PRISM

Parameters	B	SE	p
	NI		
MSS	-0.045	0.099	0.653
MS	0.203	0.089	0.027
PD	0.048	0.120	0.691
	NA/P		
BM	0.176	0.077	0.026
W	0.194	0.111	0.087
	PI		
PD	0.065	0.105	0.535
W	-0.018	0.080	0.818
ADL	0.092	0.061	0.138
SF	-0.064	0.114	0.576
EH	0.092	0.066	0.167

MSS – muscle stiffness; MS – muscle spasms; PD – pain and discomfort; BM – body movement; W – walking; AD – activities of daily life; SF – social functioning; EH – emotional health; NI – need for intervention; NA/P – need for assistance/positioning; PI – positive impact; SE – social embarrassment; B – predictor parameter

($\rho = 0.809$) (Table 4). The weakest positive correlation was obtained between the SE subscale of the PRISM and the SF subscale of the MSSS-88 scale ($\rho = 0.288$) (Table 4).

Muscle spasms are strong predictors for the NI. Furthermore, BM is a strong predictor versus W for the NA/P (Table 5).

DISCUSSION

Numerous scales used in clinical practice for spasticity measurements assessing subjective and objective parameters make it more complex to perform reliable measurements of spasticity degree presented by the patient [14].

We have demonstrated that there are significant correlations between subscales of the evaluated MSSS-88 and PRISM scales for body domains, where the highest correlation between the NA/P and W was noted. Such finding regarding the correlation between the NA/P and W could be explained by the fact that assistance over the rehabilitation treatment period reduces secondary comorbidities and influences mobility. Previous studies are in line with such observations – it was noticed that training of the locomotor system is to a certain degree beneficial for the rehabilitation outcome in patients with MS [15, 16].

Our study stressed that spasticity (MSS and muscle spasms) has the weakest correlation particularly with the NI. This could be to a certain extent explained by the fact that there are different degrees of spasticity. In the study by Haas [17], it was pointed out that 80% of MS patients in the UK study reported spasticity, with more than 50% of moderate to severe degree. However, in a study by Flachenecker et al. [18], it was stated that 74% of patients with spasticity reported stiffness. In the same study it was also noted that the need for treatment increases with the spasticity degree [19]. It should be underlined that treatment satisfaction is also variable from the perspective of both physicians and patients. Therefore, individual approach in interventional programs in the rehabilitation treatment of patients with spasticity is desirable, in order to improve efficacy of the functional outcome and spasticity reduction. This would ultimately improve the patients' quality of life long-term.

Previous studies have demonstrated that the presence of pain in patients with MS has a negative impact on daily activities and the overall quality of life [20]. Our findings are consistent with previous reports, stressing a significant positive correlation between pain discomfort and the NI.

In a study by Casetta et al. [21], it was noticed that MS in the male population has a stronger impact on disability than in the female one. Our study has demonstrated that in the domain of body function for males, there was a non-significant correlation between muscle spasms and the NI. In the participation domains, non-significant correlation was gained between the SF and SE. The same is true for the correlation between the EH and PA for the personal factors / well-being domain. In the hypothesis domain, females had a non-significant correlation between the SF and EH of the MSSS-88 scale, and the PI of the PRISM scale, while for males, a non-significant correlation was between the PD and PI. Our results stress that differences between genders of MS patients persist in different domains. Previously, the role of gender of MS patients on activities of daily living was evaluated in the study by Buchanan et al. [22], where different domains were shown to have different impact on these activities regarding gender. Such findings underline the necessity for individually-based rehabilitation programs with particular attention to the gender-based planning.

Aside the presence of MSS, we have demonstrated that muscle spasms are strong predictors for the NI. This could be justified by the fact that spasms are more severe than the presence of spasticity in terms of objective perspective. Further, BM is a strong predictor versus W for the NA/P. This is in line with the fact that W implies a certain ability of body movement and thus, in some cases, reduces the necessity for NA/P.

CONCLUSION

After comparing and considering these two scales (PRISM and MSSS-88), it is evident that each has its own characteristics and advantages. The MSSS-88 evaluates the negative impact of spasticity across eight domains, but the scale is lengthy (88 items) and does not consider possible positive aspects of spasticity. The PRISM includes 44 items and it has been developed to assess how spasticity effects the quality of the life in persons with MS. The PRISM scale is simple, accounts for both the negative and positive aspects of spasticity and it is not time-consuming. Given the facts above, we have demonstrated that both scales could be considered reliable in measuring different domains of disability for MS patients with spasticity. Because of its brevity, speed of use and simplicity, the PRISM scale can successfully compete with and replace the MSSS-88 scale in certain domains.

Thus, both should be considered valuable measuring instruments in the assessment of patients' functional status and further rehabilitation program planning.

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Повезаност између субскала *MSSS-88* и скале *PRISM* у евалуацији спастицитета код оболелих од мултипле склерозе

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САЖЕТАК

Увод/Циљ Упитници који укључују властито доживљавање болести се све више користе јер су веома важни у процени здравља и задовољства оболелих од мултипле склерозе. Циљ рада је био да се провери повезаност различитих субскала утицаја болесниковог става на измерени спастицитет (*PRISM*) и скале спастицитета код мултипле склерозе (*MSSS-88*) у процени утицаја спастицитета на различите домене активности дневног живота код болесника са мултиплом склерозом.

Методе У опсервационој студији пресека анализиране су скале *MSSS-88* и *PRISM* у пет домена: телесни домен, домен активности, домен учешћа, домен личних фактора и добробити и домен претпоставки. За статистичку интерпретацију користили смо Спирманов ρ тест, валидност тестова (конкурентну и дивергентну), линеарни регресиони метод.

Резултати Постоји значајна повезаност између субскала *MSSS-88* и *PRISM* за телесни домен. Посебно јака повезаност била је између потребе за асистенцијом, односно позиционирањем и хода. Спастицитет има посебно слабу повезаност кад је реч о болесницима са мултиплом склерозом и потребама за интервенцијом код њих. Присуство бола код болесника има негативан утицај, уз позитивну повезаност између феномена бола, нелагодности и потребе за интервенцијом. У домену телесне функције за мушкарце није било значајне разлике између мишићних спазама и потребе за интервенцијом. У домену учешћа није постигнута ρ тест значајна разлика између социјалног функционисања и социјалне непријатности, исто и између емоционалног здравља и психолошке агитације за домен добробита и личних фактора. Разлика између полова постоји у различитим доменима. Мишићни спазам је снажан предсказатељ потребе за интервенцијом. Телесна покретљивост је снажан предиктор наспрам хода и потребе за асистенцијом и позиционирањем.

Закључак *MSSS-88* и *PRISM* су поуздане у мерењима различитих домена инвалидности код којих је присутан спастицитет. Скала *PRISM* је краћа, бржа, једноставнија и може успешно да замени скалу *MSSS-88* у одређеним областима.

Кључне речи: мултипла склероза; спастицитет; скале

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Correlation between risk factors, functional recovery, and the health-related quality of life of stroke survivors

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SUMMARY

Introduction/Objective It has been estimated that 50% of strokes are preventable through the control of modifiable risk factors.
The objective of the paper was to determine the correlation between the risk factors, functional status, and the health-related quality of life (HRQOL) of stroke survivors.

Method The prospective cohort study was used. The study included 136 patients 30–79 years old. Functional recovery was assessed using the Barthel index (BI) and the Modified Rankin Scale (mRS). The HRQOL was evaluated by the generic Short Form 36 (SF-36) questionnaire. BI and mRS were determined at admission at the rehabilitation, one, three and six months after the stroke. The SF-36 was filled out at the same time. The analysis of the repeated measure variance (Repeated Measures ANOVA) was applied, as well as the correlation analysis and Spearman's coefficient of rank correlation.

Results A total number of 136 patients [66 (48.5%) male and 70 (51.5%) female] completed the questionnaire. The average age of stroke survivors was 63.72 ± 8.73. At admission, mRS was 4.75 ± 0.55, and six months after the stroke onset it decreased to 2.60 ± 1.08. The average value of BI at admission was 25 ± 24.66, and within six months it increased to 83.75 ± 18.59 ($p = 0.001$). The ANOVA showed that the values of mRS significantly decreased ($p < 0.001$) and the values of BI significantly increased (ANOVA: $p < 0.001$). All domains of the SF-36 questionnaire, except for the pain domain, significantly increased ($p < 0.001$). The physical function ($r = 0.238$; $p < 0.01$), physical role ($r = 0.199$; $p < 0.05$), and emotional role ($r = 0.237$; $p < 0.01$) were significantly lower among alcohol addicts ($r = 0.199$; $p < 0.05$). Mental health ($r = 0.244$; $p < 0.01$) and social relationships domains were significantly lower among smokers ($r = 0.272$; $p < 0.01$). The general health ($r = -0.290$; $p < 001$) and health condition change domains were significantly lower among smokers ($r = 0.225$; $p < 0.01$).

Conclusion The most important risk factor which was negatively correlated with the HRQOL was smoking. The patients who were smokers and alcohol addicts had a significantly smaller increase of the HRQOL domains compared to other patent groups. Six months after the stroke, all domains of the HRQOL significantly increased. The significant improvement of patients' functional status was positively correlated to the increase of their HRQOL.

Keywords: stroke; health-related quality of life; hypertension; smoking

INTRODUCTION

Stroke is the first cause of disability and the second most common cause of death worldwide [1, 2]. Age, sex, race, ethnicity, and heredity have been identified as markers of risk for stroke. Age is the single most important risk factor for stroke. For each successive 10 years after the age of 55 years, the stroke rate more than doubles in both men and women. An increased incidence of stroke in families has long been noted [3].

Potential reasons are a genetic tendency for stroke, a genetic determination of other stroke risk factors, and a common familial exposure to environmental or lifestyle risks. Earlier studies suggested an increased risk for men whose mothers died of stroke and women who had a family history of stroke [4]**.** In the Framingham Study, an offspring analysis revealed that both paternal and maternal histories were associated with an increased risk of stroke [5].

Hypertension is the single most important modifiable risk factor for ischemic stroke. Most estimates for hypertension indicate a relative risk of stroke of approximately 4 when hypertension is defined as systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 95 mmHg. Various cardiac diseases have been shown to increase the risk of stroke. Atrial fibrillation is the most powerful and treatable cardiac precursor of stroke. The incidence and prevalence of atrial fibrillation increase with age [5, 6].

Diabetes mellitus nearly triples, while current cigarette smoking doubles this risk. Atrial fibrillation, although often asymptomatic and undetected, is an important risk factor for stroke, increasing stroke risk about fivefold throughout all ages so that its relevance could be underestimated [7]. Patients with low concentrations of HDL cholesterol have been found to be at a higher risk of stroke [8].

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Furthermore, depressive symptoms have been increasingly recognized as a risk factor (four-fold higher) for stroke / transient ischemic attack [9].

Health is a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity [10]. This statement defines health-related quality of life (HRQOL) as a construct that focusses on the respondent's subjective perception and consists of physical, mental, and social dimensions [11].

Health-related quality of life (HRQOL) refers to the aspects of the quality of life affected by a disease, or the impact of the health condition or health care intervention on the individuals' subjective experience in functional, cognitive, social and psychological processes [12, 13].

The objective of the paper was to evaluate the correlation between the risk factors, functional status, and the HRQOL of stroke survivors after the first stroke.

METHODS

A prospective cohort study was carried out. Two hundred sixteen patients who had a stroke were randomly chosen out of the total number of 1,598 patients. The prospective study included 216 patients 30–79 years old who lived on the territory of the City of Niš. Ine hundred thirty-six of them finished the study, 11 patients left the study, and nine patients died. The observed period was from January 1, 2011 to August 15, 2013.

The criteria used for including patients in the study were the following: the first acute stroke, 30–79 years old – according to the data from the hospital-based register for stroke, hospital records, and official death certificates, persons from these age-groups have the highest specific age incidence and mortality rate for stroke in the Nišava District; the patient was hospitalized in Niš, communication was possible; written informed consent to be included in the study; inpatient rehabilitation.

The criteria used for excluding the patient from the study were as follows: previous stroke; insufficient ability to communicate; psycho-organic syndrome; aphasia; a new stroke in less than 90 days after the first one; complications after the stroke, another stroke or acute myocardial infarction; patient's death.

All of the patients were completely informed about the aims of the research. In order to include patients in the study it was necessary to receive their written consent (two copies). The permission for making the research was issued by the Ethics Committee of the Faculty of Medicine in Niš on January 18, 2010 (permission No. 01-206-8). Another permission for conducting the research was also issued on February 2, 2011 (permission No. 2280/12).

Stroke was diagnosed by a neurologist at the time the patient was hospitalized at the Neurology Clinic of the Niš Clinical Centre. This study includes the patients who were hospitalized at the Neurology Clinic of the Niš Clinical Centre at this period of time, after which they were hospitalized and treated at the Clinic for Physical Medicine and Rehabilitation of the Niš Clinical Centre.

Their functional recovery was surveyed using Barthel index (BI) and the Modified Rankin Scale (mRS) [13]. Disability was evaluated using the BI and the mRS. The BI measures the degree of autonomy in daily living activities and gives a score ranging from 0 (total dependence) to 100 (total independence). The BI mean scores were categorized as follows: BI < 30 was classified as needing "institutional care"; 30–70 was classified as "help needed," and patients having a BI > 70 were classified as being "functionally independent" [14].

The mRS assesses the patients' ability to perform the activities they carried out previously and any assistance in doing so. It ranges from 0 (no symptoms at all) to 6 (dead). Patients scoring 0–2 on the mRS were classified as independent; patients scoring 3–6 were categorized as experiencing severe disability or death [14].

The quality of life was investigated using a generic questionnaire for estimating the quality of life Short Form 36 (SF-36).

Quantitative statistical analysis was done through computer software. MS Office Excel 2010 (Microsoft Corporation, Redmond, WA, USA) computer program was used for the input, ranking, grouping, table, and graphic presentation of the obtained data. Program R version 2.12.0 (R Foundation for Statistical Computing, Vienna, Austria) was used for calculations.

The analysis of the repeated measure variance (Repeated Measures ANOVA) was applied for testing statistical importance of value changes in the characteristics of the quality of life and the characteristics of the health status during the research. The estimation of the characteristics correlation between the values of the quality of life and the factors of interest was obtained using the correlation analysis, and the values of Spearman's coefficient of rank correlation (r) were also calculated.

RESULTS

A total number of 136 patients were included in the research; 48.5% male, and 51.5% female. Their average age was 63.72 ± 8.73. The ischemic stroke was present among 77.2%, and the hemorrhage was present among 22.8% of the patients. Stroke was most often located in the left brain hemisphere (45.6%), then in the right hemisphere (44.1%), and other locations were present in 10.3% of the patients.

Twenty-seven patients (45%) had two risk factors for stroke, 17 patients (28.3%) had one, nine patients (15%) had three risk factors, four patients (6.7%) had four, and two patients (3.3%) had no risk factors present (Table 1).

Among the patients who had their first stroke, previous transient ischemic attack episodes were present in eight (5.9%), 22 (16.2%) were current and ex-smokers, diabetes mellitus was present in 46 (33.8%) patients, hypercholesterolemia was present in 48.5% of the cases, more than one third, or 48 (35.3%) patients, were alcohol addicts,118 (86.8%) had high systolic and diastolic blood pressure, and carotid stenoses were noted in 38 (27.9%) cases (Table 2).

Table 1. Distribution of risk factors among the patients

Risk factors	n (%)
Previous TIA episode	8 (5.9%)
Current smoker	22 (16.2%)
Ex-smoker	22 (16.2%)
Diabetes mellitus	46 (33.8%)
Cholesterol	66 (48.5%)
Alcohol addict	48 (35.3%)
SYS blood pressure	118 (86.8%)
DIA blood pressure	118 (86.8%)
Carotid stenosis	38 (27.9%)

TIA – transient ischemic attack; SYS – systolic; DIA – diastolic

Table 2. The values of the modified Rankin Scale (mRS) and Barthel index (BI) on admission to the Clinic for Rehabilitation and one, three, and six months after discharge

Time period	Post stroke survivors	
	mRS (n = 136)	BI (n = 136)
On admission to the Clinic for Physical Medicine and Rehabilitation	4.75 ± 0.55	25 ± 24.66
1 month later	3.82 ± 0.73	57.28 ± 24.88
3 months later	3.16 ± 0.92	74.49 ± 20.21
6 months later	2.60 ± 1.08	83.75 ± 18.59

The analysis of the repeated measure variance showed that during the research, the values of the Rankin scale significantly decreased ($p < 0.001$) among the patients, and the values of BI significantly increased, (ANOVA: $p < 0.001$). The average value of BI at admission was 25 ± 24.66, and within six months it increased to 83.75 ± 18.59 ($p = 0.001$)(Table 3).

ANOVA showed that during the investigation, all domains values of the SF-36 questionnaire, except for the pain domain, significantly increased among the patients ($p < 0.001$).

Significant positive correlation was confirmed between the value increase of physical function and the value increase of mRS ($r = 0.346$; $p < 0.01$) during the research, as well the increase of BI values ($r = 0.296$; $p < 0.01$) at admission. Significant negative correlation was confirmed between the value increase of physical function and the decrease of BI values during the research period ($r = 0.457$; $p < 0.01$), as well as the value decrease of mRS ($r = 0.207$; $p < 0.05$). The value increase of the physical function domain is significantly lower among smokers ($r = 0.238$; $p < 0.01$).

Significant negative correlation was confirmed between the value increase of the physical role domain and the value decrease of BI during the research ($r = 0.415$; $p < 0.01$) as well as the value decrease of mRS ($r = 0.397$; $p < 0.01$). Value increase of the physical role domain is significantly lower among smokers ($r = 0.199$; $p < 0.05$).

Significant positive correlation was confirmed between the value increase of the emotional role domain and the value increase of mRS ($r = 0.315$; $p < 0.01$) during the research, as well as the value increase of BI ($r = 0.203$; $p < 0.05$) at admission. Significant negative correlation was confirmed between the value increase of the emotional role domain and the value decrease of BI during the research ($r = 0.423$; $p < 0.1$), as well as the value decrease of mRS ($r = 0.287$; $p < 0.01$). Value increase of the emotional role domain is significantly lower among smokers ($r = 0.237$; $p < 0.01$) and among alcohol addicts ($r = 0.199$; $p < 0.05$).

Significant negative correlation was confirmed between the value increase of the mental health domain and the value decrease of BI during the research ($r = 0.219$; $p < 0.05$). The value increase of the mental health domain was significantly lower among smokers ($r = 0.244$; $p < 0.01$). Significant positive correlation was confirmed between the value increase of the social relationships domain and the value decrease of mRS ($r = 0.262$; $p < 0.01$), as well as the value increase of BI ($r = 0.357$; $p < 0.01$) at admission. Significant negative correlation was confirmed between the value increase of the social relationships domain and the value decrease of BI during the research ($r = 0.440$; $p < 0.01$), as well as the value decrease of mRS ($r = 0.221$; $p < 0.01$). The value increase of the social relationships domain was significantly lower among smokers ($r = 0.272$; $p < 0.01$). No significant correlations between the values of the pain domain and all other investigated factors were confirmed.

Significant positive correlation was confirmed between the general health domain and the value increase of mRS ($r = 0.220$; $p < 0.05$). Significant negative correlation was confirmed between the value increase of the general health domain and the value decrease of BI during the research ($r = 0.256$; $p < 0.01$). The value increase of the general health domain is significantly lower among smokers ($r = -0.290$; $p < 0.01$). Significant positive correlation was confirmed between the value increase of the health condition change domain and the value increase of mRS ($r = 0.443$; $p < 0.01$) during the research, as well as the value increase of BI ($r = 0.203$; $p < 0.05$) at admission.

Significant negative correlation was confirmed between the health condition change domain and the value decrease of BI during the research ($r = 0.446$; $p < 0.01$) as well as the values decrease of mRS ($r = 0.212$; $p < 0.05$). The value increase of the health condition change domain is significantly lower among smokers ($r = 0.225$; $p < 0.01$).

DISCUSSION

According to the presented results, the most frequent risk factors in stroke survivor factors were hypertension, diabetes, and smoking. There were more women among post stroke survivors than men. The stroke survivors of this study were younger at the time they had the first stroke compared to participants in other studies. The average age of stroke survivors in Taiwan was 64.5 ± 11.8 [15]. The average age of post stroke survivors in the Northern Manhattan Study was 69.2 ± 10.3 years, and of participants in Marburg 71.1 ± 11.3 years [16, 17]. In Italy, participants were 70 years old on average (age range 34–85) [18].

Women represented 51.5% of all stroke survivors in this study. In the Northern Manhattan Study, there were 62.9% of women [16]. In Taiwan there were 43.1% of post-stroke women [15].

Table 3. Correlation between the domain value changes of the SF-36 questionnaire from the inpatient rehabilitation up to six months after discharge and the values of the investigated patients' descriptive characteristics

Characteristic	Domains								
	PF	PR	ER	VI	MH	SR	Pain	GH	HCC
Sex	0.039	0.048	0.081	0.174*	0.096	0.113	0.036	0.100	0.164
Age	-0.026	-0.041	-0.001	0.013	0.017	-0.055	0.042	-0.035	0.036
BS type	0.030	0.015	0.081	0.061	-0.106	-0.024	-0.044	0.035	-0.016
Basal ganglia	0.132	0.140	0.006	-0.091	-0.086	0.134	0.127	-0.023	0.121
Right hemisphere	-0.052	-0.086	-0.017	0.060	0.158	0.031	0.005	-0.167	-0.055
Infratentorial	0.044	-0.082	-0.092	-0.122	-0.096	0.086	-0.048	0.100	0.020
Left hemisphere	-0.007	0.037	0.034	0.003	-0.099	-0.104	-0.102	0.095	-0.050
Brain stem	0.078	0.107	0.095	-0.154	-0.096	0.152	-0.048	0.080	0.078
Both hemispheres	-0.145	-0.116	-0.131	0.165	0.067	-0.115	0.100	-0.018	-0.098
Previous TIA episodes	0.011	-0.043	-0.069	-0.097	-0.129	-0.044	-0.097	0.072	0.122
Current smoker	-0.238†	-0.199*	-0.237†	-0.018	-0.244†	-0.272†	0.069	-0.290†	-0.225†
Diabetes mellitus	0.016	-0.039	-0.019	0.034	-0.011	-0.002	0.080	0.023	-0.039
HOL	0.079	-0.166	-0.025	-0.125	0.114	0.029	0.052	0.099	-0.074
Alcohol addict	-0.147	-0.127	-0.199*	-0.089	-0.137	-0.087	-0.015	-0.067	-0.123
SYS blood pressure	-0.110	-0.031	0.035	0.129	0.048	-0.087	0.019	-0.046	-0.050
DIA blood pressure	-0.110	-0.031	0.035	0.129	0.048	-0.087	0.019	-0.046	-0.050
Carotid stenosis	-0.126	-0.137	-0.096	0.094	0.012	-0.095	0.058	-0.108	-0.062
Rankin on admission	-0.207*	-0.397†	-0.287†	0.085	0.087	-0.221†	-0.031	-0.013	-0.212*
Barthel on admission	0.346†	0.341†	0.315†	-0.008	0.037	0.262†	0.167	0.220*	0.443†
Barthel changes	0.296†	0.123	0.203*	-0.032	0.013	0.357†	0.013	0.070	0.203*

PF – physical function; PR – physical role; ER – emotional role; VI – vitality; MH – mental health; SR – social relationships; GH – general health; HCC – health condition changes; HOL – cholesterol
*$p < 0.05$; †$p < 0.01$

According to the national study conducted in 2006, there were 44.5% of adults who suffered from hypertension; it was more frequent in the male population older than 45 years (48.9%), in those who lived in the southeastern part of Serbia (49.9%), those who were less educated (62.7%), and among people with less income (53.1%) In 2000, it was estimated that 40.5% of the adult population in Serbia were smokers, 46.5% being male and 30.9% female adults. Most of the smokers were up to 44 years old. Smoking habit was most frequent among male adults who lived in towns and had graduated from secondary school [19].

A review article by Carod-Artal et al. [10] provides an overview of predictors of HRQOL in stroke survivors reported by longitudinal studies. These are age, sex, stroke severity, physical impairment, functional status, and mental impairment [12].

There are conflicting data regarding sex differences and stroke outcome. While some studies found that men were more likely than women to have a poor outcome after an ischemic stroke, others found that women had worse outcomes, and still others found no significant differences in outcomes according to sex [5, 15].

The patients who had had these risk factors had a stroke two to four times more often than those who didn't [17]. Dwyer et al. [18] emphasize that smoking is a big risk factor for stroke and that stroke among young population who are passionate smokers without any other risk factor is not rare.

Patients who survive stroke need rehabilitation because of their limitations or disabilities to perform their daily activities. The efficiency of performance after stroke is very often represented by measuring the level of ability decrease (functional research). Improving HRQOL is the desired outcome for patients with stroke undergoing inpatient rehabilitation. Recovery in stroke patients receiving rehabilitation primarily occurs in the first three months after stroke and continues in the following three months [20].

In this study, the average value of BI during the inpatient rehabilitation was 25 ± 24.66, and within six months after discharge it increased to 83.75 ± 18.59. According to a similar investigation conducted in Novi Sad, after the inpatient rehabilitation, the average value of BI at the beginning of rehabilitation was 57.53, and it was 78.92 six months after discharge [21].

In this study, the patients had a significantly decreased BI at the time of admission to the hospital rehabilitation, much less than 40 (25 vs. 22). It shows a complete patients' dependence on other people's help. Stroke side predicted 11.6% of the variance in the emotion domain, which was greater than a value explained by depression. Right-hemisphere stroke has a lower HRQOL in the emotion domain than the left-hemisphere stroke in our study [22].

Regarding the sex of the patients, the physical dimension of the quality of life is more pronounced among male patients, but the differences are not statistically significant. The patients who had the increase of BI also experienced the increase of strength, movements, communication, memory, emotions, and hands domain. The increase of physical functions led to the increase of HRQOL. Post stroke survivors have significant physical and psychological sequels, which make their lives difficult or make them completely disable to perform their everyday activities. Extremity motor function predicts various HRQOL domains [22].

The researches of stroke effects that deal with basic daily activities (ADL; activities that must be accomplished

in order to live an independent everyday life) include measuring functional independence; they are the Katz index of ADL and BI [22]. A close correlation was noted between the average values of BI and the physical function, emotional role and mental health domains. Patients suffering from greater disabilities complained about the greater decrease of physical abilities, which was a bigger problem to them because of the limitation in performing their everyday activities and it led to emotional problems and lower average grades for the mental health domain.

In general, even small improvements in rehabilitation yield the feeling and perception of having reached a good level of performance [20].

Concerning the sex of the patients, both men and women experienced the increase of average values of BI after the discharge from the hospital three months after the stroke. Similar investigations made in this area in the neighboring countries showed an increase of BI among men during all of the four periods of the research; yet they become statistically important six months after the stroke ($p < 0.05$) [23].

According to results found by Granger et al. [24], greater values of BI were found among male patients than among female patients. Concerning the lateralization of the hemiplegia at hospitalization, with an average BI value of 64there is a statistically greater value among the right side hemiplegiae, and 51.17 ($p < 0.029$) among the left side ones, whereas at the time of the discharge from the hospital, three, and six months after the discharge, there are no statistically significant differences [25]. A study by Wade and Hewer [26] shows no statistically significant values of BI concerning the side of hemiplegia.

Concerning the etiology of stroke, the values of BI are smaller in hemorrhage stroke patients compared to ischemia stroke patients during all four research periods; however, these differences have no statistical significance. The data concerning the changes in the Rankin scale show that even though rehabilitation is conducted, the recovery of arms is not possible before six months have elapsed and their recovery contributes to the increase of HRQOL [25].

The decreased values of all SF-36 questionnaire domains significantly increased ($p < 0.001$) during the six months of the survey, with the exception of the pain domain. All the values of SF-36 questionnaire domain had a statistically smaller increase among smokers, and the values of the emotional role domain had a smaller increase among the patients who were alcohol addicts. Functional status was the major independent determinant affecting the quality of life [26, 27]. Factors including perceptions of overall stroke recovery are significant for the HRQOL [21, 17].

Women have worse quality of life than men at the end of the study. There are similar results in literature [28, 29, 30].

There are several limitations of this study: a small sample size, which reduces the generalizability of the results; some patients refused to participate or were excluded from the study because of dementia or aphasia; some of the patients died.

Potential limitations of other studies are different sample sizes of stroke survivors; different questionnaires used for the evaluation of the HRQOL; lack of a detailed assessment of depression, as well as the absence of data on cerebrovascular or other main new events; and the use of antidepressant medication during the follow-up.

CONCLUSION

The quality of life of post stroke survivors was significantly decreased on admission to the inpatient rehabilitation and it was lower both at the early stage of the recovery and six months after the stroke. Smoking was the most important factor which was negatively correlated to the HRQOL. The patients who were smokers and alcohol addicts had a significantly smaller increase of all of the domains of the HRQOL. Six months after the stroke, the values of all examined domains significantly increased. The significant improvement of patients' functional status was positively correlated to the increase of their quality of life.

NOTE

This paper is part of a doctoral thesis by Milan Mandić, titled "Quality of life and functional recovery of patients after the first-ever stroke."

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Повезаност фактора ризика, функционалног опоравка и квалитета живота болесника после можданог удара

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САЖЕТАК

Увод/Циљ Процењује се да се око 50% можданих удара може спречити изменом начина живота и контролом фактора ризика.

Рад је имао за циљ да утврди повезаност фактора ризика, функционалног опоравка и квалитета живота болесника после можданог удара.

Методе Примењена је проспективна кохортна студија којом је обухваћено 136 болесника старости 30–79 година. Функционални опоравак процењиван је Бартеловим индексом (БИ) и модификованом Ранкиновом скалом (МРС). Квалитет живота процењиван је генеричким упитником *SF*36. БИ и МРС одређиване су на пријему на болничку рехабилитацију један месец, три и шест месеци после можданог удара. Примењена је анализа поновљеног мерења варијансе (*Repeated Measures ANOVA*), корелациона анализа и одређиван је Спирманов коефицијент корелације.

Резултати Укупно 136 болесника ((66 (48,5%) мушкараца и 70 (51,5%) жена)) комплетно је попунило упитник. Просечна старост болесника била је 63,72 ± 8,73. Вредности МРС на пријему биле су 4,75 ± 0,55, а шест месеци после можданог удара смањиле су се на 2,60 ± 1,08. Вредности БИ на пријему износиле су 25,00 ± 24,66, а унутар шест месеци су се повећале на 83,75 ± 18,59 ($p = 0,001$). Утврђене разлике у промени МРС и БИ су статистички значајне (*ANOVA* је показала значајан пад МРС ($p < 0,001$) и значајан пораст БИ (*ANOVA*: $p < 0,001$). Сви домени квалитета живота значајно су порасли, осим домена бола ($p < 0,001$). Физичка функција ($r = 0,238$; $p < 0,01$), физичка улога ($r = 0,199$; $p < 0,05$) и емотивна улога ($r = 0,237$; $p < 0,01$) биле су статистички значајно ниже међу болесницима који пију ($r = 0,199$; $p < 0,05$). Вредност домена менталног здравља ($r = 0,244$; $p < 0,01$) и социјални односи били су значајно нижи код пушача ($r = 0,272$; $p < 0,01$). Домен општег здравља ($r = -0,290$; $p < 0,01$) и промена здравственог стања били су значајно нижи код пушача ($r = 0,225$; $p < 0,01$).

Закључак Пушење је најважнији фактор који је био у негативној повезаности са квалитетом живота болесника после можданог удара. Значајно поправљање фукционалног статуса болесника позитивно је корелилало са порастом квалитета живота.

Кључне речи: мождани удар; квалитет живота; хипертензија, пушење

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Comparative genomic fingerprinting for the subtyping of *Campylobacter jejuni* and *Campylobacter coli* biotypes

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SUMMARY

Introduction/Objective Thermophilic campylobacters, especially *Campylobacter jejuni* (*C. jejuni*) and *Campylobacter coli* (*C. coli*), are the most important causes of bacterial diarrhea in developed and developing countries. The disease can occur as a sporadic infection or as large and small outbreaks. Phenotyping and genotyping methods are in use to determine similarities between strains as well their possible common origin. The goal of the study was to compare discriminatory power of biotyping tests and comparative genomic fingerprinting (CGF) 40 (100%), as well as a combination of the two tests in detection of clonality or epidemiological relatedness between the studied strains.

Methods We investigated 23 *Campylobacter* strains using biotyping and CGF typing.

Results We found that biotyping was a more discriminatory method for *C. coli*, and CGF for *C. jejuni* strains. In the discrimination of *C. jejuni* strains, CGF had better discriminatory power [Simpson's index of diversity (ID) was 0.879] over the discrimination of *C. coli* strains (Simpson's ID was 0.389).

Conclusion Biotyping and CGF can be complementary methods in detection of similarity, relatedness and possible common origin between strains since the combination of biotyping and CGF methods gives more precise data about diversity within *C. coli* and *C. jejuni* strains.

Keywords: biotyping; molecular typing; multiplex PCR

INTRODUCTION

Campylobacter spp. (predominantly (*C. jejuni* and *C. coli*) are the most frequent causes of enterocolitis in developed and developing world [1]. Enterocolitis usually occurs sporadically. However, detected or not, small house outbreaks are more possible [2]. In order to trace the sources of outbreak or to detect epidemiologically related strains, extended biotyping or serotyping schemes based on heat labile (Lior scheme) or heat stabile (Penner) antigens can be used [3, 4, 5]. Molecular techniques, e.g. polymerase chain reaction- (PCR) based methods, provided more rapid tools for the discrimination between the strains and they are very convenient when used for detection of *Campylobacter* spp. in the specimen. However, molecular methods are not sufficiently reliable because of some *Campylobacter* genus features such as high genetic diversity, weak clonality, and high levels of intraspecies recombination. Consequently, secondary methods for the successful tracking of epidemic strains are necessary [6]. Since clusters of C*ampylobacter* have not been well defined, the detection of unreported outbreaks of food-borne diseases can be more difficult.

There are several genotyping techniques adopted for campylobacters: pulsed-field gel electrophoresis (PFGE) [7]; restriction fragment length polymorphism analysis of the flagellin gene (flaA RFLP) [8]; the DNA sequencing of the flagellin gene short variable region (flaA SVR) [9]; multilocus sequence typing (MLST) [10]; multilocus variable-number tandem repeat analysis (MLVA) – a promising tool, but still without a widely accepted protocol [11, 12]; DNA microarrays [13]; clustered regularly interspaced short palindromic repeat (CRISPR) polymorphism analysis [14]; single nucleotide polymorphism (SNP) typing [15]; and binary gene typing (BGT) [16].

The PFGE with validated protocol for *Campylobacter* spp. is superior in outbreak investigation. Yet, PFGE has numerous disadvantages: it is time-consuming and labor-intensive, and requires high concentrations of a pure culture. Contemporary requirements from a typing method as a microbiological tool are less complicated procedures on a routine basis, rapid results, inexpensiveness, better discrimination and quantitative relatedness between strains, compatibility with PFGE data, preferably automatic and portable equipment, and easy comparison within and between laboratories by the existing databases.

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In an effort to establish reproducible, discriminatory, rapid, low cost, and easy performing genotyping method for *Campylobacter*, applicable in molecular epidemiology for *C. jejuni* and *C. coli,* a 40-gene CGF assay (CGF40) at the National Microbiology Laboratory of the Public Health Agency of Canada (Winnipeg) was developed [17]. The basis for CGF is the presence or absence of genes found to be variable in previous comparative genomic studies involving multiple *C. jejuni* isolates [17]. The method involved eight multiplex PCR, each consisting of five reactions assessing alleles at multiple loci and their genetic variability. Used marker genes were those with a distribution indicative of clear presence/absence, classified as unbiased genes, with a representative genomic distribution, and the ability to capture strain-to-strain relationships and were present in two or more of *C. jejuni* genomes [17]. Data do not require querying a centralized data bank. Therefore, this type of genome analysis is exceptionally portable within laboratory networks, and exchange of information is very easy [18].

Control and prevention of disease and outbreaks are complex tasks. Of great importance is not only to develop and implement effective control measures on the identification of the sources of an infection, but also to choose an efficient microbiological tool. Nowadays, in Serbia, there are no consistent programs for surveillance and monitoring of food-borne infections and outbreaks and infections caused by enteric bacteria as well as by *C. jejuni* and *C. coli*. The methods for bacterial typing with more discriminatory power for clonality investigation can provide information on epidemiologically related strains that are more accurate.

The aim of the study was to (a) compare discriminatory power of biotyping tests commonly used in microbiological laboratories and CGF40 (100%), as well as a combination of the two tests in detection of the strains isolated in small house outbreaks, and (b) to determine the similarity, clonality or epidemiological relatedness of the strains.

METHODS

We have investigated 23 thermophilic *Campylobacter* spp. strains designated in Arabic numerals from 1 to 23, from patients with enterocolitis isolated in 2011 in Serbia. Available clinical and epidemiological data provided strain selection, and the investigation of suitability of CGF40 was conducted in relevance to epidemiology of the strains. Among investigated strains, 11 pairs (22 strains) of *Campylobacter* were identified as isolated at the same time, with the same geographical distribution and the same pattern of sensitivity to antimicrobials. We presumed that strain pairs belonged to the same species; i.e. biotype and CGF type had the same clonal pattern. Strain pairs were designated from A to K with the belonging strains as: A) 1, 2; B) 3, 4; C) 5, 6; D) 7, 8; E) 13, 14; F) 19, 15; G) 22, 23; H) 9, 10; I) 11, 12; J) 20, 16; K) 21, 17.

Strain identification and biotyping

Strains sent to the Reference Laboratory for *Campylobacter* and *Helicobacter* in Amies medium were cultured in Columbia agar [Columbia blood agar with 5% sheep blood (CBA), Liofilchem, Roseto degli Abruzzi, Italy] and *Campylobacter* agar with 5% sheep blood (CA), Liofilchem, brain heart infusion broth (BHI), (Blood agar base heart infusion, Biolife Italiana S.r.l., Milan, Italy) and Bolton medium (Fluka Chemie GmbH, Buchs, Switzerland) with 10% laked horse blood (Oxoid ltd., Basingstoke, UK), and subcultured on CBA and CA after 48 hours in the same conditions.

Previously isolated strains, stored in BHI with 15% glycerol at -70°C, were thawed at room temperature and plated on the same media at same conditions. The media were incubated for 48 hours, in a microaerobic atmosphere with 9% CO_2 at the temperature of 37°C in an incubator (pCO_2 inkubator, BINDER Inc., Bohemia, NY, USA). Colonies of *Campylobacter* were presumptively identified microscopically by stained (1% carbol-fuchsin) slides (presence of S and spiral-shaped bacteria with gullwing morphology), and by oxidase and catalase tests.

A combination of biotyping and the PCRbased RFLP test provided *Campylobacter* differentiation to the species level. In the biotyping scheme, hippurate hydrolysis, rapid H_2S production, and DNA hydrolysis tests were used [7].

In the PCR-RFLP test, in *Campylobacter*, *Arcobacter*, and *Helicobacter* species, the primer sequences amplify a 1004-bp fragment within the coding region of the 16S rRNA gene. The forward and reverse primers used were CAH 16S 1a (59 AAT ACA TGC AAG TCG AAC GA 39) and CAH 16S 1b (59 TTA ACC CAA CAT CTC ACG AC 39), respectively. Restriction endonucleases *DdeI* (Boehringer Mannheim Corp., Indianapolis, IN, USA), *TaqI* (Boehringer Mannheim Corp.), or *BsrI* (New England Biolabs Inc., Ipswich, MA, USA) were used for amplicon digestion. Distinguishing between *C. jejuni* and *C. coli* required an additional set of primers designed to amplify a portion of the hippuricase gene by using forward and reverse primers Hip 1a (5' ATG ATG GCT TCT TCG GAT AG 3') and Hip 2b (5' GCT CCT ATG CTT ACA ACT GC 3'), respectively [19].

CGF analysis

To generate CGF40, eight multiplex PCRs were performed on each isolate using forty primer sets [13]. Used loci were the following: (1) Cj0298c, Cj0728, Cj0570, Cj0181, Cj0483; (2) Cj0057, Cj0860, Cj1431c, Cj0733, Cj1427c; (3) Cj0297c, Cj1727c, Cj0264c, Cj0008, Cj1585c; (4) Cj1550c, Cj1329, Cj0177, Cj1334, Cj0566; (5) Cj0421c, Cj0033, Cj0486, Cj0569, Cj0625; (6) Cj0755, Cj0736, Cj096, Cj1141, Cj1136; (7) Cj1306c, Cj1552c, Cj1439c, Cj1721c, Cj1679; (8) Cj1294, Cj1551c, Cj0307, Cj1324, Cj0035c. Designations of multiplex PCR were 1, 2, 3, 4, 5, 6, 7, and 8, respectively. All CGF types were given in a binary format. Detected clusters were designated in Arabic numerals as 1–9 [13]. PCR reaction and its analysis were performed as described by Taboada et al. [17].

Statistical analysis

To determine discriminatory ability of typing systems, we used Simpson's index of diversity (Simpson's ID). This index indicates the probability of two strains sampled randomly from a population belonging to two different types at a 95% CI [20]. The strength and directionality of the congruence between the biotyping and CGF was assessed using the Wallace coefficient (Wi, expected Wallace coefficient value in the case of independence) according to the methods of Carriço et al. [21]. Wallace coefficients provide an estimation of how much additional information is yielded by a secondary typing method. Calculations of Simpson's ID and Wallace's coefficients were performed using an online tool at the Comparing Partitions website (http: //www.comparingpartitions.info) [17].

RESULTS

In 23 investigated *Campylobacter* strains, biochemical and molecular identification revealed the two most common species – *C. jejuni* (14 strains) and *C. coli* (nine strains), represented with three and two biotypes, respectively. All the strains belonged to nine CGF clusters.

In *C. coli*, five strains belonged to biotype I and four to biotype II (Table 1). The investigation of 14 *C jejuni* strains subdivided the isolates into three biotypes: two strains were of biotype I, eight strains of biotype II, four strains belonged to biotype III (Table 1).

C. coli clustered together: *C. coli* biotype I all fell into CGF cluster number 1 (Table 1), while *C. coli* biotype II were slightly more diverse and fell into clusters 1 and 2 (Table 1). CGF subtyping of *C. jejuni* biotype I, *C. jejuni* biotype II, and *C. jejuni* biotype III revealed that strains belonged to clusters 2, 5, and 1, respectively. While C. *jejuni* biotype I (CGF clusters 3 and 4) and *C. jejuni* biotype II were more diverse (clusters 4–8), *C. jejuni* biotype III assemble only into cluster 9 (Table 1).

Table 1. Comparative genomic fingerprinting (CGF) and cluster distribution among investigated *Campylobacter* strains

Species and biotype	N° of strains	Designations of CGF clusters	Distribution of CGF clusters
C. coli I	5	1	1
C. coli II	4	1, 2	2
C. jejuni I	2	3, 4	2
C. jejuni II	8	4, 5, 6, 7, 8	5
C. jejuni III	4	9	1

Simpson's index of diversity for biotyping of *C. coli* and *C. jejuni* strains was 0.556 and 0.615, respectively. In *C. coli strains*, typed by CGF, Simpson's ID were 0.389, while 14 *C. jejuni* strains revealed seven clusters with Simpson's ID of 0.879 (Table 2).

The two methods, biotyping and CGF of genus *Campylobacter*, gave Simpson's ID of 0.913, and in *C. coli* revealed Simpson's ID of 0.667 (Table 3). Biotyping and CGF in *C. jejuni* strains provided Simpson's ID of 0.89, while subtyping of *C. coli* I, *C. coli* II, *C. jejuni* I, *C. jejuni* II, *C. jejuni* III gave Simpson's ID of 0, 0.667, 1, 0.857, and 0, respectively (Table 3).

Assessment of congruence among applied methods revealed that the Wallace coefficient (Wi, expected Wallace coefficient value in the case of independence) for *C. coli* I it was 1 (complete congruence), for *C. coli* II 0.333 (low congruence), for *C. jejuni* I 0 (no congruence), for *C. jejuni* II 0.143 (almost no congruence), and for *C. jejuni* III it was 1 (complete congruence).

Speciation and biotyping revealed seven pairs (A–G) of *Campylobacter* spp., which were identified as being clonally related (Table 4).

Table 2. Simpson's index of diversity calculated for biotyping and CGF of *Campylobacter jejuni/coli* strains

Microorganism method	No. of strains	Method	No. of partitions	Simpson's ID	CI (95%)	CINA (95%)
Campylobacter spp.	23	Biotyping	5	0.798	0.725–0.872	0.709–0.888
		CGF	10	0.874	0.789–0.958	0.778–0.969
C. coli	9	Biotyping	2	0.556	0.482–0.629	0.375–0.736
		CGF	2	0.389	0.081–0.697	0.060–0.718
C. jejuni	14	Biotyping	3	0.615	0.433–0.798	0.412–0.819
		CGF	7	0.879	0.794–0.964	0.764–0.994

CGF – comparative genomic fingerprinting – for this analysis the online tool at the Comparing Partitions website was used (http://www.comparingpartitions.info/); ID – index of diversity; CI – confidence interval; CINA – non-approximated confidence interval

Table 3. Simpson's index of diversity calculated for CGF and biotyping in *Campylobacter jejuni/coli* strains

Microorganism	No. of strains	No. of partitions	Simpson's ID	CI (95%)	CINA (95%)
Campylobacter spp.	23	11	0.913	0.860–0.966	0.846–0.980
C. coli	9	3	0.667	0.446–0.888	0.403–0.930
C. coli I	5	1	0	0.000–0.000	0.000–0.000
C. coli II	4	2	0.667	0.667–0.667	0.258–1.000
C. jejuni	14	8	0.89	0.796–0.985	0.770–1.000
C. jejuni I	2	2	1	1.000–1.000	0.000–1.000
C. jejuni II	8	5	0.857	0.704–1.000	0.641–1.000
C. jejuni III	4	1	0	0.000–0.000	0.000–0.000

CGF – comparative genomic fingerprinting – for this analysis the online tool at the Comparing Partitions website was used (http://www.comparingpartitions.info/); ID – index of diversity; CI – confidence interval; CINA – non-approximated confidence interval

Table 4. Clonality of isolated A–G strain pairs as determined by speciation, biotyping, and comparative genomic fingerprinting (CGF) clustering

Date of isolation	Pair designation/ strain pairs	Species, biotype	CGF cluster
4/11/2011	A) 1, 2	*C. jejuni* III	both strains: cluster 9
11/21/2011	B) 3, 4	*C. jejuni* II	strain 3: cluster 7 strain 4: cluster 5
5/5/2011	C) 5, 6	*C. jejuni* II	both strains: cluster 8
7/6/2011	D) 7, 8	*C. coli* II	strain 7: cluster 1 strain 8: cluster 2
11/29/2011	E) 13, 14	*C. jejuni* II	both strains: cluster 6
4/19/2011	F) 19, 15	*C. coli* I	both strains: cluster 1
4/18/2011	G) 22, 23	*C. jejuni* III	both strains: cluster 9

However, CGF typing revealed some differences among related isolates: pairs A, C, E, F, and G showed homogenicity by CGF typing. Pair B, identified as *C. jejuni* ssp. *jejuni* II, was subdivided into clusters 7 and 5; pair D, identified as *C. coli* II, was subdivided into clusters 1 and 2. Strains of pair D differ in only one allele form of the cj1427c gene, while strains of pair B differ in 15 alleles: Cj0298c, Cj1431c, Cj1727c, Cj0264c, Cj1550c, Cj0033, Cj0486; Cj0569, Cj0755, Cj0736, Cj1306c, Cj1552c, Cj1439c, Cj1721c, and Cj1294. Expression of the gene is represented by green color squares, and the absence of expression with red squares. If same-color squares are positioned one above the other, strains either possess a particular gene or they do not (Figure 1). Strain numbers are shown at the far left of the figure, and identified species are listed at its far right.

Pairs of strains from H to K did not express species, neither biotyping nor CGF homogeneity.

DISCUSSION

In this study, we performed biotyping and CGF on 23 *Campylobacter* strains: nine *C. coli* and 14 *C. jejuni* isolates. Biotyping alone of *C. coli* and *C. jejuni* strains gave Simpson's ID of 0.556 and 0.615, respectively, while CGF typing alone of *C. coli* and *C. jejuni* gave Simpson's ID of 0.389 and 0.879, respectively. Thus, biotyping was a more discriminatory method for *C. coli*, whilst CGF was more discriminatory for *C. jejuni* strains.

The results obtained by the combination of biotyping and CGF methods indicated that application of both procedures had better discriminatory power in *C. jejuni* over *C. coli* strains.

Speciation, biotyping and CGF of investigated *Campylobacter* spp. revealed Simpson's ID of 0.913 expressing high diversity among investigated strains.

In considered *Campylobacter* species, information on temporal and spatial relatedness using biotyping revealed seven pairs of strains (14 isolates) as related. Additional CGF typing revealed that five pairs of strains also belong to the same cluster. Two closely related clusters, 1 and 2, represented one pair (*C. coli* II), which means a possible evolution of one strain. Another pair of strains (*C. jejuni* II) differs in several alleles and represents two distinct clusters: cluster 7 and cluster 5. We did not expect to find differences between pairs considering their temporal and spatial distance [22]. The presence of two pairs of clonally related strains subtyped by CGF was surprising, although it is possible that one strain underwent genetic changes, having in mind that campylobacter is an extremely genetically variable bacterium [23]. CGF expressed better discriminatory power than biotyping in determination of clonality, which can be used in investigation of outbreaks.

Using the CGF method, we found high index of diversity for the species, indicating different sources of the *C. jejuni*. Through future investigation of animal isolates, it could be answered which one of many food animal sources are in question. For the species of *C. coli*, the index of diversity was somewhat lower (0.667), indicating higher similarity between strains, and perhaps a common origin. Therefore, within one year, strains may not have much variability.

A combination of biotyping and CGF methods gave more precise data about similarity between *C. coli* and *C. jejuni* strains, having in mind that congruence between the methods as determined by Wi was 0.143 for *C. jejuni* II and 0.333 for *C. coli* II, allowing association of these two methods. These properties suggest that methods based on comparative genomics represent a better alternative to biotyping.

Detection of an epidemic strain or investigation applied in population biology of bacterial strains are an important task for microbiologists. As it was seen in this investigation, the alone application of serotyping on a strain collection can show great diversity without predominant types, when strains are selected randomly [24]. Although a disadvantage of serotyping is that many strains can be untypable, an investigation of epidemic strains may give

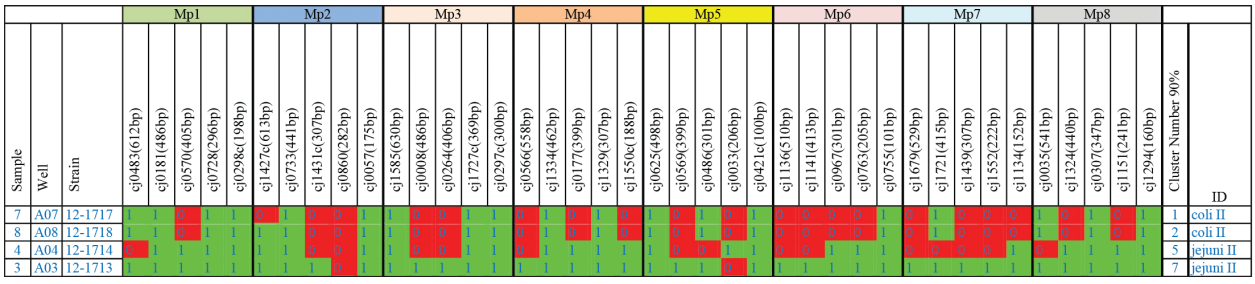

Figure 1. Algorithm of *C. coli* II (pair B) and *C. jejuni* I (pair D) with differences in gene expression; Mp1–8 – multiplex PCR 1–8; cj0483-cj1294, gene loci; ID – identification

representative and reproducible data, as in an outbreak described by DeFraites et al. [25], who detected the Lior serotype 5 in accessible isolates. The authors applied serotyping only and did not find any diversity among strains, which is possible when some subtyping methods or molecular typing methods are used.

To resolve epidemic strains, short variable regions of *C. jejuni* isolates successfully replaced serotyping [9]. One of the contemporary approaches is the multiplex PCR method for determining the capsule types of *C. jejuni*, which correlates with the Penner typing. The multiplex PCR showed sensitivities and specificities ranging 90–100% using strains of known Penner type [26]. A combination of the two methods, when primary typing method was CGF40, suggests that CGF and MLST are highly concordant. However, isolates with identical MLST profiles are composed of isolates with distinct but highly similar CGF profiles [17]. Our investigation showed that CGF and biotyping can be complementary methods in assessing clonality among *Campylobacter* spp. In addition, sequencing of the flaA gene short variable region (flaA SVR sequence typing) could supplement the CGF, with or without subsequent MLST [14].

In one investigation, several typing methods for use in the monitoring of *Campylobacter* spp. were compared [27]. The authors observed that the most discriminative combination with a Simpson's ID of 0.992 for both *C. jejuni* and *C. coli* was obtained by combining MLST with flaA-RFLP, which is feasible for short-term monitoring of *Campylobacter* spp. In our investigation, two methods, biotyping and CGF, revealed a Simpson's ID of 0.667 in *C. coli* and 0.89 in *C. jejuni* strains.

The goal of all typing and subtyping systems is a precise and efficient tracing method of infection sources. Therefore, it is a necessity to employ molecular typing approaches to quantify the contribution of different sources of human *Campylobacter* infections on the national level. Thus, it seems that the CGF method relying on the presence/absence of unbiased genes could fulfill the criteria for a modern typing method alone or in combination with other techniques.

CONCLUSION

Application of CGF alone or in combination with biotyping could reveal the clonal relationship between the strains, e.g. their participation in the same epidemic, especially when an outbreak is suspected. In the absence of the data on the outbreak, the method could reveal relatedness between the strains that could help in the outbreak detection. Introducing CGF could significantly improve investigation of clonal relatedness between strains and therefore contribute to the improvement in investigation of outbreaks. However, testing more samples will obtain more reliable results.

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Примена методе компаративног фингерпринтинга генома за суптипизацију биотипова *Campylobacter jejuni* и *Campylobacter coli*

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САЖЕТАК

Увод/Циљ Термофилни кампилобактери, посебно *Campylobacter jejuni* (*C. jejuni*) и *Campylobacter coli* (*C. coli*) најчешћи су узрочници бактеријске дијареје и у развијеним земљама и у земљама у развоју. Болест може да се јави у виду спорадичне инфекције, мале кућне или велике епидемије.
За одређивање сличности између сојева као и њиховог евентуалног заједничког порекла могу да се користе фенотипске и генотипске методе. Циљ рада је био да се упореде дискриминаторна моћ биотипизације и компаративног фингерпринтинга генома (КФГ) 40 (100%), као и комбинације ова два теста у детекцији клоналности или епидемиолошке повезаности између испитиваних сојева.

Методе Испитивали смо 23 соја бактерије *Campylobacter* применом биотипизације и типизацијом на основу КФГ.
Резултати Утврђено је да је биотипизација дискриминаторнија метода за *C. coli,* а КФГ дискриминиторна за сојеве *C. jejuni*. Дискриминација *C. jejuni* применом КФГ има већу снагу (Симпсонов индекс различитости износио је 0,879) у односу на сојеве *C. coli* (Симпсонов индекс износио је 0,389).
Закључци Биотипизација и КФГ могу бити комплементарне методе приликом детекције сличности, повезаности или могућег заједничког порекла сојева, пошто њихова комбинација даје прецизније податке о разноликости унутар врста *C. coli* и *C. jejuni*.
Кључне речи: биотипизација; молекуларна типизација; мултиплекс *PCR*

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The etiology of viral gastroenteritis in patients requiring hospitalization: differences between rotavirus and norovirus infections – practical or only academic significance?

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SUMMARY

Introduction/Objective Viral gastroenteritides (VGEs) have great infectious potential and may occur in all age groups. Loss of fluid due to vomiting and diarrhea represent a special risk, and may result in a more severe clinical presentation in children, the elderly, and people with chronic diseases.
The aim of the study was to explore the causes of VGEs among hospitalized patients, as well as to evaluate the severity of clinical symptoms in rotavirus and norovirus infections.

Methods The observational prospective study included 191 patients aged 2–88 years who were treated at the Clinic for Infectious Diseases, Clinical Center of Vojvodina, Novi Sad, over a two-year period. Testing of stool samples for viral agents was done by the reverse-transcription polymerase chain reaction method. Positive findings were found in 59 patients.

Results Of 59 patients with confirmed viral gastroenteritis, in 31 (52.5%) it was caused by rotavirus, in 17 (28.8%) by norovirus, three patients (5.1%) had other viral causes, while co-infection with two viruses was found in eight (13.5%) patients. The severity of clinical manifestations as expressed with Vesikari score did not differ with regard to infectious agents ($p = 0.353$). However, patients with rotavirus infection had a higher incidence of fever ($p = 0.043$), longer duration of diarrhea ($p = 0.015$) and dehydration ($p = 0.014$), and longer need for hospital treatment ($p = 0.030$).

Conclusion The most common cause of VGEs in our hospitalized patients was rotavirus. There was no difference in the severity of clinical symptoms between rotavirus and norovirus infections.

Keywords: norovirus; rotavirus; gastroenteritis; hospitalization; length of stay

INTRODUCTION

Acute gastroenteritis (AGE) mostly represents a mild, self-limiting disease, which usually does not require going to the doctor [1]. In a small percentage of cases, depending on the different characteristics of the pathogen and the host and their interrelationship, AGE will require hospital care. Although the proportion of severe clinical forms is small, the ubiquity and high incidence of this disease, especially in countries with poor socioeconomic conditions, gives this disease a great practical significance [2].

In triage settings, the most important is certainly the assessment of severity of the disease and indications for hospitalization, as well as the differentiation between viral and bacterial AGE. The practical importance of differentiation between causes of viral gastroenteritis is often overlooked by general practitioners [3]. The reasons for this include primarily the treatment approach that is symptomatic in all cases of viral gastroenteritis, as well as the cost of diagnostic tests, which are still based on molecular techniques and therefore usually unaffordable for patients in developing countries [4]. However, literature data indicate that there are differences in the incidence of individual causes of viral gastroenteritis, as well as in the severity of clinical symptoms, and it has been observed that these differences depend on geographic area, as well as socioeconomic status and hygienic habits of the study population [1, 3–6].

The aim of this study was to determine the distribution of causes of viral gastroenteritis among patients requiring hospitalization in our institution, as well as to determine whether there are differences in the clinical presentation between rotavirus and norovirus infections.

METHODS

Subjects

This observational prospective study included 191 patients aged 2–88 years, who were treated at the Clinic for Infectious Diseases in Novi Sad

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over a period of two years (between January 1, 2014 and December 31, 2015). Viral etiology of the disease was confirmed in 59/191 patients.

Data sources

Referrals to laboratory testing for causes of viral gastroenteritis contained basic demographic data, epidemiological data, present symptoms and signs, and disease duration. Medical records included the data on personal history, clinical course, response to treatment, and disease outcome.

Clinical definition of viral gastroenteritis

The clinical definition of a case of acute viral gastroenteritis presupposed the presence of the following symptoms: ≥ 3 watery stools/24 hours and/or ≥ 2 episodes of vomiting/24 hours, abdominal pain, and fever. The study excluded patients with comorbidities in the gastrointestinal tract, patients in whom the complaints lasted more than 48 hours prior to hospitalization, patients previously treated with antimicrobial therapy, as well as those with a stool test positive for bacterial pathogens.

Laboratory diagnostics

Stool samples for molecular diagnostics were taken not later than 48 hours after the onset of symptoms and before the initiation of treatment, and transported in a portable refrigerator (temperature between +4°C and +8°C) to the laboratory. Virus isolation from stool was done at the Center for Virology of the Public Health Institute of Vojvodina, Novi Sad. The extraction of viral nucleic acids was carried out with commercially available mini spin column kit for the isolation of RNA (Ribo Virus, Sacace Biotechnologies S.r.l., Como, Italy). The testing of samples of viral nucleic acids was done using a reverse-transcription polymerase chain reaction commercial kit by Sacace Biotechnologies. Amplification and detection of viral RNA was done using the Applied Biosystems® 7500 PCR System (Applied Biosystems, Foster City, CA, USA).

Bacteriological examination of stool for common intestinal pathogens (*Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Yersinia* spp., *E. coli* O157) was performed at the Center for Microbiology of the Public Health Institute of Vojvodina, Novi Sad.

Routine laboratory blood and urine tests were performed at the Center for Laboratory Medicine of the Clinical Center of Vojvodina.

The study was approved by the Ethics Committee of the Clinical Center of Vojvodina. All the participants in the study had signed the informed consent.

Statistical analysis

Statistical analysis was done using IBM SPSS Statistics for Windows, version 23 (IBM Corp., Armonk, NY, USA). Categorical variables were presented as numbers and percentages, and differences between groups were investigated by the χ^2 test. After the examination of distribution of continuous variables, it was found that the distribution of most of the variables differed significantly from the normal distribution, and also that most of the variables showed significant heterogeneity of variance between the groups; therefore, the data was presented as a median and an interquartile range, and differences between the groups were investigated using the Mann–Whitney U-test. Significance of correlations between the variables was tested using a non-parametric approach (Spearman rank correlation).

RESULTS

In the observed two-year period, a total of 261 patients with symptoms of acute gastroenteritis were hospitalized at the Clinic for Infectious Diseases of the Clinical Center of Vojvodina. All the patients underwent stool analysis for viruses and bacteria, and patients with proven bacterial pathogens (n = 65) and those with mixed viral and bacterial infection (n = 5) were excluded from further analysis. The remaining 191 patients were included in the study. A proven viral pathogen was found in 59/191 patients (30.9%), while stool testing for both viruses and bacteria was negative in 132/191 (69.1%) patients.

Among the 59 patients with confirmed viral gastroenteritis, in 31 (52.5%) patients the cause was rotavirus, in 17 (28.8%) it was norovirus, three patients (5.1%) had other viral causes of AGE, and co-infection with two viruses was found in eight (13.5%) patients. Patients with all stool analyses negative for pathogenic causes (n = 132) as well as patients with co-infection with two viruses (n = 8) and patients with confirmed viral etiology other than norovirus or rotavirus (n = 3) were classified as a non-rota/non-noro group (a total of 143 patients).

Further analysis included only patients with proven viral and non-rota/non-noro gastroenteritis.

Demographic data are presented in Table 1, while clinical and laboratory characteristics of the study patients are shown in Table 2.

The results showed no statistically significant difference in the age of patients in relation to the pathogen. However, when the age categories were taken into account, it was observed that rotavirus infection was more common in children under five years of age, while norovirus infection was more frequent in children aged 6–18 years. On the other hand, all three groups (rotavirus, norovirus, and non-rota/non-noro gastroenteritis) were similarly prevalent in adults (50–60%). These differences, however, did not reach the level of statistical significance.

It is interesting that of the 143 patients with non-rota/non-noro gastroenteritis, none had associated comorbidities, while in the group with norovirus gastroenteritis, as many as 35% of the patients (n = 6) had comorbidities (four patients had hypertension, and two of them chronic obstructive pulmonary disease). In the group of patients with a rotavirus infection, nine of them had comorbidities

Table 1. Demographic characteristics of patients included in the study

Parameter	Non-rota/non-noro (n = 143)	Rota (n = 31)	Noro (n = 17)	N/R	Non/n	Non/r
Age	22 (10.5-31)	19 (4.5–32)	19 (17–25)	0.627	0.831	0.400
Under 5 yrs. 5–18 yrs. Adults	21 (14.7%) 38 (26.6%) 84 (58.7%)	9 (29%) 6 (19.4%) 16 (51.6%)	1 (5.9%) 6 (35.3%) 10 (58.8%)	0.132	0.528	0.265
Sex – male	74 (51.7%)	13 (41.9%)	6 (35.3%)	0.653	0.305	0.322
Comorbidity	0 (0%)	9 (29%)	6 (35.3%)	0.654	-	-
Takeaway and restaurant food	32 (22.4%)	4 (12.9%)	2 (11.8%)	0.909	0.220	0.238
Similar symptoms in family	34 (23.8%)	8 (25.8%)	3 (17.6%)	0.776	0.793	0.994

Table 2. Comparison of clinical and laboratory characteristics between rotavirus, norovirus and non-rota/non-noro AGEs

Parameter	Non-rota/non-noro (n = 143)	Rota (n = 31)	Noro (n = 17)	N/R	Non/n	Non/r
Vomiting	118 (82.5%)	24 (77.4%)	16 (94.1%)	0.138	0.854	0.507
> 5 × per day	48 (33.6%)	11 (35.5%)	7 (40.2%)	0.696	0.532	0.838
Duration*	1 (1–2)	1 (1–2)	1 (1–1)	0.643	0.824	0.726
Diarrhea	136 (95.1%)	31 (100%)	16 (94.1%)	0.758	0.860	0.451
> 5 × per day	92 (64.3%)	17 (54.8%)	9 (52.9%)	0.926	0.406	0.213
Duration*	3 (1–4)	3 (2–4)	2 (1–3)	0.015	0.105	0.407
Fever	93 (65%)	25 (80.6%)	9 (52.9%)	0.043	0.523	0.092
Duration*	1 (1–3)	2 (1.5–3)	2 (1–2)	0.052	0.703	0.031
Max. BT	38.1 (37.2–38.8)	38.2 (37.6–38.9)	37.6 (37.2–38.3)	0.078	0.245	0.482
Bloody stool	5 (3.5%)	3 (9.7%)	0 (0%)	-	-	0.309
Abdominal pain	97 (67.8%)	20 (64.5%)	13 (76.5%)	0.597	0.653	0.884
SBP	110 (100–120)	110 (100–120)	110 (100–120)	1.00	0.534	0.638
WBC	54 (37.8%)	8 (25.8%)	4 (23.5%)	0.862	0.375	0.292
CRP	25 (8–121)	52 (7–168)	18 (15.5–21)	0.540	0.583	0.593
Potassium	4 (3.7–4.3)	4 (3.7–4.3)	4 (3.8–4.3)	0.863	0.939	0.096
Vesikari score	12 (10–14)	12 (11–13)	12 (10–12)	0.353	0.430	0.835
Mild Moderate Severe	1 (0.7%) 46 (32.2%) 96 (67.1%)	1 (3.2%) 7 (22.6%) 23 (74.2%)	0 (0%) 4 (23.5%) 13 (76.5%)	0.755	0.713	0.305
Duration of complaints before hospitalization*	1 (1–2.5)	1 (1–3)	1 (1–1)	0.154	0.262	0.481
Duration of hospitalization*	3 (2–5)	4 (3–5)	3 (2–4)	0.030	0.225	0.174
Duration of I.V.*	4 (3–5)	4 (4–5)	4 (3–4)	0.014	0.382	0.025

BT – body temperature; SBP – systolic blood pressure; WBC – white blood cells; CRP – C-reactive protein;
*The duration is expressed in days; the data are presented as the number of cases (percentage) for categorical variables, and median (interquartile range) for continued variables

(29%) – hypertension (n = 6), diabetes mellitus (n = 1), and obstructive pulmonary disease (n = 2).

With respect to the epidemiological data regarding family members suffering from similar symptoms or eating food prepared outside home, there were no statistically significant differences between the observed groups of patients. Food consumption outside home immediately prior to the onset of complaints was reported by 11.8–22.4% of patients.

An analysis of individual elements used to estimate severity of the clinical picture showed that diarrhea and fever lasted longer in patients with rotavirus infection compared with norovirus infection, while this difference was not found in patients with non-rota/non-noro gastroenteritis in comparison to noro and rota groups. However, considering the overall severity of the clinical picture as expressed by the Vesikari score, we did not find a statistically significant difference in the severity of clinical symptoms between the three GE groups.

In addition, the group with rotavirus infection had a significantly longer need for parenteral rehydration, compared to both the norovirus and the non-rota/non-noro groups, as well as a significantly longer duration of hospitalization.

Laboratory parameters did not differ significantly between the studied groups of patients.

An analysis of demographic parameters (host's characteristics) that might have potentially affected the clinical picture expressed by the Vesikari score in patients with proven viral gastroenteritis showed a statistically significant negative correlation of a moderate intensity (n = 59, r = -0.305, p = 0.033) between the Vesikari score and age. In other words, younger patients had a more severe clinical picture. Similarly, patients with comorbidities had a statistically significantly more severe clinical picture compared to those without comorbidities (n = 59, r = 0.311, p = 0.031).

Duration of hospitalization and duration of parenteral rehydration did not significantly correlate with either demographic or epidemiological factors.

DISCUSSION

Rotavirus is considered the most common cause of viral gastroenteritis and accounts for the highest percentage of patients presenting with a more severe clinical picture. This conclusion was reached by analyzing outpatients and hospitalized patients, and it has been found that the most common cause of hospitalization is rotavirus [3, 6–11]. However, studies that included only hospitalized patients have provided contradictory data about the severity of clinical picture of rotavirus and norovirus infections. Perl et al. [12], analyzing individual symptoms of viral gastroenteritis among children hospitalized in their institution, reported a more severe clinical picture in rotavirus infection compared to other pathogens. On the other hand, Kawada et al. [13] reported that in children less than 12 years of age norovirus infection caused vomiting more frequently and that vomiting and diarrhea lasted longer. A prospective study of French researchers showed that rotavirus is by its frequency and severity of the clinical picture the most important pathogen in hospitalized pediatric patients [6]. Our results showed a higher prevalence of fever in patients with rotavirus infection, as well as a longer duration of fever and diarrhea. However, considering the overall severity of the clinical picture expressed by the Vesikari score, our results did not show a statistically significant difference in the severity of clinical symptoms of gastroenteritis caused by rotavirus and norovirus. Norovirus infections require statistically significantly shorter parenteral rehydration, thereby reducing duration of hospitalization.

The discrepancy of literature data on the severity of the clinical picture and the data indicating that particular symptoms last longer in rotavirus infections may be explained by differences in the host's characteristics, rather than the characteristics of the pathogen [6, 8, 12]. In fact, our results suggest that younger children, in view of age-particular immune response, present with a more severe clinical picture, regardless of the pathogen (severity of the clinical picture does not differ with regard to the cause, but statistically significantly correlates with age). Bearing in mind that children under five years of age are the "target group" of rotavirus, this may explain the more severe symptoms and a longer hospital stay. In support of the notion that host factors are the reason for the differences in the severity of clinical symptoms is our finding showing that the presence of comorbidities significantly affected the severity of the clinical picture. It is noteworthy that we found as many as 35% of patients with associated diseases in the group with norovirus diarrhea. Considering that the incidence of norovirus increases in immunocompromised and elderly patients, this could have, besides clinical aspects, significant financial effects [13, 14]. Most literature data related to viral gastroenteritis in hospitalized patients comes from pediatric departments [6, 10–13]. Our research included patients of all ages; however, we found that rotavirus infection was significantly more common among children aged up to five years. The observed negative correlation between the age and the severity of the disease as expressed by the Vesikari score may be a result of still insufficiently-formed immunity of children who are susceptible to rotavirus infection, rather than the virulence of the pathogen itself.

Viral diarrhea in developing countries is associated with hygienic habits of the population but also with consumption of food in restaurants and takeaways [4]. Only one fifth (11.8–22.4%) of our patients consumed food outside home immediately before the onset of symptoms.

Although it is a common belief among practicing physicians that detection of causes has no great practical importance, we do not agree with this attitude. In developing countries, where the incidence of viral gastroenteritis is high, costs of hospital care for these patients are not negligible [4]. Research contributing to estimating the prevalence of rotavirus and norovirus infections as well as potential further pharmacoeconomic research are needed in order to assess the need for preventive vaccination of vulnerable groups (lower socioeconomic conditions, immunocompromised patients), which would contribute to reducing the incidence, decreasing the severity of the clinical picture, and, indirectly, to a practical increase in the budget of healthcare institutions [1, 2, 3, 15, 16].

CONCLUSION

The most common cause of AGE in patients in our institution was rotavirus. In comparison to norovirus infection, it was characterized by higher incidence of fever, longer duration of diarrhea and dehydration, as well as the need for longer hospitalization. Although the severity of clinical picture in hospitalized patients and the therapeutic approach do not differ with regard to the causes of the disease, laboratory identification of viral pathogens, in addition to the academic, has an indirect great practical significance.

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Етиологија вирусних гастроентеритиса међу хоспитализованим болесницима: разлике између ротавирусне и норовирусне инфекције – практични или само академски значај

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САЖЕТАК

Увод/Циљ Вирусни гастроентеритиси имају велики инфективни потенцијал и могу се јавити у свим узрасним групама. Нарочит ризик представља губитак течности услед повраћања и пролива, што може дати тежу клиничку слику код деце, старијих и особа са хроничним обољењима.
Циљ овог рада је да утврди дистрибуцију узрочника вирусних гастроентеритиса код хоспитализованих болесника и разлике у тежини клиничке слике између ротавирусне и норовирусне инфекције.

Методе Споведено је опсервационо проспективно испитивање код 191 болесника узраста 2–88 година лечених у двогодишњем периоду. Тестирање узорака столице на вирусне узрочнике рађено је методом *RT-PCR*. Позитиван налаз на вирусе забележен је код 59 болесника.

Резултати Од 59 болесника са потврђеним вирусним гастроентеритисом, код 31 (52,5%) узрочник је био ротавирус, код 17 (28,8%) норовирус, код три болесника (5,1%) доказани су остали вирусни узрочници, а коинфекција са два вируса забележена је код осам (13,5%) болесника. Тежина клиничке слике изражена кроз Весикари скор није се разликовала у односу на узрочника инфекције ($p = 0,353$). Болесници са ротавирусном инфекцијом имали су већу инциденцу температуре ($p = 0,043$), дуже трајање дијареје ($p = 0,015$) и дехидратације ($p = 0,014$) и дужу потребу за болничким лечењем ($p = 0,030$).

Закључак Најчешћи узрочник акутних вирусних гастроентеритиса код хоспитализованих болесника био је ротавирус. Није нађена разлика у тежини клиничке слике између ротавирусне и норовирусне инфекције.

Кључне речи: ротавирус; норовирус; гастроентеритис; дужина болничког лечења

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Frequency and distribution of scabies in Vojvodina, Serbia, 2006–2015

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SUMMARY

Introduction/Objective Scabies is a major dermatological and a public health concern worldwide. The aim of this study was to evaluate the trend of scabies, the age-specific incidence and seasonality of scabies in Vojvodina Province, Serbia.

Methods We investigated the epidemiological characteristics of scabies in Vojvodina (northern region of Serbia). We carried out a descriptive study over a 10-year period (from 2006 to 2015) and covered 21,996 patients.

Results The average incidence rate of scabies was 113.9/100,000 inhabitants with the evident increasing trend among all age groups, and especially among patients 15–19 years old. The highest incidence rate (323.9/100,000 inhabitants) was registered among children younger than 14 years. Most cases of scabies were registered during the cold months of the year, with peak activity throughout December (14.4/100,000; 95% CI: 12.2–16.6).

Conclusion A high frequency and increasing trend of scabies in Vojvodina indicates that more attention should be paid to this problem.

Keywords: scabies; epidemiology; surveillance; infection control

INTRODUCTION

Due to high morbidity, scabies is a major dermatological and a public health concern worldwide [1, 2]. Scabies occurs globally each year, leading to about 300 million cases [3]. Although scabies is considered a ubiquitous parasitism, the highest incidence rates of scabies have been registered in tropical regions [4, 5]. Taking this into consideration, the World Health Organisation has recognized scabies as one of 17 most neglected tropical diseases since 2013 [6]. In developing countries, the highest prevalence of scabies is evident among children with an average prevalence of 5–10% [7].

Low socioeconomic standard and overcrowding have contributed to the spreading of infestations. Therefore, the epidemic is more likely to occur in different collectives and refugee camps [5].

Despite the fact that scabies can be a major public health concern, in most European countries it is underappreciated, as the burden of scabies by country is unknown [4].

In our country, registration of scabies was required from 1975 to 2015, but scabies has no longer been subject to mandatory reporting since 2016 [8, 9].

The burden of scabies is also reflected in potential disease complications by secondary bacterial infections, most commonly caused by *Streptococcus pyogenes* and *Staphylococcus aureus*, with the possibility of invasive skin infection occurrence, or even sepsis [1].

Due to the global sub-registration and neglecting of the disease, the International Alliance for the Control of Scabies (IACS) was formed in 2012. IACS is a scientific forum consisting of experts from five continents with the main goal of scabies control through establishing quality monitoring of the disease through an advisory role of specialists in different areas, especially in countries with high prevalence of the disease [1].

The main goal of this study was to evaluate the trend of scabies, the age-specific incidence and seasonality of scabies in the Autonomous Province of Vojvodina (Vojvodina).

METHODS

Study area and population

Vojvodina is located in the northern part of the Republic of Serbia (situated at the crossroads between Central and Southeast Europe), with a population of almost two million. Vojvodina is bordered by Croatia to the west, Romania to the east, Hungary to the north, and Bosnia and Herzegovina to the southwest. It has a multiethnic and multi-cultural identity, with some 26 ethnic groups and six official languages. Overall, the climate is moderate continental with a mean maximum temperature in July (the average monthly temperature is 21.4°C) and mean minimum temperature during January (the average monthly temperature is -1.3°C) [10].

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Collection of data

A retrospective, observational study was conducted. The data for this study were obtained from the Communicable Disease Registration of the Institute of Public Health of Vojvodina in a 10-year period (from 2006 to 2015) [11]. Since 2005, individual registration (medical record) of scabies cases has been replaced by the aggregate reporting [8]. In accordance with law, the aggregate notification of scabies contains the data on the number of all reported cases classified by age groups (0–4, 5–9, 10–14, 15–19, 20–59, and ≥ 60 years old) during a one-week period, but do not contain information on the gender of affected people.

This aggregate registration of scabies covered only the patients who completed their first medical examination due to scabies and who were diagnosed by doctors at the primary health care level (medical examinations carried out in general medical units, occupational medicine units, dispensaries for skin diseases, and public health dispensaries for children, schoolchildren, students and adolescents).

The diagnosis of scabies is usually based on the clinical signs and symptoms with a characteristic localization of the pruritic papules, or with known epidemiological link to the person who had a similar clinical feature among the close contacts.

Confirmation of diagnosis by microscopic parasite identification is only utilized among atypical clinical cases. The participation of microscopically confirmed cases in the total number of reported cases of scabies retroactively could not be determined.

We conducted a retrospective data collection using only registration forms of patients, and the approval of an ethics committee was not required.

Statistical analysis

Incidence rates were calculated using the annual number of registered cases as a numerator and the number of inhabitants in Vojvodina according to the two censuses for the Republic of Serbia (in years 2002 and 2011) as a denominator and multiplied by 100,000 inhabitants.

Numerical data are presented through the arithmetic mean, median, minimum, maximum value, and the standard deviation. The normality assumption was checked using the Kolmogorov–Smirnov test, along with the skewed statistics. Examining the differences between the age-specific incidence rates, appropriate parametric tests such as one-way ANOVA was used, the Bonferroni post hoc test. Line chart as well as linear regression was used for estimating how the age-specific incidence rate changes over the examined period. The results were expressed through an equation of regression: $y = at + b$, where "a" is the beta coefficient of regression, "t" is the time, and "b" is the intercept of regression. The winter period (cold months of the year) was coded as "1" (October–March), while the summer period (warm months of the year) as "2" (April–September). To determine if there is a significant difference in the incidence rate of scabies between summer and winter periods over a 10-year study period, the most appropriate statistical test was the Mann–Whitney U-test, while the date (incidence rate of scabies per month) was skewed (the p-value of the Kolmogorov–Smirnov test was 0.024). The results were considered statistically significant when the p-value of the all applied models was < 0.05 and corresponded 95% confidence interval (95% CI) did not include 1. The data were analyzed using IBM SPSS Statistics for Windows, version 21 (IBM Corp., Armonk, NY, USA), and MS Office Excel (Microsoft Corporation, Redmond, WA, USA).

RESULTS

Trend of scabies in Vojvodina

During the observed period, a total of 21,996 cases of scabies were reported. The annual incidence rates of scabies ranged from 86.8/100,000 (2006) to 154.8/100,000 inhabitants (2015). In the study period, an increasing trend of the incidence rate of scabies was reported (Figure 1).

Differences in incidence rate of scabies according to age group

Table 1 shows the incidence rate of scabies by age groups. The average values of the age-specific incidence rates in patients aged 0–4, 5–9, and 10–14 years old were the highest and approximately equal (331.4/100,000, 338.3/100,000, and 302.2/100,000, respectively). A slightly lower average of incidence rate was registered among the adolescents (230.8/100,000). The lowest value of age-specific inci-

Table 1. Descriptive statistics for the incidence rate of scabies according to age group throughout the analyzed 10-year period

Age group (years)	2002 census	2011 census	Mean incidence rate of scabies	SD	95% confidence interval for mean incidence rate of scabies		Minimum incidence rate	Maximum incidence rate	ANOVA	
					Lower bound	Upper bound			F	p
0–4	92,584	88,727	331.4	42.5	301	361.9	262.5	413.6	64.8	0.000
5–9	107,834	94,809	338.3	63.7	292.7	383.9	267.1	470.4		
10–14	121,796	93,934	302.2	60.7	258.8	345.7	228.3	415.2		
15–19	137,777	109,832	230.8	77.6	175.3	286.3	125.6	355.1		
20–59	1,127,742	1,087,781	68.8	16.5	57.1	80.6	52.1	107.9		
≥ 60	444,268	456,726	45.1	14.7	34.5	55.6	33.5	81.9		

Table 2. Differences of incidence rates of scabies according to the three age groups throughout the analyzed 10-year period

Age group (year)	Mean incidence rate	Minimum incidence rate	Maximum incidence rate	95% confidence interval for mean		ANOVA	
				Lower bound	Upper bound	F	p
0–14	323.9	228.2	470.4	302.7	345.1	157.8	0.000
15–19	230.8	125.5	355	175.3	286.2		
≥ 20	56.9	33.5	107.9	47.8	66.0		

dence rate was registered among patients in the oldest age group (45.1/100,000). One-way ANOVA analysis (multiple comparison – Bonferroni test) indicated that the only age-specific incidence rate of scabies, comparing to all other age-specific incidence rates, was in patients aged 15–19 years old ($p = 0.000$). The incidence rates among the first three observed age groups (0–4, 5–9 and 10–14) did not show a significant difference between them ($p > 0.05$), and for the rest of the research we considered all three groups as one (0–14 years). For the same reason ($p > 0.05$), the similar approach was applied for age groups 20–59 and above 60, coding them as one group.

The incidence rates of scabies obtained statistically were significantly different ($p = 0.000$) among three age groups (0–14, 15–19, and ≥ 20 years old). The highest incidence rate (323.9/100,000) was registered in children 0–14 years old, followed by the incidence rate (230.8/100,000) in adolescents 15–19 years old, and by the incidence rate (56.9/100,000) in patients ≥ 20 years old (Table 2).

Trend of scabies in Vojvodina according to age groups

The steepest slope of the trend line with the highest coefficient of determination was in patients 15–19 years old, according to the annual incidence rate for the 10-year study period (2006–2015), using the equation of linear regression (the independent variable was time – year). In the subgroup analysis of trend (0–14, 15–19, and ≥ 20 years), statistically significant differences at the level $p < 0.05$ were determined for each age group. Based on the coefficients of time in the figure of linear trend, the estimated regression equation in patients 0–14 years old was $y = 14.7t + 241.7$; $p = 0.003$. In patients 15–19 years old, the equation was $y = 23.6t + 100.9$; $p = 0.000$. Among patients older than 20 years the equation was $y = 3.9t + 40.3$; $p = 0.012$. These equations and line charts indicate that during the study period, the incidence rate of scabies, at the statistically significant level, grew in 0–14-, 15–19-old patients, and in patients aged 20 years and older (Figure 2).

Seasonality of scabies in Vojvodina

Incidence rates of scabies in the period from October to March (median = 10.8; mean rank = 82.6) were higher than incidence rates of scabies in the period from April to September (median = 6.0; mean rank = 38.4). These differences were found to be statistically significant (Mann–Whitney $U = 471.5$, $p < 0.01$). The lowest average incidence rate of scabies was registered in August (5.0/100,000; 95% CI: 3.7–6.3), but peak activity was registered throughout December (14.4/100,000; 95% CI: 12.2–16.6) (Figure 3).

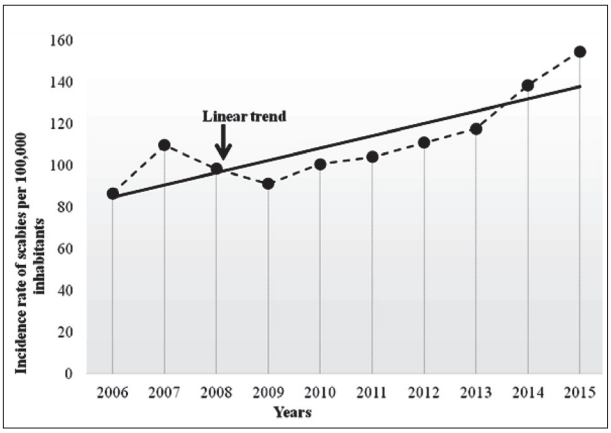

Figure 1. Incidence rate of scabies in Vojvodina, 2006–2015

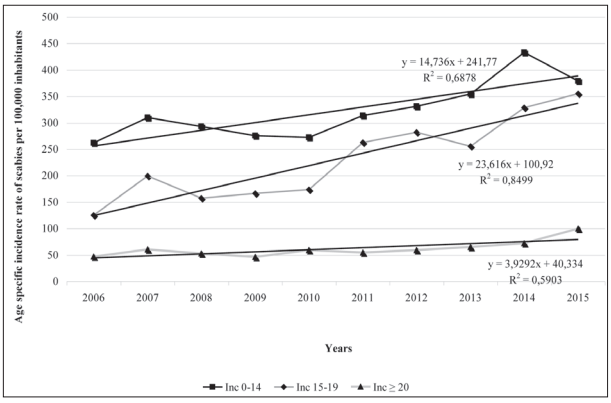

Figure 2. Age-specific incidence rate with the trend of scabies in Vojvodina, 2006–2015

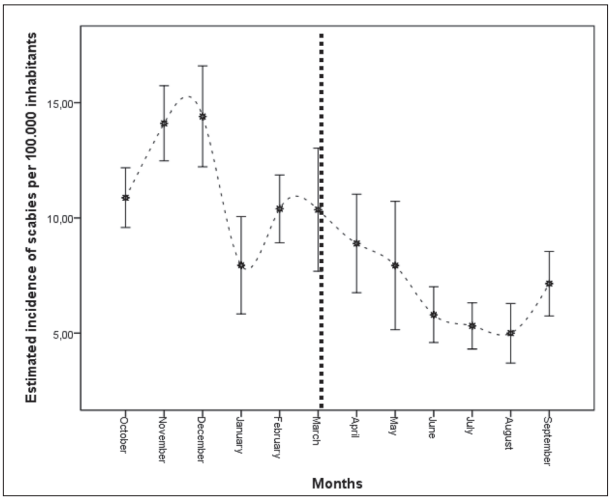

Figure 3. Seasonal distribution of scabies in Vojvodina according to 95% confidence interval for mean months' values, 2006–2015; the vertical dotted black line indicates the separation between the winter and summer period.

DISCUSSION

The prevalence studies of scabies across the world show that this disease is a major public health problem. Data from 18 published studies in the period from 1971 to 2001 show that prevalence of scabies ranged from 0.2–24% [7]. According to the recently published review of 48 publications, prevalence of scabies ranging from 0.2–71.4% [4]. The highest incidence rates of scabies have been found in the countries of tropical climate zones [4, 5, 12, 13]. Because the scabies is not included on the reportable diseases list in most countries, the real burden of scabies in European countries is unknown [3, 4, 7].

According to law, until 2015, scabies in the Republic of Serbia was included on the list of reportable diseases with obligatory monitoring [9]. During the study period, the average annual incidence rate of scabies in Vojvodina was 113.9/100,000 inhabitants.

In a study conducted in France between 2005 and 2009, Bitar et al. [14] reported that the estimated annual incidence rate of scabies was 328/100,000 inhabitants, and scabies has had an increasing trend. In 2011, the prevalence of scabies in homeless people, in those sleeping in shelters or in public places in Paris, was 0.4% and 6.5%, respectively [15].

Unlike under-reporting of scabies by passive surveillance (applied in Serbia and in most other countries), results of active (sentinel) surveillance of scabies, applied in England and Wales (with around 500,000 population) show that the average annual incidence rates of scabies during the 1994–2003 period ranged from 233/100,000 (2003) to 470/100,000 (2000) [16].

In contrast to these data, significantly lower incidence rates of scabies were registered in Belgium, although scabies was included on the list of reportable diseases since 1995. In 2005, the incidence rate of scabies was only about 3.8/100,000, which is an obvious result of under-reporting of the disease. In support to the fact of under-reporting is the additional conducted active surveillance of scabies, and this result shows that the rate of scabies was several times higher than the estimated rate of 28/100,000 inhabitants in the general population of Belgium, and a rate of 88/100,000 among the migrant population in this country [17]. However, due to implementation of the heterogeneous methodological approach in the surveillance of scabies, available data from different territories are not comparable [4, 5].

We found that the highest age-specific incidence rates of scabies were among children younger than 14 years, and the lowest rates for adults. The average age-specific incidence rates of scabies among adolescents (15–19 years old) and patients aged 20 years and older were in the ratio of 4:1, while the incidence rates in children aged up to 14 years compared to patients the same age group (≥ 20 years) were in the ratio of 5.7:1.

Data of a study carried out over a nine-year period for approximately 8.5% of the United Kingdom show that patients 10–19 years old had the highest infestation rates with slightly lower incidence among males than in females [18]. Similar to the results of the mentioned study, we found that the highest age-specific incidence rates were registered in childhood. In addition, the most evident increasing trend of scabies was among patients aged 15–19 years old. Similar results were obtained in other studies with a significant occurrence of the disease not only among younger, but even among the population aged older than 75 years [4].

In our territory, due to certain reporting procedures on scabies, all patients older than 60 years have been classified into one age group, and we could not show the trends and disease characteristics in the oldest population.

Scabies is usually spread by direct, skin-to-skin contact. In classic scabies, where 10–15 parasites are present on the skin, contaminated environment has no significant role in the transmission of these infestations, as opposed to crusted ("Norwegian") scabies, which is highly contagious and contains about two million parasites per patient [19, 20]. All of our cases were classified as classic scabies infections.

Overall, there is a trend of increasing scabies infestation. High values of age-specific incidence rates among children younger than 14 years can be regarded as a consequence of more efficient transmission through close contact in preschool and school communities. Also, the lack of maturity of children and deficit in education activity, especially in terms of scabies prevention, can be considered the main cause of this high frequency of the disease in this age group. We think that the reasons for the highest increasing trend of scabies among adolescents are perhaps in lifestyle. According to the data of one 15-year study, which was conducted at the Department of Sexually Transmitted Diseases, scabies infestation is related to lifestyle, more frequently detected in the MSM population, and among men who have sporadic sexual relations [21].

Our data showed that the average value of monthly rates of scabies was the highest during the cold months of the year (November and December, 14.1/100,000 and 14.4/100,000, respectively), similar to findings of other authors [21, 22].

Possible explanations for these seasonal variations could be related to closer contact in the population throughout cold months of the year, overcrowded rooms, and longer stay indoors, together with infrequent hygiene and change of clothes during winter months, and by the biological cycle of the mites, which prefer low temperatures for laying the highest number of eggs during the winter months. Our findings are in good agreement with those from previous studies [22, 23, 24].

As a basic preventive measure to control scabies transmission is the exclusion of patients from collectives until complete recovery [1, 5, 25]. Likewise, frequent visits to the doctor, the cost of applied therapy and the patients' contacts within the family and the collectives, as well as the social stigma, are a significant public health concern [1].

CONCLUSION

Although the results of our study come from the limited set of data, they indicate specific epidemiological characteristics of scabies that are recognized in other regions of the world,

especially among the younger population. Further studies are required to be conducted to assess the prevalence of scabies especially among the 15–19 years old age group considering that this group is the one with the most intensive increasing trend in the studied 10-year period.

Although the results of our passive surveillance clearly show that scabies are more common in younger people with an increasing trend of incidence, the future targeted research among the elderly may be focused on the estimation of potential scabies complications. Apart from primary health care doctors, this multicenter study should also include other doctors at secondary and tertiary health care level (cardiologists, rheumatologists, nephrologists).

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Учесталост и дистрибуција шуге у Војводини, Србија, 2006–2015

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САЖЕТАК

Увод/Циљ Шуга представља велики дерматолошки и јавноздравствени проблем широм света.

Циљ рада био је да се процене тренд, узрасно специфична и сезонска дистрибуција шуге у Војводини.

Методе Истраживане су епидемиолошке карактеристике шуге у Војводини дескриптивном студијом у десетогодишњем периоду (2006–2015) са 21.996 болесника.

Резултати Просечна вредност стопе инциденције шуге је 113,9 на 100.000 становника са евидентним растућим трендом инциденције у свим узрасним групама, а нарочито у узрасту 15–19 година. Највиша стопа инциденције (323,9 на 100.000 становника) регистрована је међу децом млађом од 14 година. Већина случајева шуге регистрована је током хладних месеци у години са врхунцем активности током децембра (14,4/100.000; 95% *CI*: 12,2–16,6).

Закључак Висока учесталост шуге са све израженијим трендом раста у Војводини указује на то да се више пажње мора посветити овом проблему.

Кључне речи: шуга; епидемиологија; надзор; контрола инфекције

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Clinical and autopsy findings of the homeless

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SUMMARY

Introduction/Objective The population of homeless people has been growing rapidly over the past decades, and is a part of regular repertoire in daily autopsy practice.
The paper aims to establish a contingent of autopsy findings specific for homeless persons using a cohort approach.

Methods The study group consisted of the bodies of 37 homeless men autopsied in the past 15 years. The control group consisted of 37 men and was created by a driven randomized selection following the same distribution of the causes of death. A standardized full autopsy was performed in every case, followed by microscopic examination and toxicology if indicated. Many external and internal features were compared.

Results Homeless people lived significantly shorter, and were more often unidentified at the time of autopsy ($p < 0.05$). As for external features, we found that homeless people were significantly shorter, with longer hair, beard, and nails, and worse dental status compared to the control group ($p < 0.01$); 70.3% of the homeless people were underweight; significantly more often suffered from infectious lung diseases, alcoholic liver disease and showed signs of old brain contusions ($p < 0.01$); they had higher blood alcohol concentrations at the time of death compared to the controls ($p < 0.05$), but a significantly lower atherosclerotic grade ($p < 0.01$), and were found to die significantly more often during the winter months ($p < 0.01$). Besides this, the homeless are more usually affected by specific and non-specific lung inflammations and alcohol liver diseases.

Conclusion Autopsy findings of homeless people define an almost particular presentation compared with controls**.**

Keywords: homeless; autopsy; tuberculosis; alcohol liver disease

INTRODUCTION

The progress of human civilization in developed countries has created a huge gap between social categories of the population. In all of these countries, more frequently in larger cities, we find a population of homeless people, which has been growing rapidly over the past decades [1]. Homeless people have a specific way of living, primarily hygienically unacceptable, followed by poor diet, excessive alcohol consumption, and inadequate clothing. They live in the streets, in improvised shelters, under bridges, etc. – in places which don't satisfy the minimum human needs. These are people without families, or people that have been rejected by their families, often suffering from various mental illnesses, drug and alcohol abuse, and who are often involved in fights and theft [2]. Winter time of the year represents one of the biggest challenges for their survival.

Considering the way of living, death of a homeless person is a part of regular repertoire in daily autopsy practice, almost without exception in all societies.

Even though well-known and expected, the autopsy findings of a homeless person have not been evaluated in a scientific manner to date.

The paper aims to establish a contingent of autopsy findings specific for homeless persons using a cohort approach. The study is performed under the Strengthening the Reporting of Observational Studies in Epidemiology guidelines for cohort studies [3].

METHODS

The present study is based on the comparison of autopsy findings between two groups of people. In the last 15 years, 38 bodies of homeless persons have been autopsied at the Institute for Forensic Medicine in Podgorica, Montenegro, Southeastern Europe – 37 men and one woman. The woman was excluded from the study, in order to achieve absolute homogeneity of the sample by gender. A criterion for qualifying a man as a homeless person was that he did not have a permanent accommodation in the previous10 years, but has lived in makeshift housing, often under bridges, in abandoned or demolished old houses, basements of apartment buildings, parks, and the like. The control group consisted of 37 men who have also been autopsied at the same institution in the same period. They were randomly selected from 700 men autopsied during this period, who were within the minimum and maximum age of the deceased homeless persons. In addition to the age-matched criterion, the control group was created by driven randomized selection of controls following the

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same distribution of the manner of death as it was in the group of homeless people. Putrefied bodies were excluded from the study.

A standardized full autopsy was performed in every case, followed by the microscopic examination. At the external examination of the corpse, the following characteristics were observed: nutrition status (described as underweight, normal, and overweight), body height (in centimeters), hair length (one of the longest hair strands in centimeters), length of beard/mustache (in centimeters), nail length (in millimeters – measured from the edge of the finger), descriptive feature of the overall dental status (graded in accordance with experience as poor – 0, medium – 1, and good – 2). During the autopsy, the following parameters were checked: *plaques jeunes* (old cortical contusions), coronary atherosclerotic grade (defined as Gr0 – smooth intima, Gr1 – rare small plaques, Gr2 – numerous individual plaques or confluent plaques, Gr3 – calcified plaques, Gr4 – calcified and ulcerated plaques), the lungs and pleura (for pneumonia and/or tuberculosis), and the liver (for alcohol liver diseases).

In addition to these characteristics, the age at the time of death is given, as well as the knowledge of identity of the corpse at the time of autopsy. Blood alcohol concentrations (BAC) in milligrams per gram at the time of death and the season when death occurred were also compared between the groups. The seasons were defined following the calendar: spring, summer, autumn, and winter.

The data were analyzed using descriptive statistical methods, Student's t-test and the χ^2 test.

RESULTS

Regarding driven randomization for controls, the distribution of the causes of death was the same in both groups: 24 died from a natural cause (64.9%), six from hypothermia (16.2%), four due to carbon-monoxide poisoning (10.8%), two were injured as pedestrians (5.4%), and the last one was killed by a blunt object (2.7%). Regarding driven randomization of controls, we chose 24 natural deaths, 12 accidental, and one homicidal case.

Mean age at the time of death in the homeless group was 53.2 ± 11.1, whereas in the control group it was 55.7 ± 11.65, which is not significantly different ($t = 0.169$, $p > 0.05$), but was significantly shorter regarding general male population in Montenegro ($t = 2.669$, $p < 0.01$).

There were 10 unidentified people in the homeless group at the time of autopsy, which is significantly more compared to three people with unknown identity in the control group ($\varphi = 0.249$, $p < 0.05$).

General external parameters are given in Table 1. Nutrition status is presented in Figure 1. While the controls follow the normal Gaussian distribution, homeless people are significantly more often undernourished ($p < 0.01$).

Organ-specific features are given in Table 2. Among 17 homeless persons positive for lung disease, in 65% unspecified pneumonia (bacterial of viral) was found, followed by 35% of any stage of tuberculosis (TBC).

Table 1. General external findings between the groups

Variable	Homeless	Controls	p
Mean height (cm)	172 ± 7.4	178.1 ± 7.5	< 0.01
Mean hair length (cm)	15.3 ± 11.2	6.0 ± 3.5	< 0.01
Mean beard and mustache length (cm)	2.4 ± 2.1	0.4 ± 0.7	< 0.01
Mean nail length (mm)	2.1 ± 1.3	0.6 ± 0.6	< 0.01
Dental status; (poor – 0; medium – 1; good – 2)	0.4	1.7	< 0.01

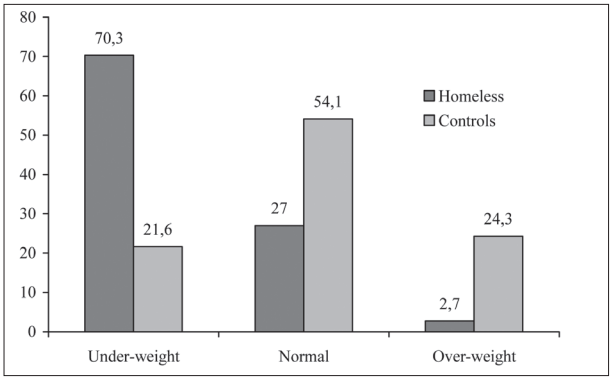

Figure 1. Nutrition status

Table 2. Internal findings between the groups

Variable	Homeless	Controls	p
Liver (number of cases with any type of alcohol liver diseases)	21	5	< 0.01
Lungs (number of cases with affected lungs)	17	3	< 0.01
Yellow plaques (number of positive cases)	11	2	< 0.01
Mean atherosclerotic grade (Gr1 = 1; Gr2 = 2; Gr 3= 3; Gr = 4)	1.19 ± 0.84	2.03 ± 0.8	< 0.01
Mean value of blood alcohol concentration (mg/g)	0.41 ± 0.97	0.09 ± 0.27	< 0.05

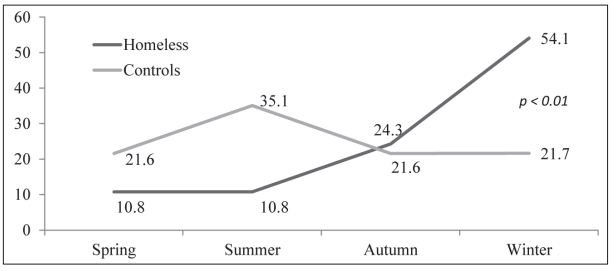

Figure 2. Seasonal distribution of deaths

Among those six homeless persons positive for TBC, five of them had active TBC and one had the signs of TBC history (caverns, fibrothorax, and calcification of the lymph nodes). Regarding controls, only three of them had lung disease and pneumonia was seen microscopically. Presentation of lung diseases between the groups is significantly different ($\varphi = -0.426$, $p < 0.001$). Similar observations were made when it comes to the alcohol liver disease, which was significantly more present in the homeless ($\varphi = -0.453$, $p < 0.001$).

Figure 2 presents the distribution of deaths over the seasons. Using the χ^2 test, homeless people were found to die significantly more often during the winter months,

compared to other seasons ($p < 0.01$) – in fact, half of them die during winter.

It was quite challenging to categorize clothes in the homeless group, but a pattern of wearing many layers of clothes (e.g. two jackets, two pullovers, three t-shirts, a pair of jeans, trousers, pajamas, and a few socks, all on the same body), often seasonally inappropriate, was observed. Moreover, the most constant fact is that the clothes are old, worn out and weathered, with an unpleasant smell. The pockets are usually full of rubbish: plastic bags, crumpled pieces of paper, different plastic and/or small metal objects, etc.

DISCUSSION

Inspired by similar researches and the rising frequency of homelessness, respecting outer appearance, external examination of the body, as well as internal specific features, we tried to characterize the prototype of a deceased homeless person.

In this study, the mean age at the time of death among homeless people was 53.2 ± 11.1, which is older compared to the results of two similar studies performed in India [4, 5]. In these studies, the average age of homeless victims in Mangalore city was 42.8, whereas the commonest age group involved in South Delhi was 31–40 years. The difference can be due to the fact that the mean age of general population in Montenegro is 39.2 versus 27 in India, according to the CIA World Factbook [6, 7]. The Cultural and socioeconomical differences, as well as the different size of gap between population classes may also be the reasons.

The majority of homeless in our study died of natural causes, which is consistent with similar studies performed in South Delhi, India, Boston, USA, Calgary Canada, and Istanbul, Turkey [5, 8, 9, 10]. On the other hand, the study conducted in the city of Mangalore showed that the most common manner of death in the population of homeless was suicide (36.6%), followed by accidents (36%). There were no cases of suicide in our study, but the percentage of accidents was almost the same. The summary of causes of death throughout different studies is given in Table 3.

Drug abuse was identified as a rising problem among homeless people in developed countries decades ago [11]. Nowadays, drug overdose is reported as one of the most common causes of death in this population in the USA and Canada [8, 9]. However, in the present study, we did not find any such case. These variations can be explained by the fact that Montenegro belongs to the EUR-B region, where prevalence of problematic illicit drug use is lower compared to other European countries (EUR-A and EUR-C regions) or the USA and Canada (AMR-A region) [12].

Table 3. Differences between the manner of death in various parts of the world

Location	Natural deaths	Accidents	Suicides	Homicides
Istanbul [9]	60.3%	39.7% belong to violent deaths in general		
Boston [3]	65.3%	30.3%	2.8%	1.6%
South Delhi [4]	61.4%	31.7%	3.4%	3.5%
Mangalore [3]	26.5%	36%	36.6%	0.9%
Present study	65%	32%	0%	3%

Considering that underweight has usually been associated with homelessness, bad nutrition status is not surprising in 70.3% of homeless people in our study. On the other hand, recent studies in the USA have suggested that obesity may be the new malnutrition of the homeless in this country, equalizing the lack of food and bad food, since a cheap "high fat / high sugar / addictive food" is plentifully produced in western countries, especially in the USA, and is available at a lower price [13, 14]. According to a public health study performed by Tsai and Rosenheck [13], 57% of chronically homeless people were overweight or obese. Another study in Boston showed that prevalence of obesity among homeless was 32.3%, while only 1.6% were underweight, suggesting the same weight distribution as for the general population [14]. Compared to western countries, the presence of unhealthy and very cheap foods in Montenegro is practically negligible and we believe that this is the reason why our results are in accordance with the stereotype of the homeless population as underweight. Another thing which suggests that homeless people in Montenegro do not consume this type of food is the mean AS grade, which is significantly lower compared to the controls.

On external examination of homeless people, we found some features typically associated with homelessness originated by their lifestyle. Hair, beard, moustache, and fingernails were significantly longer than in the controls. Also, we found that people in the homeless group were significantly shorter than the people in the control group. This may be linked with lower social and economic status of their families, leading to the food intake that was insufficient for them to reach their genetic potential for height. However, more studies are required in order to confirm the influence of suggested factors on lower height found in homeless people.

When it comes to internal examination, alcohol liver disease is a common companion of homeless people, which was shown by other studies in Canada and Japan. [9, 15] Also, homeless people had significantly higher BAC at the time of death compared to the controls. The fact that standard deviation was higher than the mean value for BAC in both the control and homeless group in our study can be explained by a small and non-homogenous sample we obtained concerning this parameter. The observation was similar with the results for beard and nail length in the control group.

The results of our study indicate that homeless people also suffer from lung diseases, especially TBC, significantly more often than the rest of the population. Similar finding was noted in studies conducted in the UK and Japan [16, 17]. Considering that TBC is closely linked to poor living conditions and malnutrition, it becomes the principal disease of poverty. Also, there are studies that suggested that alcoholism may increase the risk of developing TBC as well [18]. Knowing that TBC is common among homeless people, as well as other infectious diseases like pneumonia

and viral hepatitis, a medical examiner should be extra cautious while performing an autopsy of a person with the description given above.

Old brain contusions are often referred to as *plaques jeunes* or yellow plaques, and represent signs of previous brain injuries. The greatest risk factors for brain injury are alcohol and drug abuse [19, 20]. Socioeconomic status also appears to affect traumatic brain injury rates; people with lower levels of education and employment and lower socioeconomic status are at greater risk [21]. Since homeless people have an elevated rate of substance abuse, which may lead to falls and head injuries, as well as an increased risk of being a victim of violent assaults, the obtained result was expected.

Considering the weather conditions in Montenegro, with cold and snowy winters, and the living conditions of the homeless, a very high death rate during the winter is also expected. This is also in accordance with other studies, where homeless people usually die in the seasons with challenging and extreme weather conditions, like rainy seasons in India or winter in Japan [4, 5, 15]. Criminology documented a rise in criminal activity of homeless persons in the late autumn, in an attempt of these persons to reach prison and spend the winter there, as a way of survival.

CONCLUSION

The results of this demonstrated that a deceased homeless person is usually a male in his 50s, shorter than the average, found dead during winter, with poor hygiene and worn out smelly clothing, long hair, beard, and mustache, long dirty fingernails, poor dental status and may be presented with old cortical contusions, any stage of alcohol liver disease and/or pulmonary infection, frequently including TBC.

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Обдукциони налаз код бескућника

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САЖЕТАК

Увод/Циљ Примећен је тренд пораста броја бескућника у последњих неколико деценија, а они све чешће бивају обдуковани.

Циљ рада је да дефинише сет специфичних аутоспијских параметара присутних у популацији бескућника, коришћењем кохортног приступа.

Методе Групу испитаника чинило је 37 лешева бескућника који су обдуковани у последњих 15 година. Контролну групу, која је сачињена на основу насумичне селекције према истом узроку смрти, сачињавало је 37 мушкараца. У свим случајевима извршена је комплетна обдукција и, где је било потребно, микроскопски преглед органа и хемијско-токсиколошка анализа. Упоређени су бројни параметри спољашњег и унутрашњег налаза.

Резултати Бескућници живе статистички значајно краће и чешће су у време обдукције били неидентификовани ($p < 0,05$). У спољашњем налазу, у поређењу са контролном групом, статистички значајно чешће се среће млађа животна доб, дужа коса, брада и нокти и лошији зубни статус ($p < 0,01$). Било је потхрањено 70,3% бескућника; статистички значајно чешће су боловали од плућних болести, алкохолне болести јетре и давнашњих контузија мозга ($p < 0,01$); имали су већу концентрацију алкохола у крви у време умирања у поређењу са контролном групом ($p < 0,05$), али и значајно нижи степен атеросклеротских промена ($p < 0,01$), и статистички значајно чешће умиру у зимским месецима ($p < 0,01$). Поред наведеног, они чешће болују од специфичних и неспецифичних запаљења плућа и алкохолне болести јетре.

Закључак Опис случајева смрти бескућника, упоређен са контролном групом, даје готово специфичну слику.

Кључне речи: бескућник; обдукција; туберкулоза; алкохолна болест јетре

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Delayed diagnosis of homocystinuria presenting as bilateral congenital lens subluxation

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SUMMARY

Introduction Homocystinuria is an autosomal recessively inherited defect leading to hyperhomocysteinemia and associated with ocular manifestations, mainly myopia and ectopia lentis.

Case outline A 26-year-old male with secondary glaucoma due to bilateral lens subluxation was admitted to the Department of vitreoretinal surgery. Horizontal nystagmus, bilateral lens subluxation, and bilateral amblyopia were first discovered at the age of three years. Preoperative laboratory workup revealed elevated levels of homocysteine. Bilateral pars plana lensectomy and vitrectomy followed by a sulcus fixation of the intraocular lens (ALCON MA60 Acrysof IOL) were performed. The patient was prescribed folic acid, methionine, and pyridoxine, and was urged to maintain a methionine-low diet. After a bilateral lensectomy and sulcus fixation of the intraocular lens and a methionine restriction therapy combined with vitamin B_6, B_9, and B_{12} supplementation, his condition improved greatly.

Conclusion In this report of a rare case we emphasize the importance of examining differential diagnoses of lens subluxation, since early intervention can prevent serious complications.

Keywords: lens subluxation; homocystinuria; glaucoma

INTRODUCTION

Homocystinuria is an autosomal recessive defect in metathione metabolism leading to hyperhomocysteinemia. It is associated with mental retardation, seizures, marfanoid habitus, and ocular manifestations, mainly myopia and ectopia lentis (EL) [1]. It has an estimated incidence of 1:50,000–200,000, sufficiently high to consider it for screening in newborns [2, 3]. After the condition is suspected based on physical findings, personal and family history, a workup is done for confirmation, including measuring homocysteine levels in blood and urine. Treatment consists of pyridoxine, vitamin B_{12}, folic acid, anticoagulation agents for stroke prevention, and low-methionine diet in drug-resistant cases [4].

Since treatment can reduce mortality and severity of complications, early diagnosis is crucial. Neonatal screening tests used for testing other similar metabolic disorders lack sensitivity in detecting homocystinuria. In most cases, the condition is confirmed after three years of age, presenting with lens subluxation [5]. We report a case of homocystinuria diagnosed in a 26-year-old, who had experienced ocular manifestations of the disease since early childhood.

CASE REPORT

A 26-year-old Caucasian male was referred to the Department of Vitreoretinal Surgery, Osijek Clinical Hospital Center. The reason of the referral was the need for surgical treatment of a subluxated lens that had caused secondary glaucoma. At the age of two, he underwent a left nephrectomy and subsequent chemotherapy due to Wilms tumor. At the age of three, he was diagnosed with horizontal nystagmus, bilateral subluxation of lenses, and bilateral amblyopia, and was scheduled for periodical exams. During high school education he experienced learning difficulties.

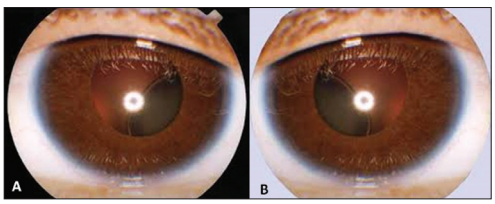

Figure 1. Biomicroscopic finding of the right (A) and left (B) eye shows bilateral inferotemporal subluxation of the lenses protruding in the inferior part of the vitreum

On admission, light hair, short stature (height of 162 cm, weight of 73 kg, BMI 27.8 kg/m^2), and bradydactylia were noted. The biomicroscopic ophthalmic examination showed bilateral inferotemporal subluxation of the lenses protruding in the inferior part of the vitreum (Figure 1). Zonular fibers were partially visible. Myopic changes were found on the fundus. Vitreal liquefaction was present.

Best corrected visual acuity was 0.4 LogMAR (Snellen acuity 6/15, decimal acuity 0.4) in the right eye and 0.7 LogMAR (Snellen acuity 6/30, decimal acuity 0.2) in the left eye.

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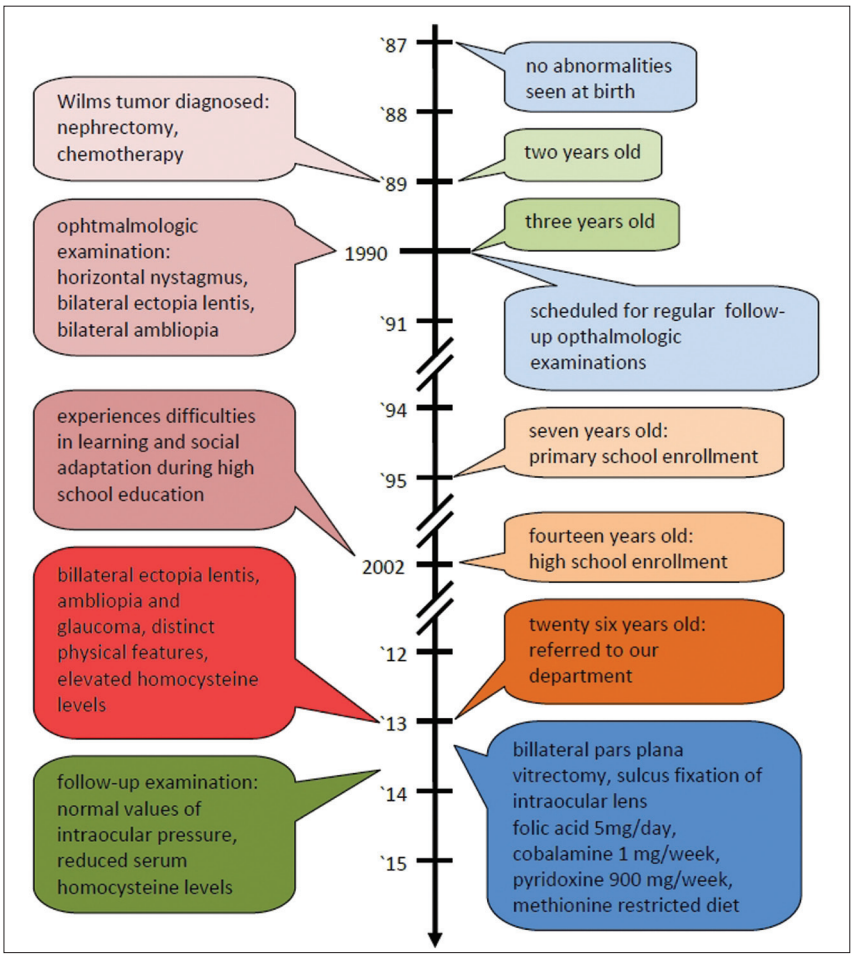

Figure 2. Flowchart of events

Applanation tonometry showed increased intraocular pressure (IOP; 33/31 mmHg).

The patient was referred to an internal medicine specialist. He underwent a physical examination and a complete ophthalmologic examination. Routine laboratory tests, including plasma homocysteine measurement, were ordered. Homocysteine level was 15 μmol/L.

Systemic signs and elevated homocysteine levels suggested homocysteinema as the most probable underlying condition. Blood dyscrasia, Fabry disease, and acidemias were ruled out, since there was no history of thromboembolic events and coagulation test results were normal. There was no family history of serious diseases, including homocysteinemia.

He was administered timolol/dorzolamide, brimonidine, and latanoprost in order to relieve elevated intraocular pressure. Since secondary bilateral glaucoma was unresponsive to treatment, a bilateral pars plana lensectomy, and vitrectomy followed by a sulcus fixation of the intraocular lens (ALCON MA60 Acrysof IOL; Alcon Inc., Hünenberg, Switzerland) were performed.

Also, a therapeutic regimen was established consisting of folic acid (5 mg/day), cobalamin (1 mg/week), pyridoxine (900 mg/day), and a methionine-restricted diet.

Three months upon the initiation of therapy, homocysteine levels were reduced to 10 μmol/L. Ophthalmologic examination showed cIOL 0.1 LogMAR (Snellen acuity 6/7.5, decimal acuity 0.8) in the right eye, cIOL 0.3 LogMAR (Snellen acuity 6/12, decimal acuity 0.5) in the left eye, and normal values of IOP.

The timeline of events is shown in Figure 2.

DISCUSSION

Early detection and treatment are of paramount importance in homocystinuria patients. Timely interventions can reduce the number and severity of complications. Abnormally high and progressive myopia at a young age combined with systemic complications are signs of suspected homocystinuria. Nevertheless, significant delays in diagnosis happen [6].

EL occurs in around 80% of patients and it is the most common involvement in homocystinuria [7, 8]. About 70% of patients will develop EL by eight years of age, and 82% by the age of 10 [9].

Signs that might suggest EL include very high myopia, abnormally progressive myopia, myopia at a young age, or high myopia without a myopic fundus [6]. Later signs include decreased vision, monocular diplopia or pain secondary to pupillary glaucoma, and vascular signs [10].

Even though EL is one of the most prominent symptoms of homocystinuria, and 5% of all lens dislocations may be attributed to this metabolic condition, homocystinuria is often neglected in the differential diagnosis of

EL, which leads to a delay in, or a lack of, correct diagnosis and treatment with a mean of 11 years from the onset of major signs until the diagnosis [6, 10]. In the case of our patient, the delay of diagnosis was 23 years.

Every EL requires a broad differential diagnostic approach since it is often a presentation of a systemic disease. It can be etiologically divided into two groups: hereditary and secondary to other causes [7]. The latter include trauma, infections, a large eye, anterior uveal tumors, pseudoexfoliation syndrome, and hypermature cataract. Hereditary EL occurs in systemic disorders such as Marfan syndrome, homocystinuria, Weil–Marchesani syndrome, Ehlers–Danlos syndrome, deficiency in sulfite oxidase, and hyperlysinemia. Hereditary EL without systemic associations includes aniridia, congenital glaucoma, familial EL, and ectopia lentis et pupillae.

Homocystinuria is divided into two groups based on the therapeutic response [9]. About 50% of patients respond well to vitamin B_6 (B_6-responsive homocystinuria) supplements in high doses [10]. Vitamin B_6-responsive patients have lower incidence, and later occurrence of complications [7, 11]. B_6-non-responsive patients require a methionine-restricted diet with daily intakes of methionine not exceeding 40 mg/day [9]. An alternative therapeutic approach can be considered in these patients, which involves the use of methyl donors, betaine or its precursor choline, that reduce homocysteine levels by promoting its conversion to methionine [6, 10]. A combined therapy was prescribed in our patient.

Treatment from infancy with pyridoxine, folic acid, and betaine reduces cardiovascular risk by 80–90% [12]. To prevent thromboembolism, antiaggregant treatment with acetylsalicylic acid should also be considered in cases of immobilization or after surgery [8].

Because of the increased probability of thromboembolism, conservative treatment of EL is advised when possible [10, 13]. Lensectomy is performed in cases of secondary complications, such as progressive lens subluxation, cataract formation, lens instability, retinal detachment, or pupillary block glaucoma, as was the case in our patient [13, 14].

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Касна дијагноза хомоцистинурије приказана обостраном конгениталном сублуксацијом сочива

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САЖЕТАК

Увод Хомоцистинурија је аутозомно рецесивни наследни поремећај који води у хиперхомоцистенемију и повезан је са очним поремећајима, у првом реду кратковидошћу и ектопијом сочива.

Приказ болесника Мушкарац старости 26 година са секундарним глаукомом због обостране сублуксације сочива примљен је на Одељење витреоретиналне хирургије. У доби од три године откривени су хоризонтални нистагмус, обострана сублуксација сочива и обострана амблиопија. Преоперативна лабораторијска обрада показала је повишен ниво хомоцистеина. Урађена је обострана *pars plana* лензектомија и витректомија и сулкус фиксација интраокуларног сочива (*ALCON MA60 Acrysof IOL*). Прописана му је фолна киселина, витамини $Б_6$, $Б_9$ и $Б_{12}$ и саветована дијета са ниским садржајем метионина. После оперативног захвата и примењене терапије стање му се значајно побољшало.

Закључак У овом приказу ретког случаја наглашавамо важност диференцијалне дијагнозе сублуксације сочива, пошто рана интервенција може спречити озбиљне компликације.

Кључне речи: сублуксација; хомоцистинурија; глауком

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Jejunal tumor made of primary gastrointestinal stromal tumor and metastatic breast carcinoma – an extremely rare case

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SUMMARY

Introduction The occurrence of synchronous or metachronous malignant epithelial and mesenchymal tumors is rare. Infiltrating ductal breast cancer rarely produces metastasis in the gastrointestinal tract, and when it does, it represents a significant differential diagnostic problem. Morphologically, they can mimic primary cancers localized in the gastrointestinal tract or peritoneum.

Case outline In this paper, we present a female patient with primary, synchronous bilateral breast cancer, which after five years of follow-up had given metastases to the lungs, bones, peritoneum and mesentery, and in a node localized in the small intestine. The node was composed of two malignant components – a mesenchymal one and an epithelial one. The mesenchymal component had histologic and immunophenotypic characteristics of a gastrointestinal stromal tumor and the epithelial component was morphologically and immunohistochemically identical to the diagnosed primary breast cancer. Because of all this, the nodal tumor mass was interpreted as a primary gastrointestinal stromal tumor of the small intestine, in which the deposit of metastatic ductal breast carcinoma was observed.

Conclusion Metastases of breast cancer in organs of the gastrointestinal tract are encountered rarely, mainly in the terminal stage of the disease. In available literature, a case of metastasis of breast cancer (metastasis of malignant epithelial tumors) in gastrointestinal stromal tumor has not been found.

Keywords: breast cancer; gastrointestinal stromal tumor; metastasis

INTRODUCTION

Breast cancer is the most common malignant tumor and accounts for about 27% of all malignancies in women [1, 2]. Invasive lobular carcinoma accounts for 5–15% of all breast cancers. Infiltrating lobular carcinoma is often multicentric, bilateral, more commonly gives local relapse and distant metastases, and is increasingly being diagnosed in postmenopausal women. Synchronous occurrence of two tumors is rare – especially rare are cases of synchronous malignant epithelial and mesenchymal tumors. Takeuchi et al. [3] described synchronous lobular breast cancer and gastrointestinal stromal tumor (GIST) in a patient with neurofibromatosis type 1. Adim et al. [4] published a case of synchronous and metachronous occurrence of GIST with other malignant tumors in the gastrointestinal tract (GIT). They found that GIST could be synchronously or metachronously present with malignancies out of the GIT, most commonly in the breast. Afif et al. [5] described a rare synchronous bilateral breast cancer and gastric GIST. Infiltrating ductal carcinoma of the breast gives metastases to the lungs, bone and liver, and metastases of infiltrating lobular carcinoma frequently involve GIT, the peritoneal surface and retroperitoneum [2]. Isolated adrenal metastases originating from invasive ductal carcinoma of the breast are extremely rare [6]. Metastases in the GIT can be clinically manifested as obstruction, bleeding, and often mimic a primary carcinoma [2]. Metastasis of breast cancer in the GIT are very rare. Borst and Ingold [7] followed 2,604 subjects for 18 years and found metastases in 17 patients (less than 1%). The analysis of Mourra et al. [8] showed that out of 35 patients with verified metastatic disease in the colon and rectum, in 17 cases the metastasis of breast cancer was present, which is almost half of all metastases that were analyzed by these authors.

Metastases of breast cancer in the GIT and peritoneum are an important differential diagnostic problem. Morphologically, they can mimic primary cancers localized in the GIT or peritoneum (mesothelioma). In some cases, metastases of breast cancer in the GIT may occur after several years (more than ten years), and the primary breast cancer sometimes becomes forgotten [1, 9]. All of the above may lead to the misinterpretation of cancer as primary cancer in the GIT [9].

We report a case of a patient with primary, synchronous bilateral breast cancer, which after five years of follow-up gave metastases to the lungs, bones, and peritoneum, and metachronous (after five years) GIST in the small intestine. We particularly emphasize that, at the same time, GIST of the small intestine and metastasis of breast cancer in GIST were present.

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CASE REPORT

A 57-year-old female patient had been diagnosed with a bilateral synchronous breast cancer five years previously. In the treatment of cancer of the right breast, the patient underwent mastectomy with dissection of axillary lymph nodes. Invasive ductal carcinoma of the breast (no special type – NST), grade 2, pT2N3aM0 was diagnosed (Figure 1.A). Immunohistochemical analysis of invasive components of the tumor revealed that the tumor had a positive staining for the estrogen receptor alpha (ER) and negative staining for the progesterone protein receptor (PR) and receptor of epidermal growth factor 2 (HER2). In the treatment of tumor of the left breast, the patient underwent a modified mastectomy. Analysis indicated the presence of invasive ductal carcinoma (NST), grade 2, pT1cN1aM0 (Figure 1.B). Immunohistochemically, the tumor of the left breast had the same characteristics as the tumor of the right breast. The patient received six cycles of chemotherapy. After chemotherapy, the patient received radiotherapy of both axillary regions and right pectoral region, followed by hormone therapy with tamoxifen.

Two years after the first surgery, a local relapse in the right pectoral region was confirmed, 7 × 5 mm in size, which was surgically removed and histologically identified as a relapse of the previous disease. The patient refused a specific oncologic therapy. Three years after the diagnosis of tumors in both breasts, further progression of the disease was found. Metastases were verified in both lungs and vertebrae. The patient still refused a specific oncological treatment.

Five years after the diagnosis of bilateral breast tumor, the patient was hospitalized with the clinical picture of acute ileus. A laparotomy was performed, which revealed the presence of masses in the small intestine (jejunum) and the mesentery. The two masses were resected.

One mass was located in the small intestine (jejunum) and was resected with a part of the small intestine in the length of 3.6 cm. On the opposite side of the mesentery (antimesenterically), a nodal tumor mass was present, with smooth surface and 4.5 × 3 × 2.5 cm in size. On the section, the nodal tumor mass was largely a solid, homogenous structure, whitish, and to a lesser extent cystic, brown and black. The mucosa of the small intestine over the nodal tumor mass was not changed (Figure 2). Histologically, the nodal tumor mass was located in the muscle and subserous layer of the wall of the small intestine and made of a mixed population of cells (mesenchymal and epithelial). The dominant component of the tumor (about 80%) was mesenchymal (Figure 3). Mesenchymal component of the nodal tumor mass was made up of uniform spindle cells with oval nuclei, granular chromatin, and eosinophilic cytoplasm. Mitotic figures were rare (3/50 HPF). The mesenchymal component of the tumor was immunohistochemically positive for the following antibodies: vimentin, CD117, CD34, Ki-67 (nuclear positivity was present in about 2% of the mesenchymal component of the tumor) (Figure 4). The epithelial component of the tumor was diffusely mixed with mesenchymal component

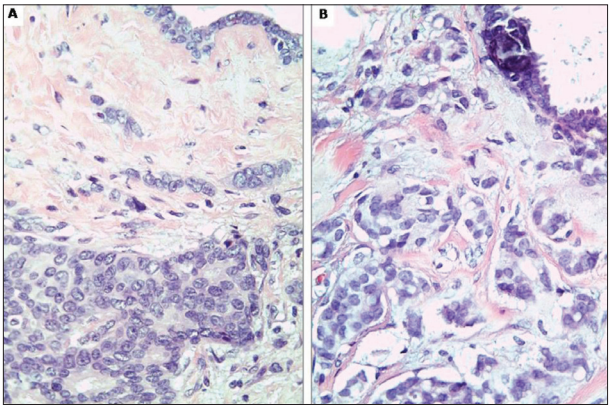

Figure 1. Bilateral ductal breast cancer – A: microscopic image of ductal cancer in the right breast; B: microscopic image of ductal cancer in the left breast (H&E, × 400)

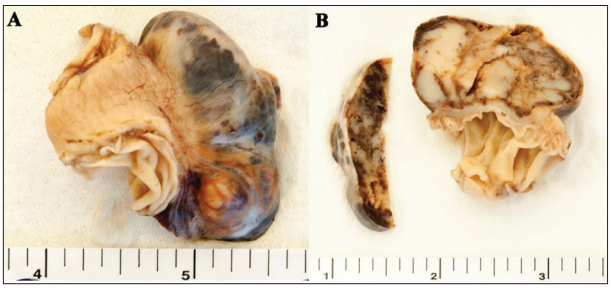

Figure 2. The macroscopic appearance of resected nodal tumor mass localized in the small intestine (jejunum) – A: external surface of the node; B: appearance on node section

and built of solid, trabecular, cribriform, atypical adenoid and tubular formations. Tumor cells had a moderate degree of polymorphism, oval nuclei, and eosinophilic cytoplasm. In the lumen of adenoid and tubular formations, there was an eosinophilic content. Mitotic figures in the epithelial component of the tumor were rare (3/10 HPF). In the stroma, there were lymphocytes, histiocytes, and areas of bleeding. Epithelial component made approximately 20% of the tumor. Immunohistochemically, the epithelial component was positive for the following antibodies: CK (AE1/AE3), CK7, CEA (m), ER, GCDFP15, and E-cadherin (Figure 5). The negative reaction of both components of the tumor was found for the following antibodies: CK (HMW), 5/6 CK, CK20, CDX2, ESA (BerEp4), calretinin, chromogranin A, synaptophysin, and CD56. The nodal tumor mass was well circumscribed, with expansive growth, and on the surface there was a complete connective tissue pseudocapsule. In the vicinity of the described tumor, the small intestine did not show morphological changes. Based on the morphological and immunohistochemical characteristics, the nodal tumor mass localized in the small intestine was interpreted as a primary GIST of the small intestine, low risk, in which the previously diagnosed ductal breast cancer metastasized.

The second tumor change that was removed during the same procedure was localized in the mesentery. Histologically, it was interpreted as a metastatic deposit of previously diagnosed breast cancer.

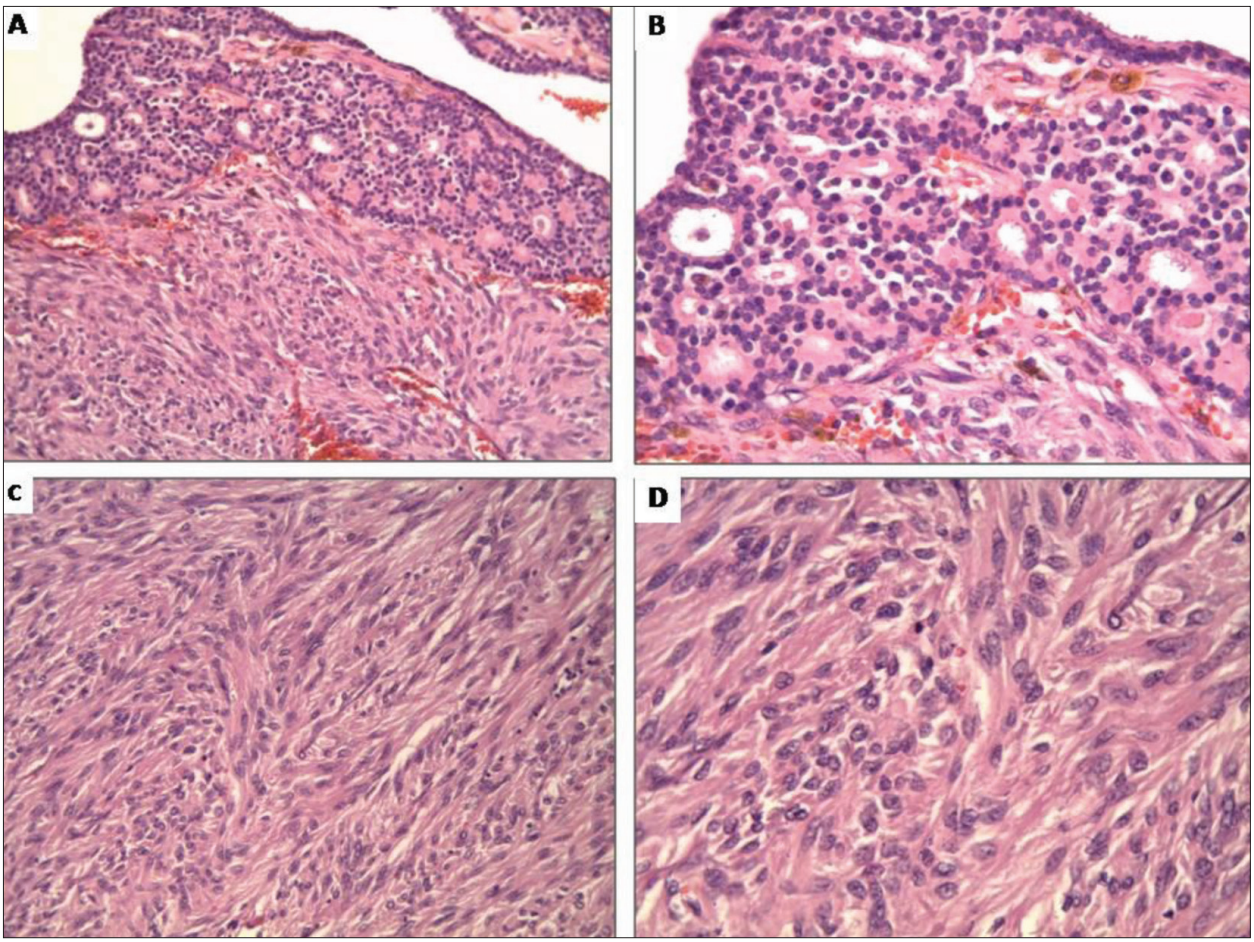

Figure 3. Histological image of the small intestine tumor, made up of a mesenchymal and an epithelial component; A: in the upper part of the image there is an epithelial component of the tumor, and in the lower part there is a mesenchymal component (H&E; × 200); B: H&E; × 400; C: histologic appearance of the mesenchymal component of the small intestine tumor (H&E; × 200); D: H&E; × 400

DISCUSSION

Gastrointestinal stromal tumors are usually solitary tumors in patients aged 50 to 60 years, with no association to tumors of another histogenetic origin. Associated occurrence of GIST and other tumors is present in patients with syndromes such as neurofibromatosis type 1, Carney triad, and familial GIST. The association of GIST with other tumors, in patients who do not have the aforementioned syndromes, is rare and reports in the literature vary from 4.5% to 33% [10]. Gonçalves et al. [10] followed 101 patients with GIST and established the existence of other tumors (other than GIST) in 14 patients (13.8% of cases). In this study, there was a case of association of GIST and ductal breast cancer. In most cases, GIST was less than 5 cm in size and had a low or very low malignant potential. It is diagnosed accidentally during surgery or follow-up of other malignancies. In the study by Gonçalves et al. [10], there was one case of a relation between GIST and ductal breast cancer. The most common localization of the coexistence of GIST and other tumors are stomach and colon [11]. Coincidence is certainly not the only explanation of the phenomena of synchronous and metachronous neoplasms with GIST. Possible reasons may be the presence of tumor syndromes, new genetic changes, and exposure to carcinogenic agents. A significant number of authors concluded that there is a greater incidence of gastrointestinal tumors in patients with GIST than in the general population [10]. AbdullGaffar [12] in his research established the relation between GIST and tumors out of the gastrointestinal system. The study included 21 patient, four of which (24% of cases) had GIST and another tumor outside the GIT.

Agaimy et al. [11] analyzed 4,813 cases and found the synchronous or metachronous presence of other malignancies in 486 cases. They showed the association of most types of GIST with malignancies localized in the GIT (47%). Lymphoma/leukemia and breast cancer were associated with GIST in 7% of cases each, cancer of the prostate was associated with GIST in 9% of the cases, kidney cancer in 6%, cancers of lungs and female genital system in 5% each, carcinoid and soft tissue tumors including osteosarcoma in 3% each, melanoma in 2%, and seminoma were associated with GIST in 1% of the cases. Similar results were obtained by Adim et al. [4], who studied 78 cases and found another malignancy in 13 patients (16.6% of cases).

Takeuchi et al. [3] presented a patient who suffered from neurofibromatosis type 1 and who had been diagnosed with invasive ductal carcinoma of the left breast, and after seven years with invasive lobular carcinoma of the right breast and synchronous GIST of the small intes-

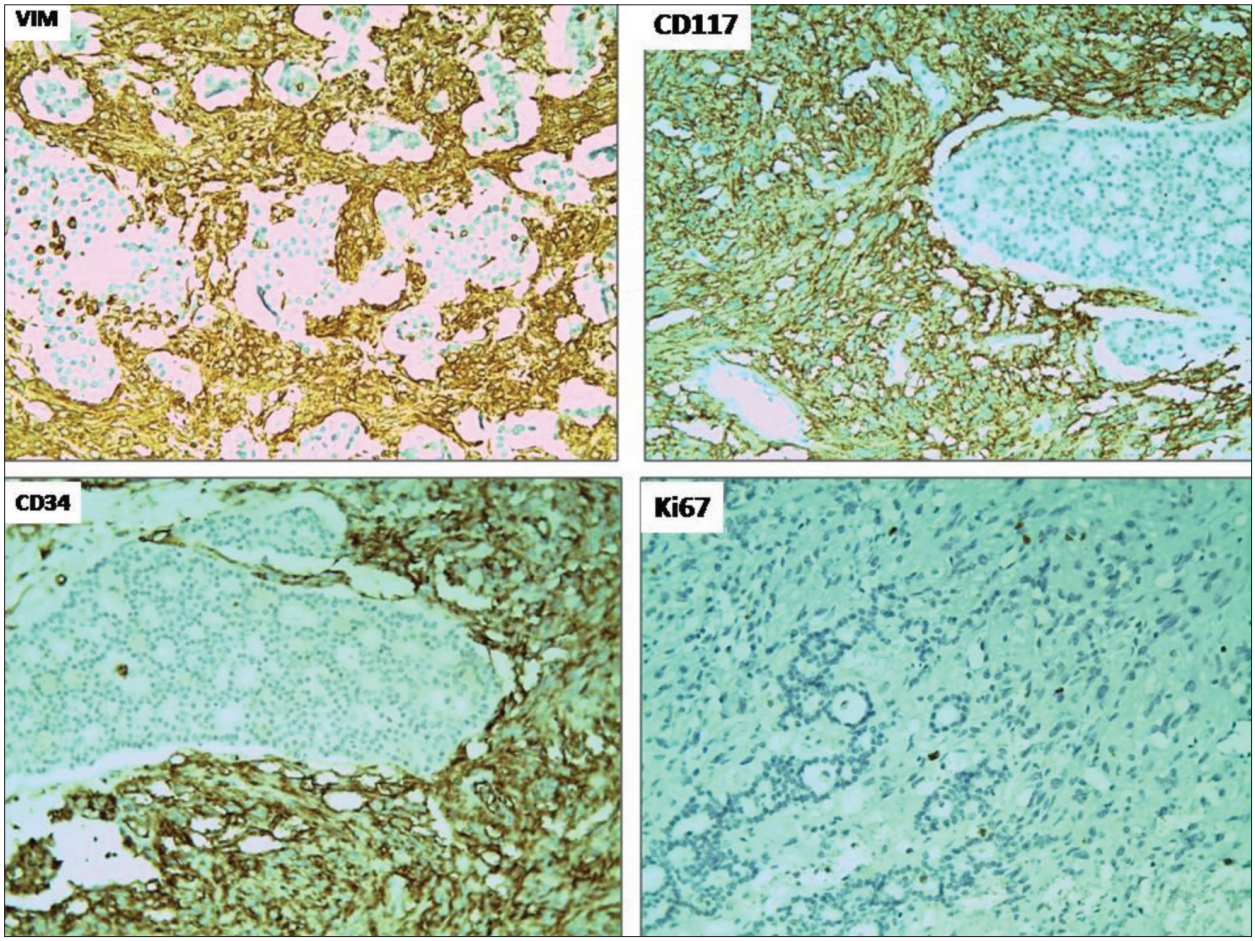

Figure 4. Immunohistochemical image of the small intestine tumor; the mesenchymal component is positive to vimentin, CD117, and CD34; Ki67 positivity is seen in rare mesenchymal cells (× 200)

tine. In the present case, metastases of breast cancer in the peritoneal cavity and GIST were not found.

The incidence of breast cancer metastases in organs of the GIT is rare. In the literature, the frequency is up to 0.34%. McLemor et al. [13] have identified the presence of metastases in the GIT in 41 patients, out of the total number of 12,001 patients. Invasive lobular carcinomas more often give metastases to the GIT compared to the ductal carcinoma [13]. Metastases of breast cancer in the GIT may occur many years (usually between five and 20 years) after primarily diagnosed breast cancer. The authors note that metastases occur after an average of seven years. The most common localization of metastatic breast cancer in the GIT are stomach and small intestine, rarely the colon [13]. Metastases of breast cancer in the GIT are usually associated with metastases in other organs (up to 90% of cases) [13]. Mourra et al. [8] analyzed tumor metastases in colon and rectum. The total number of analyzed cases was 35, and in as much as 48.6% of the cases it was a metastatic breast cancer. The cause of metastatic breast cancer in organs of the GIT is not entirely clear. A possible reason is a certain tropism of tumor cells of lobular or ductal carcinoma. The synchronous or metachronous occurrence of GIST and various other tumors is not clear. It is possible that there is a common pathogenesis or a cause, especially in women. It is necessary to conduct more clinical, epidemiological, and genetic studies to determine the clinical significance of the association among GIST and extraintestinal tumors.

In the literature, we did not find any information about the presence of a synchronous invasive ductal cancer, metachronous GIST in the small intestine, and the presence of metastatic ductal carcinoma in GIST tumor. In our patient, five years after the diagnosis of bilateral, synchronous breast cancer the presence of metastases in the lungs, bones, and organs of the GIT (mesentery) was showed. At the same time, GIST of the small intestine (jejunum) was diagnosed, in which the histological and immunohistochemical analysis showed the presence of ductal carcinoma of the breast. Due to the presence of metastasis of epithelial tumor (ductal breast cancer) in a malignant mesenchymal tumor (GIST), this case is unique.

Miller et al. [14] described the case of a patient with a synchronously diagnosed adenocarcinoma of the colon and metastatic lobular carcinoma in the colon and small intestine. Colon adenocarcinoma was localized in the sigmoid colon. Strictures in the small and large intestine, including the sigmoid part, were occupied by the tumor tissue with the histological image consistent with lobular breast cancer. Breast cancer had not been previously diagnosed. The authors point out that this is the only case in the available literature of coexistence of colon adenocarcinoma and metastatic lobular breast cancer in the same part

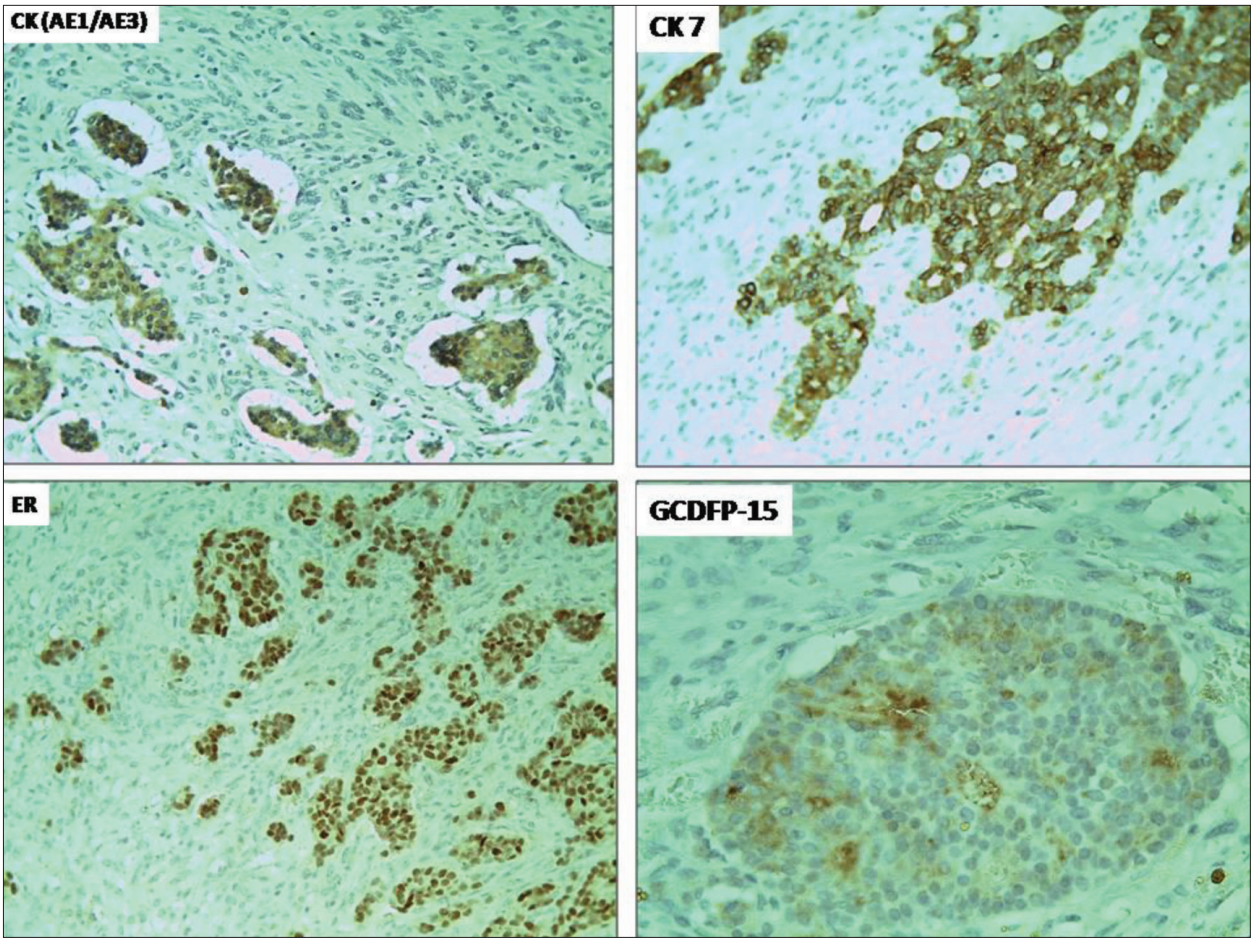

Figure 5. Immunohistochemical image of the small intestine tumor; the epithelial component of the tumor shows immunopositivity to cytokeratin (AE1/AE3), cytokeratin 7, estrogen receptor alpha, and GCDFP-15 (× 200)

of the colon, where breast cancer had not been diagnosed previously [14]. The authors described the presence of one tumor next to another (coexistence) and did not describe the presence of metastatic deposits of lobular carcinoma of the breast in the primary adenocarcinoma of the colon.

Macías-García et al. [15] were the first to describe a collision of GIST and prostate cancer. The authors described a high-risk GIST of the spindle cell variant that originated in the anterior rectal wall and that exhibited perirectal extension and wide infiltration of the adjacent peripheral prostate lobules, as well as a prostatic acinar adenocarcinoma.

The diagnosis of metastatic breast cancer in the GIT can be difficult due to several reasons. The first reason is a long period from the diagnosis of the primary breast tumor (usually more than five years), which can lead to the neglect of the primary disease. The second reason is the clinical presentation of the disease, which can mimic a disease of the GIT. Clinically, it can be manifested as the primary tumor, followed by abdominal pain, anorexia, vomiting, bleeding, obstruction, perforation, etc. The following possible reasons are difficulties in obtaining appropriate material: usually, the tumors are located in the subserous and muscle layer of the wall and are inaccessible to endoscopic sampling, as well as the morphological similarities with primary tumors localized in the GIT [8, 16, 17, 18]. In order to overcome the difficulties in differentiating, it is necessary for all participants in the diagnostic and the treatment team to have information about previous interventions and diagnosed diseases. It is necessary to have information on their progress and to have the possibility to access the previous diagnostic procedures (radiological, histological) that can be compared to the morphological, immunophenotypic image of the previously diagnosed disease with samples that are subsequently obtained. In this way, the possibility of misinterpretation of tumor process should be minimized.

All of the above has helped us in the differentiation of the nodal tumor mass in the small intestine, which was morphologically composed of two malignant components (epithelial and mesenchymal). The mesenchymal component had histological and immunophenotypic characteristics of GIST, while the epithelial one was morphologically and immunohistochemically identical to the primary diagnosed breast cancers. The nodal tumor mass was interpreted as a primary GIST of the small intestine where the deposit of ductal metastatic breast cancer was observed.

The prognosis of survival in cases of metastatic breast cancer in the GIT is poor and is less than two years [5, 17]. Appropriate systemic therapeutic approach for metastatic breast cancer in the GIT has a positive effect. Total therapeutic response to the systemic therapy is between 32% and 53%. Systemic therapy has a beneficial effect on survival, while surgical treatment has no significant effect on survival [18].

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Тумор јејунума сграђен од примарног гастроинтестиналног стромалног тумора и метастатског карцинома дојке – изузетно редак случај

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САЖЕТАК

Увод Појава синхроних или метахроних малигних епителних и мезенхимних тумора је ретка. Инфилтративни дуктални карцином дојке ретко даје метастазе у гастроинтестиналном тракту, а кад се појаве, представљају значајан диференцијално-дијагностички проблем. Морфолошки, могу имитирати примарне карциноме локализоване у гастроинтестиналном тракту или перитонеуму.

Приказ болесника Приказана је болесница са примарним, синхроним билатералним карциномом дојке, који је након петогодишњег праћења дао метастазе у плућа, кости, перитонеум, мезентеријум и у чвор локализован у танком цреву. Чвор је грађен од две малигне компоненте – мезенхимне и епителне. Мезенхимна компонента је хистолошких и имунофенотипских карактеристика гастроинтестналног стромалног тумора (ГИСТ), а епителна компонента је морфолошки и имунохистохемијски идентична примарно дијагностикованим карциномима дојке. Због тога је туморски чвор интерпретиран као примарни ГИСТ танког црева, у којем је уочен метастатски депозит дукталног карцинома дојке.

Закључак Метастазе карцинома дојке у органе гастроинтестиналног тракта се срећу ретко, углавном у терминалном стадијуму болести. Метастаза карцинома дојке у ГИСТ није описана у доступној литератури.

Кључне речи: карцином дојке; гастроинтестинални стромални тумор; метастаза

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Pulmonary embolism as the first sign of the nephrotic syndrome

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SUMMARY

Introduction Pulmonary embolism (PE) is a serious complication of deep venous thrombosis, with a significant morbidity and mortality. More often, PE complicates the course of the nephrotic syndrome (NS), in particular when the disease is active, but it may occur as the first sign of illness when the diagnosis of the NS is being delayed as a result. Membranous nephropathy is, generally speaking, the most commonly reported glomerulonephritis associated with the increased risk of thrombosis.

Case outline This report summarizes our experience with three young male patients (a 26-year-old, a 22-year-old, and a 45-year-old), in which PE was the first presenting feature of the NS. All of them were admitted to the hospital experiencing chest pains, dry cough, and shortness of breath. One of them had high temperature and the other two swelling of the lower parts of legs. Computed tomography of the thorax showed pulmonary artery thrombosis in all three patients. Diagnosis of the NS was confirmed by laboratory analysis, while renal biopsy showed membranous nephropathy. The treatment was based on the pulse of methylprednisolone (1.5 g over a period of three days), with alternating therapy of oral corticosteroids and cyclophosphamide on a monthly basis during six months. After six months, two patients reached incomplete remission, while the third one still has the NS and normal renal function.

Conclusion Not so rare occurrence of thromboembolic events in the NS suggests that one should always suspect the NS in all patients with deep venous thrombosis or PE.

Keywords: nephrotic syndrome; membranous nephropathy; pulmonary embolism

INTRODUCTION

Pulmonary embolism (PE) is a serious complication of deep venous thrombosis (DVT), with a significant morbidity and mortality [1, 2]. PE most commonly occurs from DVT of legs or renal venous thrombosis, although in many cases the location of thrombosis hasn't been found in other areas. Thromboembolism is among the most serious complications of the nephrotic syndrome (NS) [3, 4]. PE may complicate the course of the NS, especially when the disease is already active, or, less commonly, it may appear as the first sign of illness and fail to be identified, in which case usually delays the diagnosing of NS.

We shall present three cases of NS, where PE was the first sign of membranous nephropathy (MN).

Case report 1

A 26-year-old man was admitted to the Clinic for Lung Diseases complaining of chest pains, dry cough, high temperature, and shortness of breath. The initial chest radiography was normal. Bronchopneumonia was suspected and treatment with antibiotics was initiated. Two days upon admission, additional deterioration of breathing occurred. Electrocardiogram showed sinus tachycardia. In laboratory analysis, an elevation in D-dimer (36 mg/l) was observed, as well as a decrease in antithrombin III activity (76%). Computed tomography of the thorax showed thrombosis of the pulmonary arteries and also in the branches of the lower lobes. Anticoagulant therapy was introduced (low-molecular-weight heparin, then oral anticoagulation). Other sites of thrombosis were excluded after performing the Doppler sonography of the lower limbs and renal veins. On cardiac echography, there were no signs of pulmonary hypertension. Ultrasound examination revealed enlarged kidneys (13 cm in diameter) with normal parenchymal thickness and echogenicity. Immunology tests were normal. Blood analysis: hemoglobin 156 g/l, urea nitrogen 3.4 mmol/l, creatinine 57 umol/l, total protein 36 g/l, albumin 14 g/l, total cholesterol 8.2 mmol/l, and triglyceride 3.3 mmol/l. Urine sediment analysis revealed 10–15 red blood cells. Urinary protein excretion was 12 g / 24 hours, clearance of creatinine was 181 ml/min. A nephrologist was consulted and the diagnosis of nephrotic syndrome was confirmed. Percutaneous renal biopsy was done and the specimen showed glomeruli with mild thickening of the glomerular basement membrane with granular deposition of IgG, compatible to MN (Figures 1 and 2). A detailed examination

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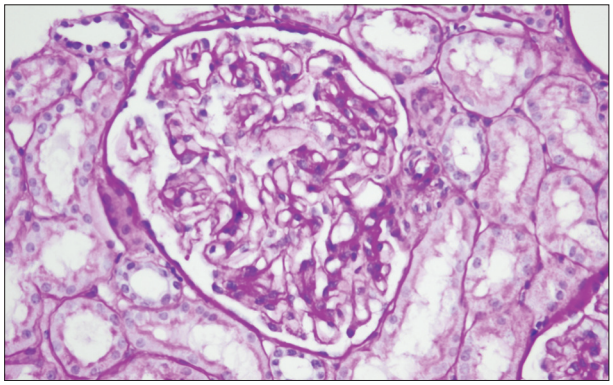

Figure 1. Light microscopy – mild thickening of the glomerular basement membrane (periodic acid – Schiff reaction, × 400)

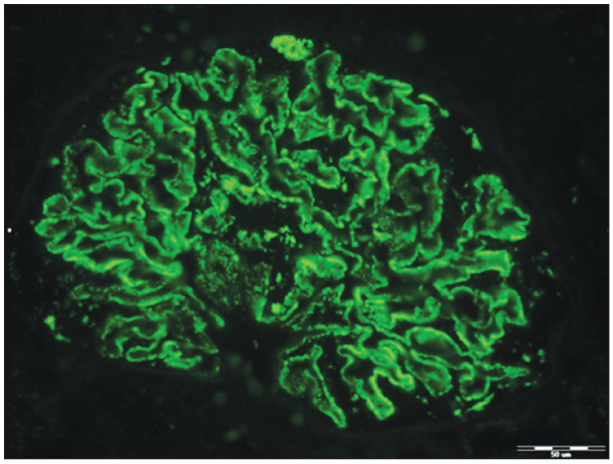

Figure 2. Immunofluorescence microscopy – granular deposition of immunoglobulin alongside glomerular basement membrane (× 400)

excluded secondary causes of MN. The treatment was based on the pulse of methylprednisolone (1.5 g over a period of three days), with alternating therapy of oral corticosteroids and cyclophosphamide on a monthly basis during six months. The symptomatic therapy included ACE inhibitors, a diuretic, and statin. After six months of treatment, we registered partial remission of NS, and after 12 months, complete remission with proteinuria 0.3 g / 24 hours.

Case report 2

A 22-year-old man was admitted to the Coronary Intensive Care Unit with chest pain and shortness of breath. Several days before the admission to the hospital, the patient noticed a swelling of his legs, which disappeared quickly. In initial laboratory tests, D-dimer was high, while cardiac enzymes were normal. Electrocardiogram showed sinus tachycardia. Blood gases in arterial blood were normal. Computed tomography of the thorax showed thrombosis of the pulmonary artery. The patient was treated with anticoagulant therapy (low-molecular-weight heparin, then oral anticoagulation). On cardiac echography there were no signs of the pulmonary hypertension. Other sites of thrombosis were excluded after performing the Doppler sonography of the lower limbs and renal veins. Thrombophilia screening tests (antiphospholipid antibodies, protein C and S, antithrombin III, factor V mutation) were normal.

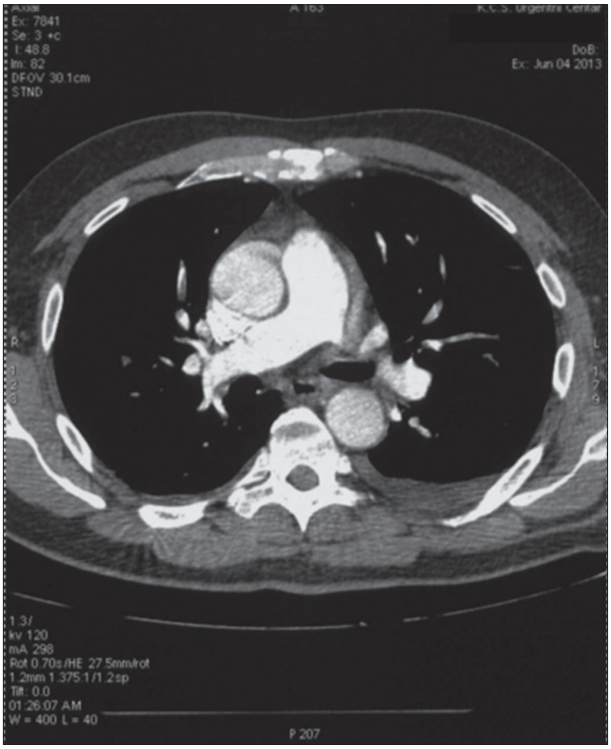

Figure 3. Computed tomography of the thorax – thrombosis in lobar branches of the right pulmonary artery

The laboratory analysis showed that the renal function was normal, while total cholesterol was high. Analyses of urine were not done. Upon full recovery, he was discharged from the hospital with oral anticoagulation. After four months, the patient got respiratory infection with secretions from the nose, followed by cough and high temperature. He suddenly began to swell (swelling of the eyelids and legs, stomach distension) and became oliguric, when he went to the Emergency Room. A nephrologist was consulted and he was admitted to the Clinic for Nephrology. Laboratory analysis showed hemoglobin 136 g/l, urea nitrogen 6.2 mmol/l, creatinine 78 umol/l, total protein 34 g/l, albumin 17 g/l, total cholesterol 9.2 mmol/l, and triglyceride 2 mmol/l. Urine sediment analysis revealed 5–7 red blood cells. Urinary protein excretion was 10 g / 24 hours, clearance of creatinine was 171 ml/min. Ultrasound examination revealed enlarged kidneys (12 cm in diameter) with normal parenchymal thickness and echogenicity. The diagnosis of the NS was confirmed. The patient's treatment was changed to low-molecular-weight heparin, and percutaneous renal biopsy was done. The specimen showed glomeruli with diffuse thickening of the glomerular basement membrane with granular deposition of IgG, compatible with MN. A detailed examination excluded secondary causes of MN. The treatment was based on the pulse of methylprednisolone (1.5 g over a period of three days), with alternating therapy of oral corticosteroids and cyclophosphamide on a monthly basis during six months. Symptomatic therapy included ACE inhibitors, a diuretic, and statin. After six months of treatment, we registered partial remission of NS, proteinuria decreased to 3 g / 24 hours. After 12 months, the proteinuria continues to maintain the same level.

Case report 3

A 45-year-old man was admitted to the Clinic for Lung diseases complaining about the stabbing pain in the left half of the thorax (that intensifies during the intake of air), shortness of breath, and swelling of lower legs. The symptoms began two days before the admission. Auscultation of the lungs revealed impaired breathing on both lower sides. Electrocardiogram showed sinus tachycardia. The arterial blood gases were normal, with a PH of 7.47, partial pressure of oxygen of 8.3 kPa, partial pressure of carbon dioxide of 5.5 kPa, oxygen saturation of 93%. Laboratory analysis showed that the D-dimer was elevated (16.5 mg/l). Computed tomography of the thorax showed partial thrombotic mass in both lobar and segmental branches of the medial segment of the right middle lobe and smaller pleural effusions in laterobasal segment of the lower lobe (Figure 3). PE was diagnosed. He was treated with low-molecular-weight heparin (80 mg of enoxaparin twice per day), oxygen, and antibiotics. Other sites of thrombosis were excluded by Doppler sonography of the lower limbs and renal veins. On cardiac echography there were no signs of pulmonary hypertension. Immunology tests were normal. Laboratory analysis showed elevated white blood cell count and C-reactive protein (12.7×10^9/l and 117 mg/l), creatinine 69 umol/l, total protein 51 g/l, albumin 19 g/l, total cholesterol 14.6 mmol/l, and triglyceride 2.9 mmol/l. Urine protein was quantified at 7.4 g / 24 hours, clearance creatinine 128 ml/min. A nephrologist was consulted and the diagnosis of NS was confirmed. Renal biopsy was performed and specimen showed glomeruli with mild thickening of glomerular basement membrane with granular deposition of IgG, compatible with MN. A detailed examination excluded secondary causes of. The treatment was based on the pulse of methylprednisolone (1.5 g over a period of three days), with alternating therapy of oral corticosteroids and cyclophosphamide on a monthly basis during six months. ACE inhibitors, a diuretic, and statin were prescribed. After six months of treatment, proteinuria continues to maintain the high value of 9.6 g / 24 hours. After 12 months, cyclosporin was introduced and we have registered clinical improvement (without leg edema) and incomplete remission of NS with proteinuria of 4.5 g / 24 hours.

DISCUSSION

Membranous nephropathy is the most common cause of the NS in adults [5]. The etiology of approximately 75% of MN cases is idiopathic [6]. The peak incidence occurs in the fourth to fifth decade of life, with predominance in men [7, 8]. Proteinuria is the typical presentation of MN and the NS occurs in 70–80% of patients [9]. Thromboembolism is the most significant life-threatening complication of NS [3, 4]. It can be found in any major blood vessel and incidence varies from 8% to 36% in literature [10, 11]. Most of venous thromboses occur within the first six months after the NS diagnosis [12].

Kayali et al. [13] found that patients with NS had greater risk for both DVT and PE, with a relative risk of 1.72 and 1.39, respectively. In contrast to them, Suri et al. [14] showed that PE was more common than DVT (25.7 versus 16.6%, respectively); however, this study included only 34 pediatric patients with the NS. Kumar et al. [15] confirmed in their examination that idiopathic MN is a prothrombotic state, particularly in the first six months of the diagnosis, and that PE was the most common thromboembolic event in their patients.

According to Annual Report of Kidney Biopsies in Serbia, incidence of the MN in Serbia (observed period 2010–2014) was 9.4–11.7% [16, 17, 18]. In our cases, PE was the first presenting feature of the NS. No other site of thrombosis was detected in our patients. Only one patient experienced, in addition to respiratory symptomatology, swollen legs on admission to the hospital, and the second one reported the known history of swelling. Two of the patients were very young men, and the third one was a middle-aged man. In one patient, urine analysis wasn't done during the first hospitalization, thus delaying confirming the diagnosis of the NS.

Several specific clinical markers are being used for stratifying patients with the risk of thrombotic events, such as a biopsy-proven diagnosis of MN and albumin level < 28g/l in patients with MN.

Barbour et al. [19] analyzed patients with the idiopathic NS and showed that the diagnosis of MN was associated with an increased risk of thromboembolism compared to FSGS and IgAN. Lionaki et al. [20] showed that an albumin level < 28 g/l was independently associated with a higher thrombotic risk. Kumar et al. [15] found that the 24-hour proteinuria > 10 g/day could be regarded as an independent risk factor for thromboembolic events in patients with idiopathic MN, irrespective of the serum albumin. In all of our cases, all the patients had serum albumin < 20 g/l. Two of them had proteinuria > 10 g/day. All the patients had a biopsy-confirmed diagnosis of MN. Considering that they were all treated with anticoagulation therapy, the kidney biopsy was done with great caution, and we didn't detect any relevant complications. By detailed examination, secondary causes of MN were excluded (diabetes mellitus, infection, autoimmune disease, malignancies, effect of drugs). In addition to anticoagulation therapy by heparin or warfarin, they were treated with immunosuppressive protocol for MN. We didn't detect a repeated thromboembolic event. Full remission of the NS was achieved in one patient, while partial remission occurred with other two patients.

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Емболија плућа као први знак нефротског синдрома

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САЖЕТАК

Увод Емболија плућа (ЕП) представља компликацију дубоке венске тромбозе коју карактерише значајан морбидитет и морталитет. Често се јавља код болесника са већ дијагностикованим нефротским синдромом (НС), посебно када је болест у активној фази, али може се јавити и као први знак болести и тада се лако превиди. Мембранозна нефропатија (МН) најчешћи је тип гломерулонефритиса који се повезује са повишеним ризиком за тромбозу.

Приказ болесника Код три мушкараца ЕП је дијагностикована као први знак НС. Сви болесници су на пријему у болницу имали бол у грудима, сув кашаљ и осећај недостатка ваздуха. Један болесник је имао повишену температуру, а друга два су дали податак о отицању потколеница. Компјутеризованом томографијом грудног коша постављена је дијагноза тромбозе плућне артерије. Додатним анализама откривен је НС, а биопсијом бубрега код сва три болесника утврђена је МН. Болесници су лечени пулсевима метилпреднизолона (1,5 *g* током три дана) и наизменичном месечном применом кортикостероида и циклофосфамида *per os* током шест месеци. После завршене шестомесечне терапије, код два болесника је постигнута инкомплетна ремисија НС, а код трећег болесника одржао се НС са нормалном функцијом бубрега.

Закључак Имајући у виду честу појаву тромбоемболијских компликација код НС, код свих болесника са дубоком венском тромбозом и ЕП треба мислити на НС.

Кључне речи: нефротски синдром; мембранозна нефропатија; емболија плућа

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Dysgerminoma and pregnancy

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SUMMARY

Introduction Dysgerminomas are germ cell ovarian tumors. They affect young females, prevalently during childhood. The problem arises when dysgerminoma is diagnosed in women of reproductive age who have never given birth and require a surgical procedure.

Case outline A 28-year-old patient was admitted to hospital in week 26 of her first pregnancy. The reason for patient hospitalization was the growth of the isthmic myoma diagnosed by her obstetrician-gynecologist in the primary care unit. By examining the medical history of the patient, the following was revealed: A year and a half before pregnancy she was diagnosed with left ovary dysgerminoma. The patient's medical history led us to conclude that uterine myoma was a misdiagnosis and that the actual diagnosis was dysgerminoma of the right ovary. The surgery was performed after the fetal viability had been achieved.

Conclusion Malignant ovarian tumours may occur in young women during pregnancy and increase in size significantly in a short period of time, although their recurrence is not expected in such a short period of time after surgical treatment. This poses a great challenge for obstetricians.

Keywords: dysgerminoma; malignant; ovarian germ cell tumor; pregnancy

INTRODUCTION

Dysgerminomas are germ cell ovarian tumors. About 20% of all ovarian tumors originate from germ cells, whereas only 3% of them are malignant. Dysgerminomas account for about 1% of all germ cell tumors but they are frequently malignant [1]. They affect young females, prevalently during childhood, and the vast majority of them need and respond well only to chemotherapy. The problem arises when dysgerminoma is diagnosed in women of reproductive age who have never given birth and require a surgical procedure. A more serious situation occurs if dysgerminomas develop in young patients during pregnancy, which poses many medical and ethical dilemmas. We present a case of a young patient with a previous history of a dysgerminoma managed by left adnexectomy. The patient conceived two years after the surgery. However, a dysgerminoma of the right ovary was diagnosed in the sixth month of pregnancy. The surgery was performed after the fetal viability had been achieved.

CASE REPORT

A 28-year-old patient was admitted to hospital in week 26 of her first pregnancy. The reason for hospitalization was the growth of the isthmic myoma diagnosed by her obstetrician-gynecologist in the primary care unit. Ultrasonography revealed a viable pregnancy at 24 weeks of gestation and a solid mass of uncertain origin, 100 × 100 mm in size, detected on the right side of the uterus. An ultrasound examination performed four weeks earlier showed a mass 60 × 60 mm in size.

By examining the medical history of the patient, the following was revealed: a year and a half before pregnancy, the patient was diagnosed with a left ovary dysgerminoma. She had undergone left adnexectomy and the partial resection of the right ovary. Dysgerminoma with a negative immunoprofile (alpha-fetoprotein, inhibin, and epithelial membrane antigen) and a positive immunoprofile of the tumor (reticulin and a high level of Ki67) was confirmed by histopathology. The pathology of a part of the right ovary showed only corpus luteum. The surgery was the only management option. The control ultrasound as well as tumor markers six months before pregnancy were normal. The patient's hystory led us to conclude that uterine myoma was a misdiagnosis and that the actual diagnosis was dysgerminoma of the right ovary. The laboratory findings were as follows: D-dimer 5,022 ng/mL, lactate dehydrogenase 12,715 IU/L, aspartate transaminase 95 U/L, alanine transaminase 174 U/L. The magnetic resonance imaging (MRI) finding in week 27/28 of the pregnancy showed a giant tumor in the pelvis, 200 × 200 mm in size, ascites, lymphomegaly, and bilateral hydroureteronephrosis (Figure 1). The pregnancy was terminated at week 31/32 of gestation by Caesarean section and a viable preterm female baby was

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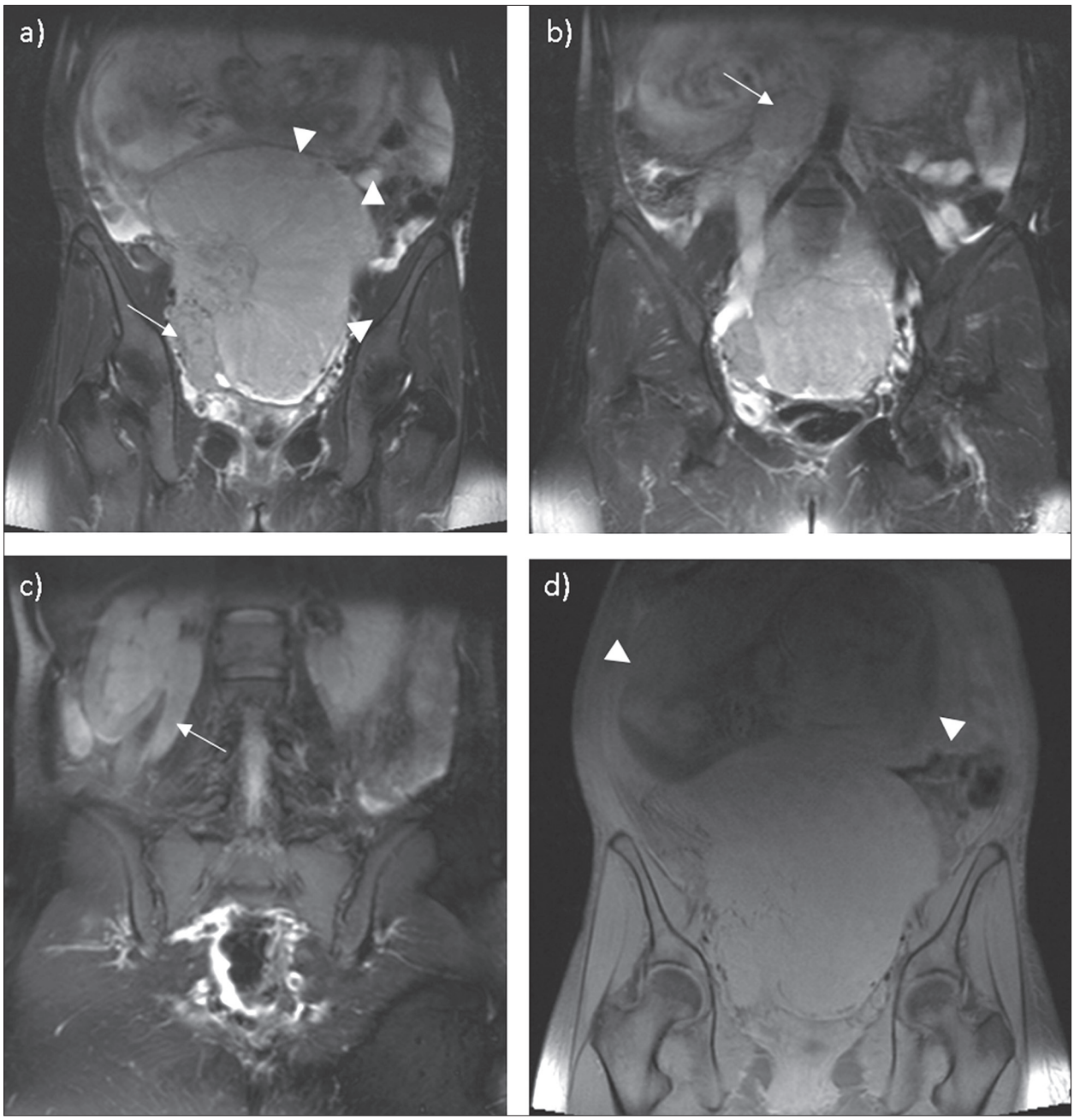

Figure 1. Pelvic MRI; coronal reconstructed T2-weighted images show: a) a large intermediate signal solid mass with prominent fibrovascular septa, occupied pelvic cavity with upward extension into abdominal cavity (arrowhead); in the caudal part of the mass another lobulated mass corresponding to a conglomerate of lymph nodes is shown (arrow); b) para-aortic lymph node mass (arrow); c) mass effect on adjacent structures with consequent hydroureteronephrosis; the diameter of the ureter measured up to 13 mm (arrow); d) coronal fat-saturated T1-weighted image shows the displacing of fetus cranially (arrowhead)

born with birth weight of 1,630 g and the Apgar score of 7/10, 8/10 at the first and fifth minute of life, respectively. Afterwards, the total abdominal hysterectomy with right adnexectomy, omentectomy, para-aortic, iliac, and obturator lymphadenectomy was done. The distal part of the right ureter was also resected and the ureterocystostomy was performed. The patient subsequently underwent chemotherapy with bleomycin, etoposide, and platinum (BEP, four cycles). The patient is currently free of the disease two years post-treatment, with a healthy baby.

DISCUSSION

As mentioned above, dysgerminomas are tumors originating from the primordial ovarian germ cells. Dysgerminoma has a classic correlation with seminoma of the testis, having an identical histological structure. Germ cell tumors account for about 70% of ovarian neoplasm cases during the first decades of life, and are rarely found after this period [2]. Approximately 80% of cases are reported in patients under 30 years of age (mean age: 21 years), which is a finding consistent with our case.

The incidence of adnexal masses associated with pregnancy varies from 1 in 80 to 1 in 8,000 pregnancies, based on different studies. The frequency of ovarian tumors in such adnexal masses is between 1 in 80 and 1 in 2,200 pregnancies [3]. The reported rate of malignant tumors in the total number of ovarian tumors associated with pregnancy was from 1.3% to 7.9%. In fact, in a study by Ueda et al. [4], among 106 cases of ovarian tumors discovered during pregnancy, only five (4.7%) were malignant. The most common diagnosis was dermoid, while dysgerminoma was noted in only one case.

Over a 10-year period, only two cases of dysgerminoma during pregnancy have been diagnosed at our clinic, which represents less than 1% of cases. This fact may lead to a conclusion that the rate of the malignant ovarian tumors associated with pregnancy is very low. This discrepancy of ovarian malignancy incidence between pregnant and non-pregnant women can be explained by the age difference among women [5]. Most patients with a malignant ovarian tumor were over 40 years of age, and these patients were rarely pregnant. Thus, dysgerminoma may be the only malignant ovarian tumor to be kept in mind when detecting adnexal mass during pregnancy.

Taking into account the rarity of this tumor, a misdiagnosis during pregnancy is not uncommon, as it was the case here. A literature review reveals that it is not unusual to misdiagnose dysgerminoma by an ultrasound examination and diagnose uterine fibroids instead. In our case, dysgerminoma was misdiagnosed as a fibroid uterus not only by ultrasound but also by the MRI. MRI has a sensitivity of about 98% for detecting the origin of an ovarian tumour. However, there have been reports of mistaking a malignant ovarian tumor for pedunculated uterine fibroid with areas of cystic degeneration, as in our case [6]. Ovarian tumors generally remain asymptomatic, until they are discovered due to their large size or related complications.

In the current case, dysgerminoma was diagnosed as a result of the enlargement of the pelvic mass thought to be a uterine fibroid. The 14-week obstetric ultrasound showed a corpus luteum cyst in the enlarged right ovary, but all diameters were within the normal range. The 20-week obstetric ultrasound showed a pelvic mass diagnosed as uterine fibroid with a diameter of 60 mm, while the mass was twice as large four weeks later. The specialist literature indicates that certain neoplasms may undergo geometric growth of up to 20% of their original size in a very short period of time (one to two months). The structure, consistency, and contiguity with the uterus all pointed to uterine fibroid, as was demonstrated by the ultrasound examination. For this reason, the above finding was perceived as uterine myomatosis, and if there is a suspicion of a uterine myoma in pregnancy, the diagnostic procedure is not the same as in a case of a malignant ovarian tumor, when testing for tumor markers, pelvic MRI, and other diagnostic methods are performed. Although dysgerminoma is highly suspected when a patient has phenotypic signs of certain syndromes associated to states like Cowden syndrome, ataxia telangiectasia syndrome, Swyer syndrome (pure gonadal dysgenesis associated with the XY 46 karyotype), and Apert syndrome (an autosomal dominant disorder), in this particular case, the suspicion should be based on the previous medical history of the patient [7–10].

Our patient was diagnosed with the left ovary dysgerminoma one and a half years before the pregnancy and she underwent surgery. No precise recommendations for further outpatient follow-up are known based on any randomized controlled trials. However, follow-up should maximize the ability to identify recurrences while minimizing risks. Follow-up care depends on the stage of the disease, which is typically predictive of recurrence risk. Ovarian dysgerminomas tend to recur most often in the first two to three years after treatment. Therefore, most authors suggest follow-up observation and a physical examination every three to four months for the first three years, every six months during the fourth and fifth year, and annual surveillance thereafter. Typically, the authors do not recommend any adjuvant chemotherapy for stage Ia dysgerminomas as was the case with the first surgery. Although 10–15% of stage Ia tumors may recur, essentially all of them are salvaged with chemotherapy [11]. This patient underwent all postoperative checkups. However, since she conceived afterwards, she was probably not provided with an adequate follow-up. It may also be hypothesized that pregnancy induces rapid growth of tumor, although further studies are needed to confirm the hypothesis.

By publicizing this case, we aim to raise awareness of malignant ovarian tumors possibly affecting young females in pregnancy, the volume of which may rapidly increase within a very short period of time although the recurrence of previous malignant disease is not expected in such a short period after surgery. This poses a great challenge for obstetricians.

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Дисгермином и трудноћа

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САЖЕТАК

Увод Дисгерминоми су тумори герминативних ћелија јајника. Најчешће се јављају код млађих особа и то у дечјем добу. Проблем настаје када се постави дијагноза дисгерминома у репродуктивном периоду и када је потребно лечити га хируршки а болесница још увек није остварила своје потомство.

Приказ болесника Примигравида, 26 година стара, примљена је у клинику у 26. недељи гестације због сумње на експанзиван раст истмичног миома откривеног ултразвуком од гинеколога у примарној здравственој заштити. Код болеснице је годину и по дана раније урађена левострана аднексектомија због оваријалног дисгерминома. Дијагноза миома материце је била погрешна и радило о дисгерминому десног јајника. Хируршко лечење дисгерминома обављено је након постизања феталне одрживости.

Закључак Малигни тумори јајника могу се јавити код младих жена током трудноће убрзо после хируршког лечења и значајно увећати у кратком периоду.

Кључне речи: дисгермином; тумори герминативних ћелија оваријума; трудноћа

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

First Macedonian child with tyrosinemia type 1 successfully treated with nitisinone and report of a novel mutation in the *FAH* gene

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SUMMARY

Introduction Hereditary tyrosinemia type 1 (HT1) is a severe hereditary metabolic disorder of tyrosine metabolism due to fumarylacetoacetate hydrolase (FAH) deficiency and accumulation of toxic products in tissues. More than 80 mutations in the *FAH* gene are presently reported on the Human Genome Mutation Database. To date, no molecular genetic defects of HT1 in Macedonia have been described.

Case outline A female infant two and a half months old presented with failure to thrive, anemia, edemas, and severe coagulation disturbances. The diagnosis of HT1 was based on high levels of serum α-fetoprotein, increased serum tyrosine, and positive succinylacetone in urine. Nitisinone treatment with tyrosine-restriction diet was immediately introduced. The patient, currently aged five years, has normal growth, psychomotor development, and no focal lesions on abdominal MRI. A screening of the *FAH* gene revealed two heterozygous mutations – c.[1A>G];[784T>A]. The mutation c.784T>A is a novel one (p.Trp262Arg), and was predicted to be the cause of the disease by an in silico analysis.

Conclusion To date, this case is the first and only child with HT1 successfully treated with nitisinone in our country. Also, this is the first report of an HT1 patient caused by the c.784T>A mutation.

Keywords: hereditary; tyrosinemia type 1; nitisinone; mutation

INTRODUCTION

Hereditary tyrosinemia type 1 (HT1) is a rare but severe hereditary metabolic disorder of tyrosine metabolism. The worldwide prevalence of HT1 is 1 in 100,000 newborns, but is more common in some regions, notably in Quebec, Canada [1, 2]. It results from fumarylacetoacetate hydrolase enzyme (FAH) deficiency, encoded by the *FAH* gene and an accumulation of toxic products in many tissues, particularly in the liver, kidneys, and the brain. Molecular genetic testing by targeted analysis for the common *FAH* pathogenic variants and sequence analysis of the entire coding region can detect pathogenic variants in more than 95% of affected individuals [3]. More than 80 mutations in the *FAH* gene are presently reported on the Human Genome Mutation Database (HGMD® Professional 2016.2, http://www.hgmd.cf.ac.uk). Patients from different ethnic groups with HT1 have different common mutations in the *FAH* gene [4].

HT1 patients typically present in infancy with acute liver failure, cirrhosis, neurologic crises, and renal tubular dysfunction with hypophosphatemic rickets. If untreated, death typically occurs before two years of age, although chronic forms allowing longer survival have been reported [5].

Biochemical findings include elevated succinylacetone in the blood and urine; elevated serum concentrations of tyrosine, methionine and phenylalanine, and elevated tyrosine metabolites in urine. The evolution of the disease has improved considerably since the introduction of nitisinone (NTBC) treatment depending on the age of the patient at diagnosis and at the start of the treatment [6].

Herein we report the first HT1 child from Macedonia successfully treated with nitisinone therapy. Due to the low incidence, as well as difficulties in diagnostics of rare diseases in our country, all previous cases were diagnosed with an advanced liver disease and had unfavorable outcome, either lethal or required urgent liver transplantation. Also, this is the first patient in whom the diagnosis of tyrosinemia was confirmed by a genetic analysis.

CASE REPORT

A female infant two and a half months old, the second child of healthy nonconsanguineous parents, presented with failure to thrive, anemia, and edemas. The infant was born after 39 weeks of gestation, with the birth weight of 3,100 g and had normal postnatal course. No genetic diseases had been reported in the family. The child was exclusively breastfed, but experienced difficulties in gaining weight. Several days prior the admission, swelling of the abdomen, feet, and wrists was noticed. Physi-

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cal examination revealed a pale, unhealthy-looking infant, with abdominal distension and peripheral edemas. The weight was on the fifth percentile for the age. The liver was enlarged 4 cm below the costal margin, non-tender, and had firm consistency. The spleen was palpable 2 cm below the left costal margin.

A laboratory analysis showed anemia, hemoglobin level of 80 g/l, and red blood cell count of 2.83×10^{12}/l.

There was significant hypoproteinemia and hypoalbuminemia, with values of 32 g/l and 18 g/l, respectively. The bilirubin level was slightly elevated, total bilirubin was 39 μmol/l, and conjugated 12 μmol/l. Serum transaminases were within normal limits (65 U/l for aspartate transaminase, 59 U/l for alanine transaminase), and alkaline phosphatase was 970 U/L (normal 120–450 U/l). Coagulation screening showed prolonged prothrombin time of 46 seconds, and partial thromboplastin time was 33 seconds. Alpha-fetoprotein was > 10,000 IU/ml (normal < 87 IU/ml). Blood gases and electrolytes were normal, as well as the blood urea nitrogen and serum creatinine values. Serum amino acid analysis showed elevated tyrosine of 396 mmol/L (normal value < 200 mmol/L). Urine organic acid analysis revealed elevated succinylacetone. Ultrasonography of the abdomen showed hepatosplenomegaly, ascites, and the hypoechogenic structure of the renal medulla.

According to the findings, the infant was diagnosed with tyrosinemia type 1 and nitisinone therapy (1 mg/kg/day) was initiated combined with tyrosine-restricted formula (Tyrex®, Abbott Nutrition, Lake Forest, IL, USA). The patient received several plasma and albumin transfusions and vitamin K supplementation.

Following the NTBC treatment, there was a significant improvement of the liver function. Coagulation improved two days after treatment initiation.

The child was followed-up regularly without further hospitalizations. Parameters remained normal during the follow-up. The serum tyrosine levels were frequently measured and maintained 200–400 mmol/L, as recommended. Succinylacetone was negative two weeks after starting the treatment and was determined yearly afterwards. Alpha-fetoprotein was 8,298 IU/ml at the age of six months, and 312 IU/ml at 12 months. NTBC concentration at the age of three years was 36.2 μmol/l (target values 40–60 μmol/l); thus, the nitisinone dose was increased. Annual follow-up liver MRI has shown no focal lesions to date. The ophthalmological examination was scheduled every six months and has always been normal. The child is now five years old and has normal growth and psychomotor development.

Molecular analysis was performed. Genomic DNA was isolated from the patient's whole blood leucocytes and from her parents' afterwards. Fourteen coding exons of the *FAH* gene (ENSG00000103876) and their flanking intronic regions were amplified in 13 fragments by polymerase chain reaction. The products of the polymerase chain reaction were sequenced in both directions on ABI 3500xL genetic analyzer (Applied Biosystem, Foster City, CA, USA). In the patient's DNA, genetic testing showed two heterozygous mutations: c.1A>G in exon 2, inherited from the child's father, and c.784T>A in exon 10, inherited from the child's mother. The c.784T>**A** mutation has never been reported previously in HT1 patients, is not present in the 1,000 genome database (http://www.1000genomes.org/home), and was predicted to be disease causing (in silico analysis, MutationTaster, Charité – Berlin University of Medicine).

DISCUSSION

Hepatorenal tyrosinemia or tyrosinemia type 1 is a rare autosomal-recessive disorder of tyrosine metabolism with an incidence of 1:125,000 in central Europe [7]. Because of the low global occurrence of HT1, a considerable number of cases may go unrecognized especially in absence of an established newborn screening.

Our case presents the first report and the only HT1 patient from Macedonia diagnosed in early infancy and successfully treated with nitisinone. Due to the limitations of diagnostic tests in our country, many HT1 patients had been unrecognized.

A recent study from Macedonia included four patients with HT1 diagnosed over a three-year period; two of the patients had an unfavorable outcome with death occurring at the mean age of 126 days, and one patient was transferred for a liver transplantation. The authors emphasize the initial promising results of nitisinone treatment started at that time [8].

HT1 children presenting before the age of six months typically have acute liver failure with initial loss of synthetic function for clotting factors. Our child presented with liver dysfunction (edemas, jaundice, bleeding tendency), an important feature for diagnosing hereditary tyrosinemia type 1. The prothrombin time was markedly prolonged and did not correct after vitamin K and plasma supplementation. Paradoxically, serum transaminase levels were normal and serum bilirubin concentration was only slightly elevated, in contrast to most forms of severe liver disease in which there is marked elevation of transaminases and serum bilirubin concentration. This discrepancy in the liver function is described in the literature; resistance of affected liver cells to cell death may be a possible explanation [9].

Mayorandan et al. [7] in a recent study analyzed 168 patients with HT1 from 21 centers with the average age of the diagnosis being 12.9 months; most of them were symptomatic at diagnosis, with a combination of liver and renal dysfunction. In their study, the acute liver failure was significantly higher in the group of patients between two and six months of age. Our patient had preserved renal function. High serum tyrosine in combination with increased α-fetoprotein level and severe coagulopathy raised the suspicion of tyrosinemia in our patient. Detection of succinylacetone in urine is the most reliable biochemical diagnostic method for HT1. However, there is a reported unusual case of a four-month-old infant with HT1 presenting with severe liver disease and negative succinylacetone in urine. Fumarylacetoacetase protein and activity was decreased, but not absent [10].

Nitisinone, or 2-(2-nitro-4-trifluoromethylbenzyol)-1,3-cyclohexanedione (NTBC), a potent inhibitor of 4-hydroxyphenylpyruvate dioxygenase, a step in the tyrosine degradation pathway, has revolutionized the management of tyrosinemia type 1 [6, 11].

Nitisinone administration usually results in a remarkable clinical improvement within a few days in more than 90% of patients; thus, the treatment should commence as soon as the diagnosis is confirmed, or even suspected because of liver disease [12].

If coagulation improves within one week, recovery can be assumed; otherwise, an increase of the nitisinone dose or liver transplantation should be considered [12]. Our patient showed rapid improvement. Delayed NTBC treatment is associated with an increased risk of liver carcinoma and a requirement of the liver transplantation. Mayorandan et al. [7] in their study point out the necessity of newborn screening programs to allow an early diagnosis and access to adequate treatment, as they report a 2–12-fold higher risk for developing hepatocellular carcinoma depending on the age at the time the treatment was started compared to patients treated as neonates. Also, psychomotor impairments, attention-deficit hyperactivity syndrome and behavioral disorders, neurological disturbances or learning difficulties were present in very few patients when NTBC treatment was initiated in the newborn period [7].

Our patient was monitored regularly in one-month intervals during the first year of life, according to the recommendations, and every three months after achieving good control and stability, as well as the parents' understanding and compliance. The metabolic control was assessed by determining succinylacetone concentration in dried blood or urine and the level was always below the detection limit.

Nitisinone tolerance of in the child was good, without any side effects. Mayorandan et al. [7] reported side effects of NTBC treatment in very few patients: transient thrombocytopenia, leukopenia, and transient ocular symptoms. Patients with side effects seemed to have higher range of NTBC values compared to those with no side effects; however, because of the small sample size, statistical analysis was not possible.

Unfortunately, we were not able to determine the nitisinone level more frequently. Monitoring of nitisinone plasma levels permits individual dosing, minimizing treatment costs and side effects without hampering metabolic control. However, the target level of nitisinone is not well defined and varies among centers [13, 14].

Simoncelli et al. [15] provided a cost–consequence analysis for all children with HT1 treated in Quebec, Canada, between 1984 and 2009, concluding that nitisinone treatment significantly improved the outcomes of patients with tyrosinemia type I, while decreasing the utilization of health care resources by significant reductions in the number and duration of hospital admissions, admissions to a pediatric intensive care unit, and the number of liver transplants.

Although molecular testing is not essential for diagnosing HT1, it has greatly improved the diagnostic power for the disease and is useful for prenatal diagnosis and genetic counselling. Despite the fact that the spectrum of the FAH gene mutation has been expanded, current knowledge is not adequate for establishing the disease's genotype–phenotype correlation.

Angileri et al. [4] in a recent study described the 95 mutations reported so far in HT1 with special emphasis on their geographical and ethnic distributions, concluding that such information should enable a preferential screening for mutations most predominant in a certain region or ethnic group.

Our patient represents the first case from Macedonia with genetically confirmed HT1. She was a compound heterozygote for two mutations – c.[1A>G];[784T>A]. The c.1A>G mutation is a missense previously known mutation in codon 1 which changes the initial Met into Val (p.Met>Val) and negatively affects the initiation of FAH protein translation [16]. This mutation in a homozygous state was also reported in patients with HT1 from Emirates, Greece, and Saudi Arabia [17–20].

Georgouli et al. [18] reported a five-month-old infant with HT1 presenting as Escherichia coli sepsis and severe coagulopathy due to liver dysfunction. The patient was homozygous for c.1A>G. Despite the early diagnosis and NTBC treatment, the patient died from multi-organ failure.

Imtiaz et al. [19] reported five homozygous carriers of the c.1A>G mutation in a cohort of 43 HT1 patients originating from the Middle East.

The other c.784T>A mutation detected in our patient is a novel mutation, which changes highly conserved Trp^{262} into Arg (p.Trp262Arg). By an in silico analysis (MutationTaster; PolyPhen-2 – public domain; SIFT – the University of British Columbia, Vancouver, BC, Canada), this mutation was predicted to be disease causing.

In conclusion, our patient presents the first experience with nitisinone treatment in our country. Despite the excellent results, the child needs further careful monitoring because of possible long-term complications, particularly hepatocellular carcinoma.

Also, reporting of underlying mutations in HT1 patients who belong to different ethnic groups is helpful not just for genetic counseling but also for further research.

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Прво дете из Македоније са тиросинемијом тип 1 успешно лечено нитисиноном и приказ нове мутације у *FAH* гену

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САЖЕТАК

Увод Хередитарна тирозинемија тип 1 (ХТ1) озбиљан је наследни поремећај метаболизма тирозина који настаје као последица недостатка ензима фумарилацетоацет-хидролазе и нагомилавања токсичних продукта у разним ткивима. До сада је описано више од 80 мутација у *FAH* гену, а ниједан случај мутација са ХТ1 у Македонији.

Приказ болесника Женско одојче старо 2,5 месеца није напредовало у тежини, имало је анемију, отоке и тешке поремећаје коагулације. Дијагноза ХТ1 је заснована на повишеним вредностима α-фетопротеина и тирозина у серуму, а позитивним сукцинилацетоном у урину. После постављања дијагнозе уведено је лечење са нитизиноном и ограничење уноса тирозина у исхрани. После пет година дете има нормалан раст и психомоторни развој, као и уредан налаз МР абдомена. Молекуларном анализом *FAH* гена откривене су две хетерозиготне мутације – c.[1A>G];[784T>A]. Мутација c.784T>A је нова (p.Trp262Arg) и сматра се одговорном за појаву болести (*in silico analysis*).

Закључак Ово је први и једини случај детета са ХТ1 који је до сада у нашој земљи успешно третиран нитизиноном. Такође, ово је први извештај за c.784T>A мутацију код ХТ1 болесника.

Кључне речи: наследна; тирозинемија тип 1; нитисинон; мутација

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REWIEV ARTICLE / ПРЕГЛЕД ЛИТЕРАТУРЕ

Population ageing alongside health care spending growth

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SUMMARY
The Silver Tsunami or population ageing has become a globally widespread phenomenon. The purpose of this review is to observe its dynamics and consequences from a local Balkan perspective. The main drivers of this unique demographic evolution are extended longevity, improved early childhood survival, absorption of women into the labor markets, and consequences of sexual revolution leading to falling female fertility. This process lasting well over a century is taking its toll on contemporary societies. Major side effects are shrinking young labor force and growing pool of elderly and retired citizens in many countries. This equation tends to worsen further in the future threatening long-term financial sustainability of public social and health insurance funds. Notable health expenditure growth, accelerating worldwide since the 1960s, is to a large degree attributable to ageing itself. Growing share of senior citizens increases demand for medical services and costs of health care provision. Home-based care provided by the family caregivers presents another important reality putting a huge burden on modern communities. Serbs are no exception in this landscape. Historical demographic evolution of this nation gives a clear evidence of advanced and accelerated ageing, which is well documented in post-World War II era. This synthesis of rich published evidence shows clear upward parallel trend between the pace of population aging and the growth of health expenditure. National authorities shall be forced to consider reform of the current health care financing pattern inherited from the demographic growth era. This might be the only way to smooth out the impact of population ageing on the financial sustainability of the health system and long-term medical care in Serbia.
Keywords: population; ageing; health expenditure; trend; Serbia; aged

INTRODUCTION

Population ageing or the so-called "Silver Tsunami" presents a unique phenomenon in written demographic history of the mankind over the past eight millennia [1]. Traditional societies, regardless of the dominant ethno-religious pattern or the way of life, were young societies [2]. These were dominated by at least 15% of children younger than five years and with the portion of elderly aged over 65 significantly less than 5%. In contemporary momentum, as we approach year 2020, the growing portion of senior citizens and the decreasing portion of children are meeting a melting point, where these two trend lines are about to cross each other for the first time ever [3]. How did it all happen?

The social circumstances changed essentially since the dawn of European Industrial Revolution [4]. Although some of these nations, such as the French one, entered the aging process almost two centuries ago, this hadn't become a noticeable social and public-health issue almost until the 1980s [5]. The fall of female fertility was caused by the sexual revolution, female education, and the absorption of women into the labor markets worldwide [6, 7, 8]. These changes created effective financial incentives for women to give birth to fewer children. The second side of the equation were successes of modern medicine. Early childhood survival became far more successful and human longevity gains were bold [9]. Combined effects of extended life expectancy of an average citizen at birth coupled with lower fertility effectively created the conditions for the increase of median age within the society [10].

Once upon a time, poor agricultural nations on European soil had a median population age far below the age of 20. This landscape resembles very few remaining contemporary countries, such as Afghanistan or the states of Sudanese Africa. These countries are marked by the United Nations Population and Social Affairs Division as eighteen „demographic outliers." Unlike these, vast majority of nations around the world, led by the earliest historical shift across industrialized Northern Hemisphere, belong to the dominant ageing pattern [11]. Nowadays, their median population age is either approaching 40 years or even slightly crossing this threshold.

RESHAPING THE POPULATION PYRAMID OF MODERN-DAY NATIONS

Important part of the aforementioned far-reaching changes is not only moving upward the median population age bur rather reshap-

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ing the entire population pyramid. Once dominated by the youth and children, today we face gradual but unidirectional spreading of community share of senior citizens. Although it affects both sexes, due to natural higher longevity of women in most cultures, there is an effect called "feminization" of the senior population. This refers to the domination of women among the elderly. Besides this, it is possible to observe not only the growing percentage of persons aged 60/65 or more, but also the growing portion of persons in deep senium – older than 80. Actually, while observing the landscape of changes, we see crystal clear evidence supporting the accelerated population ageing across the globe [12]. This means that the percentage point share of the elderly has grown far more quickly during the past three decades as compared to the previous three decades.

Some regions of the world entered this demographic transition earlier than others and today find themselves in a more advanced stage of ageing. Europe and Asia are typical examples, each one in its own peculiar conditions. So far, Europe remains the oldest continent, but as we approach 2050, regardless of the recent one-child policy shifts, it is obvious that China will become the fastest ageing large nation [13, 14]. A recent research on top ranked emerging markets led by the BRICS and the Next Eleven nations gives a hint on how this phenomenon is spreading from the traditional high-income countries towards the so-called newly-industrialized economies [15]. In geographic terms, it usually means migration of decreasing fertility pattern from the rich North towards the rising nations of the global South. Notable exception in this group of countries is the Russian Federation with its early historical roots of ageing in the late imperial Romanov era and its exceptional industrial legacy of the former Soviet Union [16]. Since the end of the Cold War in 1989 and accelerated globalization in many world regions such as the Eastern Europe, there is direct evidence of accelerated population ageing in these new social circumstances [17].

THE IMPACT OF AGEING ON MEDICAL SPENDING

How does population ageing affect the demand for medical services and work load for national health systems worldwide? There are several sides to this equation [18].

Firstly, there is the fact of simple labor market shrinking and the serious issue of long-term financial sustainability of national health systems [19]. Since the late 19th century Bismarck-style initiatives, European tradition has introduced modern risk-sharing arrangements and the very concept of health insurance [20]. The target groups during these early decades were industrial workers and their families. The concept gradually became applied to most layers of societies throughout the long course of history [21]. Surprisingly for many scholars, the first nation to deliver universal health coverage for the entire nation inclusive of the poor was the Soviet Union as early as back in early 1930s with its renowned Semashko system [22]. The standard way of funding massive and hierarchical modern-day health systems was imposing broad revenue base for the health insurance funds burdened on the shoulders of employees and employers alike [23]. Be it this or the general taxation model, a variety of different patterns of health care funding in most contemporary societies rely on a massive body of employed citizens. These people at their best working life age are effectively supporting the needs of elderly and retired citizens [24]. Most of these financial strategies were historically derived many decades ago, from the so-called population growth mode. They have one important assumption: that lower younger floors of the demographic building supporting the heavy upper floors consisting of senior citizens will always prevail in numbers and strength. Unfortunately, our time witnesses putting this axiom to the limit. Lower floors are becoming ever thinner and weaker and upper floors are becoming more massive. The work force is shrinking while the pool of retired citizens receiving and consuming all kinds of social benefits is expanding [25].

The second fact refers to testified medical needs of the elderly. Searching through the scholarly literature we find an abundance of evidence that senior patients tend to suffer from expensive chronic non-communicable diseases. They do more frequent laboratory tests and imaging examinations, have more outpatient physician visits, frequent and lengthier hospital admissions, and consume more prescription and over the counter medicines [26]. Furthermore, their need for occasional medical implants, physiotherapy treatments, and psychotherapy is far more exposed compared to younger counterparts. The crown on the medical spending attributable to the age group of 60 and above is probably the last year of life. It is well documented that the last year palliative and/or terminal care, particularly the one referring to cancer, usually costs as much as that individual's entire lifetime medical consumption [27].

The third contributing cost driver lies outside the entire hospital sector and is frequently heavily underestimated. It refers to the home-based care for the elderly, exhausted, and sick persons [28]. Only a minor part of this care is provided for by professional facilities and nursing staffs. To a large extent, this burden relies on family caregivers. The social costs of such an engagement are hidden, and visible ones present only the tip of the iceberg [29]. Examples from Israel and Japan witness the massive pool of people in the community working hard at full-time jobs, with all further ramifications for their families and their workplace [30, 31]. Among many related ongoing developments, the exploding pandemic of dementia worldwide will probably make this burden far heavier in the foreseeable future, with Asia and Europe in the lead.

An essential part of the global transformation of health expenditures among the regions with entirely different economic models is the fact that low- and middle-income countries are overtaking an increasingly growing share of the World's total health spending [32]. This fact becomes most obvious when we compare the leading among the traditional free-market high-income economies such as G7 and the leading emerging markets such as the BRICS [33]. In a three-decade time horizon it is clear that partici-

pation of the latter led by China is getting bigger at the expense of the former group of countries led by the US [34].

AGEING AMONG SERBS

Serbs as one of the traditional nations of Europe since antiquity began to age almost a century ago [35]. Prior to World War I, there had been the 1870–1910 time window testifying of exceptionally high birth rates and top-ranked fertility in most of Europe. Since those days, fertility has been falling faster or slower, depending on historical circumstances and overall social welfare [36]. Most authors recognize constant negative migration rates as a contributing factor to the ageing process [37]. Nevertheless, decreasing fertility rates and extended longevity remain as the major drivers [38]. Life expectancy at birth was growing in Serbia as in most other similar Eastern European countries significantly in the decades immediately following World War II. These successes were partly attributable to the established methods of preventive and clinical medicine, but probably far more to the improved welfare and living standards. Upward trend characteristic of the socialist era of peaceful prosperity in the former Yugoslavia ended with civil wars of 1990s [39]. Consecutively, in these years, there was a peak of total population size in Serbia, which continued to shrink further in the upcoming years marked with poverty. Partial economic recovery since the early 2000s shaken by the global recession reaching Serbia only in 2010/2011 had some visible impact on fertility rates. In the meantime, government population policies proposed some measures of support to the childbearing families. Heavy emphasis was on the third-child policy, whose implementation was poor and heavily dependent on frequent government mainstream priority changes in the country. Regardless of some temporary successes, the downward trend remains persistent in the long run in all major demographic trends [40].

Official UN Population and Social Affairs registries provide data on ageing indicators for most countries for the 1950–2015 period and a medium scenario forecasts up to year 2100. It is tempting to observe some of these data in comparison with the data on health spending in Serbia. Unlike demography, it was only since establishing the National Health Accounts System in 1995 that financial flows within the national health systems became measurable in an internationally comparable manner. Therefore, we can consider some of these data in Table 1 presented below. Both sets of population and economics variables refer to the Republic of Serbia within the 1995–2015 time window or the closest years available. Among the most remarkable changes is the increase of the median age, from 34.1 to 20.6 years in only two decades (Table 1). At the same time, total per capita health spending in the purchasing power parity terms grew from $246 in 1995 to $1,312 in 2015 (Table 1). The latter changes are far more dynamic and dependent on the affordability issues and the overall welfare in the country. Nevertheless, there remains one important indicator of growing priority of health spending for the national policy makers. Share of gross domestic product available devoted to health care jumped from approximately seven to 10 percentage points. Unlike in some mature economies as in the case of Japan, Serbian official statistics have no insight into the part of medical consumption attributable to the elderly

Table 1. Demographic indicators of ageing in Serbia and health care expenditure indicators 1995–2015 according to the United Nations and the World Health Organisation estimates

Demographic indicators of ageing in Serbia*	1995 (or the closest year available)	2015 (or the closest year available)	Difference
Population aged less than 15 years (%)	17.05	14.36	-5.8
Population aged over 60 years (%)	17	24.4	+7.4
Median age (years)	34.1	40.6	+6.5
Total fertility rate (per woman)	1.92 (1995–2000)	1.56 (2010–2015)	-0.36
Number of live births (thousands)	650.41 (1995–2000)	458.76 (2010–2015)	-191.65
Number of deaths (thousands)	524.34 (1995–2000)	566.83 (2010–2015)	+42.49
Ratio between the number of live births and deaths	1.24 (1995–2000)	0.81 (2010–2015)	-0.43
Life expectancy at birth, male/female (years)	69/75 (1995–2000)	72/77.5 (2010–2015)	+3.0 / + 2.5
Old-age dependency ratio (ratio of population 65+ per 100 population 15–64)	17.2	25.6	+8.4
Potential support (ratio of population 15–64 per population 65+)	5.8	3.9	-1.91
Life expectancy at birth (both sexes combined) (years)	71.91 (1995–2000)	74.65 (2010–2015)	+2.74
Life expectancy at age 60 (both sexes combined) (years)	17.71 (1995–2000)	19.12 (2010–2015)	+1.41
Health care expenditure indicators**			
Total health expenditure % gross domestic product	7%	10% (2014)	+3%
Total expenditure on health per capita at PPP (NCU per US$)	$246	$1,312 (2014)	+$1,266
General government expenditure on health per capita PPP (NCU per US$)	$162	$812 (2014)	+$650
Private expenditure on health in current PPP, per capita (NCU per US$)	$85	$500 (2014)	+$315
Out of pocket expenditure in current PPP per capita (NCU per US$)	$73	$480 (2014)	+$407
Total expenditure on health in million current PPP US$	$2,441	$9,358 (2014)	+$6,917
Total expenditure on health in million current US$	$814	$4,514 (2014)	+$3,700

PPP – purchasing power parity; NCU – national currency units;
Sources: * United Nations Department of Population Economic and Social Affairs Division: The World Bank: http://data.worldbank.org/indicator/SP.POP.TOTL?display=default&locations=RS
**World Health Organization – Global Health Expenditure Database: http://apps.who.int/nha/database/Select/Indicators/en

[41]. This is by far the most comparable indicator of medical spending in international terms. Thus, it is clear that medical and long-term home care is gradually becoming an area of great national interest. Further on, as we might see from the published literature, similar patterns of population ageing are becoming familiar to all of the Southeast European nations inclusive of some of the traditionally younger ethnic communities [42]. The myriad of these diverse health care legacies are now forced to adapt to the new circumstances. Rapid and extensive development of legislative framework devoted to genders, retirement, and elderly health insurance issues in Serbia are good examples of what is happening in the entire region [43, 44].

HEALTH EXPENDITURE IN THE COUNTRY OVER THE PAST TWENTY YEARS

Health spending patterns in Serbia since the early 1990s were marked by notable health reforms [45]. Impetus for such efforts came externally by supranational authorities such as the World Health Organisation, European Commission, World Bank, and UN agencies, and was adopted by a series of local governments [46]. Changes from socialist health care establishments of the former Yugoslavia towards the pre-World War II free market traditions began in the 1990s. We should bear in mind that most former Yugoslav republics, with the exception of Slovenia, entered this process with a one-decade delay due to civil wars of Yugoslavia [47]. Yugoslavia health care financing model was not a typical Semashko system, unlike in most of Central and Eastern European societies (CEE), but rather mixed Bismarck with a municipally-funded health care [48]. Regardless of many cycles of capacity building in health care and institutional changes, in most of CEE and Serbia alike, central state-owned health insurance funds survived to date. These funds remain the pillars of public health care funding in a setting with rather underdeveloped private health sector. Although governmental financial responsibilities increased during the past two decades in Serbia, these were effectively overwhelmed by the out-of-pocket spending [49]. The growth of private expenditure on health is probably the single most concerning fact in the Balkans and even the top emerging BRICS markets as well [50]. Such a trend depicts actually the inability of local authorities to increase investments in health to compensate the vulnerability of at least the poorest citizens against the catastrophic health spending. Impoverishment due to illness remains common throughout the Southeast European region. Parts of these medical care costs incurred to the patient's family are legal mandatory payments, while others represent informal payments and corruption of sorts. Regardless of the nature of excessive medical spending by the ordinary citizens, most is attributable to the leading non-communicable prosperity diseases. Cancer, diabetes, depression, fertility assistance, hepatitis, AIDS – these are some of the top morbidity causes with a huge budget impact and work load for the Serbian hospital and outpatient sector [51, 52].

An indirect indicator of the transforming cost matrix within the national health care system is actually the local pharmaceutical market. Although it doubled in size in terms of the value-based turnover of prescription medicines, some Anatomical Therapeutic Chemical code groups have gained momentum well over 2,000% in only a decade while others have virtually disappeared. A prominent budget impact belongs to expensive monoclonal antibodies and targeted biologicals used in oncology and autoimmune diseases [53]. This simple fact points out to the slowly-reshaping morbidity structure of the local population and a changed demand for certain pharmaceuticals [54]. The balance between brand name drugs and generic medicines plays a great role, which is most obvious in the case of large markets [55]. Here we may see that reimbursement rules for drugs prescribed by the attending physician were evolving towards a more strict control, cost containment, and greater participation by the patients in the costs of treatment. Unlike in the socialist era, the inability of the public funding to cover the needs for medicines outside essential ones led to the vulnerability of poor citizens and households [56, 57]. Many studies indicate that the level of poverty among the retired elderly citizens in Serbia is by far the highest compared to the national average [58]. These gaps and insufficiencies are frequently covered by their employed children and out of revenues other than pension.

CONCLUSION

Population ageing is a phenomenon so widespread and far-reaching that it will mark the spirit of the 21st century and all domains of life of diverse communities across the globe. Besides promising gains in longevity, it leads to substantial growth of medical care needs in all societies. Contemporary health systems have been historically built on the demographic growth model. Such systems will not be capable to cope with the sky-rocketing costs of medical and long-term care associated with the ever-larger share of the elderly. Serbia is no exception to these rules. Adopting national policies of support to the healthy ageing might release some of the financial pressure. Other strategies could involve personalized medical care and higher involvement of cost-effectiveness criteria in priority allocation of medical resources [59]. Without a bottom-up rethinking of national health coverage and social support traditions, burden of ageing itself will remain virtually unbearable even for the richest of nations [60].

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Старење становништва и раст издвајања за здравствену заштиту

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САЖЕТАК

Сребрни цунами или старење становништва је постало раширена светска појава. Циљ овог рада је да пружи увид у динамику процеса и његове последице у балканским условима. Неки од водећих чинилаца ове јединствене демографске еволуције су већа дуговечност, побољшано преживљавање у раном детињству, веће запошљавање жена и последице сексуалне револуције у смислу опадајуће женске плодности. Овај процес је отпочео пре више од једног века и сада је видљив његов траг у већини савремених друштава. Међу водећим нежељеним последицама су опадање младе радно способне популације и растући удео старијих и пензионисаних особа у многим земљама. Ова једначина тежи да дубље поремети финансијску одрживост јавних здравствених и социјалних фондова. Раст издвајања за здравствену заштиту, који се убрзава на светском нивоу од шездесетих година, у великој мери се може приписати самом старењу становништва. Растући проценат грађана у сенијуму увећава тражњу за медицинским услугама и трошкове здравствене заштите. Кућна нега, коју пружају превасходно чланови породице, представља другу важну реалност са огромним теретом по данашње заједнице. Српски народ није изузетак у овим питањима. Историјска демографска еволуција овог народа даје јасне доказе одмаклог и убрзаног старења становништва, посебно у периоду после Другог светског рата. Прилог пред нама синтезом богатог корпуса објављених доказа показује јасан паралелни тренд између брзине процеса старења популације и пораста потрошње за здравствену заштиту. Национална политика Републике Србије ће бити приморана да преиспита садашњи систем финансирања здравства, историјски сазидан на моделу демографског раста. Тиме ће се моћи ублажити утицај старења становништва на одрживост пружања здравствене заштите и дугорочне неге у овој земљи.

Кључне речи: старење становништва; потрошња за здравствену заштиту; дугорочни тренд; Србија; старије особе

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CURRENT TOPIC / АКТУЕЛНА ТЕМА

Ethics and marketing in esthetic dentistry

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SUMMARY
Contemporary dentistry is, first of all, characterized by diverse accelerated development, owing to improvements of information and other technologies, as well as the development of dental materials (shape-memory biomaterials, nanomaterials, biomaterials for application in tissue engineering, etc.). Expert doctrinaire attitudes move from the direction of operative interventions, whereby disease and acute symptoms are primarily treated, towards the strengthening of oral health by minimally invasive procedures. A particular place in patients' total rehabilitation belongs to numerous esthetic procedures which, to a large extent, make up a *wants-based* service, led by the patients' needs and affinities.
This paper deals with differences between cosmetic and esthetic dentistry. The complexity of esthetic dentistry, which favors therapy with the change of function parameters in care for the patient, is emphasized. On the other hand, more attention is paid to the need to know and respect ethical and marketing principles that follow any activity of dentists, starting from the first contact with the patient, the selection of certified materials, to the implementation of the appropriate treatment plan.
Well-directed communication and comprehensive awareness of the patient, the use of the visual analog scale, consideration of realistic resources in therapy, and the acceptance of de Bono model of adopted parallel thinking are determinants which help dentists define a problem adequately, find quality solutions, open alternative solutions, and reduce the potential risks in patients' therapy.
Keywords: esthetic dentistry; cosmetic dentistry; ethics; marketing

ETHICS AND MARKETING IN ESTHETIC DENTISTRY

Dental practice has been dramatically changed in the last 30 years. Many analysts call these changes a real revolution, but a revolution always denotes current and essential changes. However, tumultuous development of dentistry could rather be characterized as an evolutionary, gradual progress, supported by different factors. It should be emphasized that the present practice, implemented by an increasing number of practitioners, engendered from situations in which solely acute symptoms are treated, as well as operative interventions, whereby the disease is rehabilitated (*needs-based service*).

Nowadays, a significant emphasis is put on preserving and strengthening oral health by minimally invasive procedures, whereby patients are, to a large extent, treated with respect to their wishes and expectations (*customer-driven*, *wants-based service*). Patients have open access to information on overall health, and increased awareness of their problems results in numerous questions and great expectations. Attractiveness and youthful appearance represent a part of the vitality of an individual and a symbol of personal success. In addition, the development of different modern restorative systems makes everyday application more complex, creating new possibilities in esthetic dentistry. The point that should be considered with much care is the fact that most information accepted by patients originates from mass media, not from professional authorities. This undoubtedly increases unrealistic expectations of patients, discrediting the rational and possible therapeutic results.

Modern trends favor esthetic dentistry in its complete expansion with a colorful range of processes and procedures demonstrated by everyday practice. Only one simple, inevitable didactic question for the profession remains open – what dentistry is non-esthetic?

According to the above mentioned statements, the purpose of this paper is to point out the differences in understanding the concepts of cosmetic and esthetic dentistry, discuss the ethical quality of procedures and therapeutic modalities present in esthetic dentistry, and to explain in a critical manner the importance of marketing in dentistry practice.

ESTHETIC OR COSMETIC DENTISTRY

It must be admitted that there is confusion in terms and essence of perception of esthetic and cosmetic dentistry. This is due to the fact that there is an overlap of different esthetic and cosmetic treatments, and to the fact that esthetic and cosmetic procedures in medicine, particularly in surgery, are defined as cosmetic practice.

It is useful to familiarize ourselves with the etymology of the words esthetics/esthetic and cosmetics/cosmetic. The noun 'cosmetics' de-

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rives from the Greek word '*kosmetike*', which means 'the art of dressing and decoration', while the adjective 'cosmetic' derives from the Greek word '*kosmetikos*', which means 'the skill of decorating and ornamenting' or a superficial touch-up of the face and body in order to presents oneself as better, more beautiful, and more impressive [1, 2, 3]. The word 'esthetics' is of similar origin (the Greek word '*aisthetikos*') but means 'sensitive,' 'perceptive.' Esthetics also represents the branch of philosophy concerned with beauty as a quality determined by all that satisfies senses, realized by line, color, shape, proportion, gesture, behavior, and attitude. Therefore, the concept of esthetics is more complex than the concept of cosmetics, which implies that esthetic dentistry is more comprehensive and more complex than cosmetic dentistry.

When it comes to treatment of patients, cosmetic treatment would be the treatment that includes reversible procedures undertaken to provide the so-called optimum patients' appearance, which is sociological, cultural, and limited in time. Thus, the diagnostic composite mock-up, which directly polymerizes with non-etched surface of the teeth enamel, and provides the assessment of the future reconstruction, could be considered a cosmetic treatment. Used in this way, it is temporary, superficial, reversible, and does not damage or changes the structure of supporting tissues. On the other hand, esthetic procedures are adapted to the individual priorities of patients. They are dynamic, coordinated to the expectations of patients, their subjective criteria, but with morphophysiological determinants. It seems that the creation of a patient's smile by appropriate restorations represents the most controversial segment in dentistry. There are data stating that out of 10 dentists who assess a patient's attractiveness of smile, 11 different answers can be obtained, starting from therapists favoring natural appearance to those who point out the beauty of artificially designed compositions [4, 5, 6]. Also, the fact is that the existence of trends and determinants in culture inevitably leaves a mark in the dentistry practice.

It can be stated that procedures in cosmetic dentistry repair patients' appearance only, without changing or enhancing the function, while treatments in the domain of esthetic dentistry imply the application of biological parameters and procedures by which the patients' ideal form, function, and appearance with long-standing effects is achieved [7]. However, in practice, cases that imply esthetic and cosmetic procedures with the same objective are frequent, so that their practical interweaving can still seem confusing.

Terminological ambiguity often leads to a deeper confusion, where 'cosmetic' and 'esthetic' are understood as 'beautiful.' In addition, the subjective aspect should be added, by which, for example, very white teeth are deemed a feature which makes an individual attractive, and, therefore, whitening protocols are the domain of esthetic dentistry. Alternatively, such a view of things seems variable, because it has beautifying as the sole objective, and therefore is of cosmetic character. Finally, it must be admitted that different starting attitudes are personal choices and may be deemed neither right nor wrong.

Perhaps it is more to point out the need of observing esthetic dentistry as a bioesthetic discipline which emphasizes the beauty of living beings and things in their original forms and functions [1].

ETHICS IN ESTHETIC DENTISTRY

Ethics is a branch in philosophy dealing with study and analysis of moral values, which essentially means the standardization of practical life effects of humans [8]. It is deemed that ethical considerations related to procedures indicated and realized in patients' esthetic rehabilitation are extremely complex and severe. Such complexity is the result of a large number of problems that become obvious by examination, while the severity reflects in the different perception of patients addressing the therapists. Numerous real dangers should be added to this sensitive field, where the facts are rarely perceptible. Sometimes it is possible to quickly notice the physical problem, but more frequent is the situation in which the cause that brought the patient to the doctor remains unclear. In addition, it seems that this group of patients is not average, and it is not easy to discern their wishes, aspirations, and expectations. Changes brought by 'the new teeth and new appearance' have an improving effect on the quality of life, i.e. easier selection of a partner, or easier access to better paid jobs, in general making people happier. Such patients' contemplations should be respected, but marked and practically implemented only within the limits of what is realistic and agreed upon.

However, it is completely unethical to provide treatment to patients whose whishes are completely unrealistic, particularly if professional procedures are destructive. Undoubtedly, it is necessary to understand the wishes of patients, but it is also essential, with discussion, analysis of the model for studies, and other diagnostic procedures, to explain to them realistic therapy frameworks. Alike, it should not be forgotten that the patient is the only person who can assess the success of the treatment long-term. A potential danger for suggestible patients are comments, even minor criticisms, of persons in their immediate surroundings. It is known, for example, that major esthetic reconstructions in plastic surgery require previous psychiatric examination of patients and appropriate assessment by a medical specialist.

Prior to the patient's consent, it is ethical to inform him/her about potential risks and consequences borne by the selected esthetic procedure. This primarily refers to indications that require creating ceramic and metal-ceramic dental crowns and bridges as complex esthetic and functional solutions. Particular attention should be paid to young patients, in whom solutions that are "more aggressive" can result in unfavorable future effects. There are data showing that in patients younger than 30 years of age such treatments cause numerous complications [6, 9]. Failure to familiarize the patient with the data on the quantity of tooth substance that will be removed in tooth preparation to accept, for example, ceramic restoration is

deemed unethical. In such cases, there is around 20% risk that the pulp will be damaged or that abscesses or painful sensibility will appear, while the patient's reactions can be anger, disappointment, or lawsuits [10]. Subgingival localized demarcations of preparation, and consequential recession around artificial crowns, particularly in patients with a thin gingival biotype, are also realistic complications with poor esthetic outcome to which the patient should be warned prior to the beginning of treatment.

Unfortunately, destructive (esthetic) procedures without real cause, with unethical biological scenario, are sometimes unjustifiably advised to patients. In the profession, attitude by which teeth are depicted as parts of a group are well known. In the reconstruction of a single central incisor it is not uncommon to perform restoration of the neighboring incisors without real cause. Sometimes, all incisors are reconstructed with the same material during one session to achieve the effect of group esthetic matching. Such situations can often get out of hand and therapists-enthusiasts easily expand their philosophy to canine teeth and the lateral teeth region. It is not uncommon that teeth from the first molars on one side to the first molars on the other side are fitted with porcelain veneers as a popular esthetic modality with uncertain model of occlusion. Recently, "non-aggressiveness" of porcelain veneers has been criticized when a tooth should be sacrificed due to minimum malposition or a group of teeth should be sacrificed in order to attain a wide buccal corridor, which is not considered a natural esthetic quality. Such indications are characterized as "cosmetic crime" and are deemed unethical procedures. In addition, researches following the described trend are short-term, insufficiently objective, and frequently sponsored by dental manufacturers [5, 10, 11]. An even more dramatic example is "forced" implant placement (previous pretreatment in further prosthetic therapy) of appropriate manufacturers followed by insufficiently verified clinical certification.

In search of proper communication with patients whose esthetic problems are in their own focus, it is useful to ask some questions and consider their attitudes through objective self-evaluation of the desired treatment. To what extent dental tissues will be "scarified" by the appropriate esthetic protocol and what will remain for possible reconstructions (*self-preservation*), will the recommended treatment of teeth improve their appearance (*self-improvement*), and has the objective of the implemented destructive therapy on teeth brought an esthetic change of smile and face (*self-destruction*) – these are the questions that should be answered by the patient during discussion. Unfortunately, awareness of possible side effects and later consequences of esthetic therapeutic procedures, which is in close relation with the wide variety of information in dental advertising brochures neglecting this aspect of notification, often do not exist in patients.

In everyday practice, it would be useful to adopt and use the possibility that the patient himself/herself puts forward his/her system of values concerning several essential questions. The *visual analogue scale* (VAS) is a known instrument of esthetic analyses in scientific researches; however, it is rarely used in routine work. The essence is to obtain brief visual answers on the scale from 1 to 100, where the patient's personal attitude to asked questions is demonstrated through the following positions: not important, important, and very important. The VAS scale questions are similar to those already mentioned, when the patient thinks about the importance of existence of strong and healthy teeth, about the possibility that teeth appear more beautiful, and about methods to avoid major damage to teeth as well as possible future complications. An addition includes questions representing the patient's concern regarding teeth color and position. Potential conflict between the patient's wishes and clinical reality is the situation which is discussed in detail and confirmed in writing prior to starting the therapy.

In order to find the best compromise between esthetic solutions which unite patients' desires and sophisticated professional therapy with minimum biological complications, it is necessary to answer in a critical manner these open questions: 1. What is the benefit of the proposed therapy, and how great are the risks?; 2. What are realistic problems that follow the appropriate esthetic modality?; 3. What are the real motives that determine the therapy?; 4. Is the realistic description of the procedure and long-standing effects that the patient can expect given?; 5. Are there alternatives for the proposed therapy?; 6. What are material costs?; 7. What are the possibilities of correction/change if the treatment gives rise to unforeseen developments?; 8. Does the doctor propose the same treatment to himself/herself and his/her loved ones if he/she is in the role of the patient? [12].

The fact is that the public, as well as every patient, expects appropriate service from dental profession. Observance of ethical standards within the profession is deemed dentists' basic duty, with emphasis on elementary principles of preserving health: to do good, work in the best interest of patients (*beneficence*), and not to harm the patient (*non-maleficence*). Essentially, a set of ethical principles, bearing moral prefix, determines the behavior of the profession in solving patients' esthetic problems. Having free framework of the most important determinants, ethics nevertheless differs from law, but also from absolute freedom in activities, and is described as "devotion to inapplicable" [12]. In the profession, ethical behavior is deemed mandatory, not optional.

Almost every decision, diagnosis or set-up indication in esthetic or cosmetic segment bears the ethical and legal component. Though there are cultural specificities, specifically within races, it seems that there is a largely established hegemony in creating very white teeth arranged in the ideal composition without pronounced individuality of each tooth. It should not be forgotten that the social codices in the modern world have been changed, compared with the past, and the loss of teeth is no longer accepted as a sign of natural aging, but as a situation which is prevented or successfully solved in different accessible ways. There is a consideration that cosmetic dentistry in its 'noninvasiveness' is a great threat to the preservation of health of natural teeth [12].

MARKETING IN ESTHETIC DENTISTRY

Viewing inevitably present marketing postulates, it should be pointed out that there are complex relations in social couplings between dental science and practice, which additionally contribute to confusion, foremostly in patients [13]. Apart from esthetic criteria, which are inherited or acquired through the influence of the environment, dentists and patients (directly or indirectly) are being satiated with numerous recommendations they receive from mass media and guerilla actions of corporative marketers. Mass media through contents in different forms (announcements, advertisements, recommendations, testimonies, presentations, etc.) launch information which is most often the result of paid campaigns of corporations or organizations aiming at earning profit. All other guerilla actions relate to numerous alternative methods whereby the patient is reached through an intermediary – a dentist or a dental technician. These actions are sometime foreseeable (they include workshops, accompanying programs on symposiums, distribution of flyers and other material), and sometime quite innovative.

Surrendering to such information, patients, thinking that they make decisions on certain desirable characteristics (appearance of their teeth) independently, in fact implement the dictated instructions [14]. This takes place on two levels: the first one is clear, perceptible, aware of the plan, and the other very subtle, subconscious, and emotional [15, 16, 17]. By these messages, the intelligence or differentiation of patients' is in no way diminished; the messages simply appeal to parts of personality that are not cognizable [18, 19].

Thus, marketing (as science, discipline, philosophy, theory and practice, aiming at market research, recognition or creation of needs, design of products, goods, services, information, and all-comprehensible, satisfying determined needs, promotion, marketing, and sale at a certain price) and ethics joined together imply relatively simple cognition: that all processes of research, creation, and marketing of services/products must be conceptualized and realized to the benefit of all involved parties [8, 20, 21, 22].

Taking into account that all technological innovations and advanced possibilities of digital media change the nature of interaction between dental companies and dentists, today it is possible to communicate with a patient by name and surname, to develop long-term relations with him/her, to forge partnerships, and to enable him/her to come to therapy whenever it suits him/her. In digital strategy, it is known as 'Martini principle' – anytime, anywhere, anyhow. In addition, digital channels enable new interventions that simply could not exist without the Internet. Interactivity and *real-time* dialog created the digital marketing – more flexible, more precise, and more measureable than the traditional one. Also, new technologies have brought new possibilities: marketing specific for the location of the message recipient, a completely new method of interaction in markets, and the movement of advertising from the model called 'interruption marketing' ("interrupts" uncalled into our life) to the so-called 'permission marketing' (in which we give permission so that the advertising message we want can be sent to us when we want it) [23].

The interweaving of ethical and marketing spheres is regulated by law (the Law on Advertising), standards, and codices, but corporations, dental companies, private doctors' offices, clinics, and creative individuals are given certain freedom in representing and advertising [24].

Nowadays, the research of pathology and oral health includes systematic collecting, registering, and analyzing of available information on patients, identifying possible solutions and potential added problems (Figure 1). Consequential examinations in the sphere of presenting esthetic dentistry lead to the following three conclusions:

1. The application of simple but aggressive marketing tools, which are on the one hand directed at dentists and on the other one at patients, is observed. By lobbying and exposing to constant activity of promoters and training on courses and workshops, companies inspire the primary target group, dentists, to become dominant leaders (*opinion leaders*), who thereby obtain exclusivity (or an illusion thereof in the domain of prestige, the public, visibility, popularity) in certain markets [25, 26];

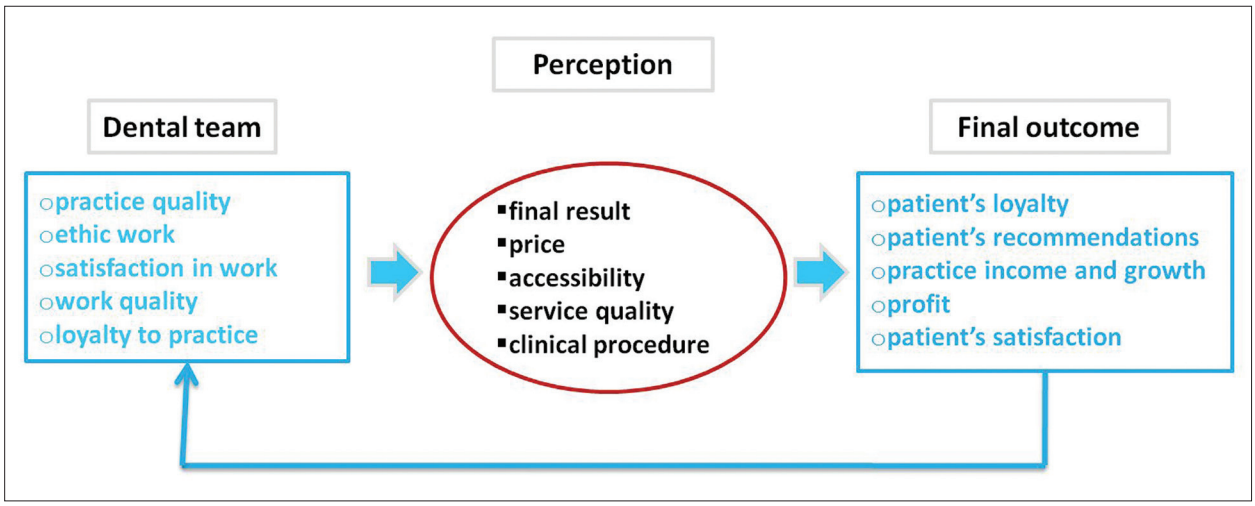

Figure 1. Profit in dental practices as a result of the combination of several factors [13]

2. The secondary target group, patients, attain the *digest* version of services in commercials (toothpastes, antiseptics, denture adhesives, etc.) with permanent pressure that lifestyle is determined not only by healthy, but white, evenly spaced teeth which form the charming smile (Hollywood or Bollywood film stars). Information is offered by attractive photographs / visual content, and important facts are brushed aside (e.g. inscriptions at the end of commercials in small letters, or fast pronunciation for the purpose of shorter duration of commercials, and therefore lower price);

3. Dental companies, dealing with the production of dental materials implement very short researches (e.g. frequent one-year retrospective studies, instead of desirable years-long prospective studies), rebranding the existing products and changing only some particular ingredients. Due to aggressive campaigns of marketing "new" products in the market, by general hyperproduction, companies overwhelm specialists, who heavily follow fast, more formal than essential, changes of trends. The most frequent outcomes of such a strategy in practice is either hanging on to one manufacturer or consuming seemingly the most attractive or the most accessible product in an utterly unfair competition.

The largest damage which marketing can directly inflict on dentistry reflects in the visible effect: "Intellectual and professional adaptations of patients and dentists who invest their skills into something, wherein perhaps they personally do not fully believe, and which helps dissipating and final destruction of the most precious human spiritual goods – trust in existence of significant objective of human activity and respect of the integrity of man" [27]. Besides all internal needs of patients to resist to the society of spectacle, the society wherein image and money dominate, in constant quixotic struggle with hyper-commercialization, it is clear that marketing is an "omnipresent aspect of economic system and withdrawal would represent the capitulation of the main principle of capitalism – expansion," and is unavoidable [27]. For this reason, it is necessary to arm ourselves with knowledge which would capacitate patients and dentists to distinguish faster and wiser in favor of their health, using marketing only as one of fashionable methods of presenting dental procedures. Beautiful is not only that what is as such presented and accepted by the society, but is, in most general esthetic terms of the word, pleasant, good, harmonious, of quality, valuable, worthy, seductive, and consistent [28].

In this direction, one of the useful models of parallel thinking is de Bono model of Six Thinking Hats. The model explains the possible strategy of mental thinking through processes of hat color by the use of different tools [29].

With all dilemmas, it should be concluded that a positive shift of the complete dental profession is present, which, by developing and changing known attitudes, puts a focus on respecting wishes and needs of patients [30]. Alike, esthetic dentistry, largely marketing oriented, bears an obligation of the therapist to recognize the patient being uninformed, familiarize him/her with different cosmetic and esthetic procedures, really point out possible negative consequences of proposed therapeutic modality, and propose the most optimum therapy.

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Етика и маркетинг у естетској стоматологији

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САЖЕТАК

Данашњу стоматологију карактерише убрзани развој захваљујући унапређењу информационих и других технологија, као и развоју денталних материјала (биоматеријали са меморисаним обликом, наноматеријали, биоматеријали за примену у ткивном инжењерингу и др.).

Стручни доктринарни ставови крећу се из правца оперативних интервенција, којима се примарно санирају болест и акутни симптоми, ка јачању оралног здравља мало инвазивним поступцима. Посебно место у свеукупној рехабилитацији болесника припада бројним естетским процедурама, које у великој мери чине праксу вођену потребама и афинитетима болесника.

У раду се дискутује о разликама у поимању козметске и естетске стоматологије. Наглашена је сложеност естетске стоматологије, која даје предност терапији са променом функцијских параметара у збрињавању болесника. С друге стране, акценат се ставља на потребу познавања и поштовања етичких и маркетиншких начела која прате сваку активност лекара, почев од првог контакта са болесником, одабира сертификованог материјала до реализације одговарајућег терапијског плана.

Добро усмерена комуникација и свеобухватна информисаност болесника, употреба *BAC* скале, као и сагледавање реалних ресурса у терапији одреднице су које помажу стоматологу да дефинише проблем на прави начин, изнађе квалитетна решења, отвори алтернативне солуције и смањи могуће ризике у терапији болесника.

Кључне речи: естетска стоматологија; козметска стоматологија; етика; маркетинг

IN MEMORIAM

Марина Божић (1962–2017)

Др Марина Божић је рођена 14. фебруара 1962. у Сурдулици, градићу на југу Србије. Њени родитељи, мајка Радмила, која је завршила економски факултет, и отац Трифун, правник, преселили су се у Ниш, док је Марина, њихово друго дете по реду, била мала. Тако се Марина Божић после завршене основне школе и гимназије у Нишу уписала на Медицински факултет у истом граду. Дипломирала је 1986. године и две године после тога запослила се у Лесковцу као лекар опште медицине. Није наставила професионалну каријеру у Дому здравља, окренула се радиологији и 1998. започела специјализацију из ове области медицине. Током трајања специјализације прихватила се волонтерског рада на Институту за радиологију Клиничког центра у Нишу. Очигледно да су Маринине колеге веровале у њен таленат, препознавши да је током специјализације рођен изузетни стручњак. Било је сасвим природно што се по положеном специјалистичком испиту са одликом запослила на Институту.

Др Марина Божић се можда и колебала коју ће област рада изабрати у радиологији, али се ипак одлучила за дијагностику болести дојке. Врло брзо је овладала техникама тзв. *breast imaging* процедуре док је била на челу одељења за мамографију своје матичне установе (2002–2010). Предано је радила готово читаву деценију на мамографији, ултразвучној дијагностици и, као круни свега, магнетној резонанци дојки. Наредне године (2011) отишла је на стручно усавршавање у трајању од шест месеци у САД, у Њујорк (*Memorial Sloan-Kettering Cancer Center*). Потпуно је овладала најсуптилнијим биопсијским техникама: стереотаксичном и ултразвуком вођеној вакуум асистираном биопсијом дојки, као и тзв. *core needle breast* биопсијом, биопсијом дојке широком иглом. Своју установу је тиме учинила једном од водећих у Србији у тој области радиологије.

Догађа се да неки лекари, медицински радници или научници добију још за живота различита признања. Некима се то догоди позно, некима, нажалост, иако су то заслужили по свим мерилима, никада. Дело оних медицинских прегалаца које смрт покоси у напону снаге често може бити ненамерно заборављено или запостављено.

За др Марину Божић показало се потпуно друкчије. Видело се одмах колико је њен рад у области радиологије цењен и поштован у широком кругу истинских професионалаца, али и код оних у потпуности посвећених том одговорном послу. Али то се не односи само на колеге из уже струке, радиологе, који су ову оцену, још за њена живота, изрекли 2012. прогласивши је за најбољег радиолога Института за радиологију Медицинског факултета у Нишу. Напротив, својом личношћу и стручношћу остављала је снажан и неодољив утисак и на хирурге, и патологе или онкологе, на многе друге колеге разних специјалности... Мишљење свих њих који су знали за њено предано прегалаштво је неподељено: најмање што следи делу др Марине Божић је незаборав. Такав допринос српској радиологији, развоју дијагностике и раног откривања рака дојке не само да није пролазан и кратковечан него је посебан и изузетно значајан. Обуку коју је обавила са изванредним успехом у Њујорку, Салцбургу, Бечу или Љубљани учинили су је великим ауторитетом у овој области. Дивило се њеној радној посвећености, несебичности у преношењу знања и обучавању колега у болницама широм Србије, Новом Саду, Крагујевцу, Врању... Кроз обуку за скрининг дојке прошло је више од двадесет радиолога из свих градова наше земље. Марина Божић се пела ка врху трновитим стазама, тако је и научно сазревала. Али у

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тој нежној, тактичној, лепој и паметној жени било је исконске снаге, знања, енергије и амбиције да се посвети једној од најосетљивијих дијагностичких проблема у радиологији. И у томе је била једна од најбољих у Србији.

Медицина, радиологија, и у оквиру ње – мамографија, одредиле су живот др Марине Божић.

После Маринине трагичне смрти у саобраћајној несрећи у марту ове године, њена кћи је на столу у ординацији пронашла један цитат који је докторка одштампала са интернета, последњих дана живота. „Онај тко је задовољан собом не успоређује се с другима. Онај тко се не успоређује с другима није завидан. Онај тко није завидан није огорчен. Онај тко није огорчен је сретан. Онај тко је сретан знат ће усрећити још некога. Онај тко себе и још некога поред себе усрећује открио је смисао живота, не тражећи га."

Природно је да се ово присећање на живот и дело др Марине Божић завршава управо овим цитатом, који остаје као њен *memento*.

Вукашин М. АНТИЋ
Општа болница Врање,
Хируршко одељење, Врање, Србија

Пре подношења рукописа Уредништву часописа „Српски архив за целокупно лекарство“ (СА) сви аутори треба да прочитају Упутство за ауторе (*Instructions for Authors*), где ће пронаћи све потребне информације о писању и припреми рада у складу са стандардима часописа. Веома је важно да аутори припреме рад према датим пропозицијама, јер уколико рукопис не буде усклађен с овим захтевима, Уредништво ће одложити или одбити његово публиковање. Радови објављени у СА се не хонорарашу. За чланке који ће се објавити у СА, самом понудом рада Српском архиву сви аутори рада преносе своја ауторска права на издавача часописа – Српско лекарско друштво.

ОПШТА УПУТСТВА. СА објављује радове који до сада нису нигде објављени, у целости или делом, нити прихваћени за објављивање. СА објављује радове на енглеском и српском језику. Због боље доступности и веће цитираности препоручује се ауторима да радове свих облика предају на енглеском језику. У СА се објављују следеће категорије радова: уводници, оригинални (научни и стручни) радови, метаанализе, прегледни радови, претходна и кратка саопштења, прикази болесника и случајева, слике из клиничке медицине, видео-чланци, радови за праксу, актуелне теме, радови из историје медицине и језика медицине, лични ставови, наручени коментари, писма уреднику, прикази књига и други прилози. Оригинални радови, претходна и кратка саопштења и прикази болесника и случајева публикују се искључиво на енглеском језику, а остале врсте радова се могу публиковати и на српском језику само по одлуци Уредништва. Радови се увек достављају са сажетком на енглеском и српском језику (у склопу самог рукописа). Текст рада куцати у програму за обраду текста *Word*, фонтом *Times New Roman* и величином слова 12 тачака (12 *pt*). Све четири маргине подесити на 25 *mm*, величину странице на формат А4, а текст куцати с двоструким проредом, левим поравнањем и увлачењем сваког пасуса за 10 *mm*, без дељења речи (хифенације). Не користити табулаторе и узастопне празне карактере (спејсове) ради поравнања текста, већ алатке за контролу поравнања на лењиру и *Toolbars*. За прелазак на нову страну документа не користити низ „ентера“, већ искључиво опцију *Page Break*. После сваког знака интерпункције ставити само један празан карактер. Ако се у тексту користе специјални знаци (симболи), користити фонт *Symbol*. Подаци о коришћеној литератури у тексту означавају се арапским бројевима у угластим заградама – нпр. [1, 2], и то редоследом којим се појављују у тексту. Странице нумерисати редом у доњем десном углу, почев од насловне стране.

При писању текста на енглеском језику треба се придржавати језичког стандарда *American English* и користити кратке и јасне реченице. За називе лекова користити искључиво генеричка имена. Уређаји (апарати) се означавају фабричким називима, а име и место произвођача треба навести у облим заградама. Уколико се у тексту користе ознаке које су спој слова и бројева, прецизно написати број који се јавља у суперскрипту или супскрипту (нпр. ^{99}Tc, *IL*-6, O_2, $Б_{12}$, *CD*8). Уколико се нешто уобичајено пише курзивом (*italic*), тако се и наводи, нпр. гени (*BRCA1*).

Уколико је рад део магистарске тезе, односно докторске дисертације, или је урађен у оквиру научног пројекта, то треба посебно назначити у Напомени на крају текста. Такође, уколико је рад претходно саопштен на неком стручном састанку, навести званичан назив скупа, место и време одржавања, да ли је рад и како публикован (нпр. исти или другачији наслов или сажетак).

КЛИНИЧКА ИСТРАЖИВАЊА. Клиничка истраживања се дефинишу као истраживања утицаја једног или више средстава или мера на исход здравља. Регистарски број истраживања се наводи у последњем реду сажетка.

ЕТИЧКА САГЛАСНОСТ. Рукописи о истраживањима на људима треба да садрже изјаву у виду писаног пристанка испитиваних особа у складу с Хелсиншком декларацијом и одобрење надлежног етичког одбора да се истраживање може извести и да је оно у складу с правним стандардима. Експериментална истраживања на хуманом материјалу и испитивања вршена на животињама треба да садрже изјаву етичког одбора установе и треба да су у сагласности с правним стандардима.

ИЗЈАВА О СУКОБУ ИНТЕРЕСА. Уз рукопис се прилаже потписана изјава у оквиру обрасца *Submission Letter* којом се аутори изјашњавају о сваком могућем сукобу интереса или његовом одсуству. За додатне информације о различитим врстама сукоба интереса посетити интернет-страницу Светског удружења уредника медицинских часописа (*World Association of Medical Editors – WAME; http://www.wame.org*) под називом „Политика изјаве о сукобу интереса“.

АУТОРСТВО. Све особе које су наведене као аутори рада треба да се квалификују за ауторство. Сваки аутор треба да је учествовао довољно у раду на рукопису како би могао да преузме одговорност за целокупан текст и резултате изнесене у раду. Ауторство се заснива само на: битном доприносу концепцији рада, добијању резултата или анализи и тумачењу резултата; планирању рукописа или његовој критичкој ревизији од знатног интелектуалног значаја; завршном дотеривању верзије рукописа који се припрема за штампање.

Аутори треба да приложе опис доприноса појединачно за сваког коаутора у оквиру обрасца *Submission Letter*. Финансирање, сакупљање података или генерално надгледање истраживачке групе сами по себи не могу оправдати ауторство. Сви други који су допринели изради рада, а који нису аутори рукописа, требало

би да буду наведени у Захвалници с описом њиховог доприноса раду, наравно, уз писани пристанак.

НАСЛОВНА СТРАНА. На првој страници рукописа треба навести следеће: наслов рада без скраћеница; предлог кратког наслова рада, пуна имена и презимена аутора (без титула) индексирана бројевима; званичан назив установа у којима аутори раде, место и државу (редоследом који одговара индексираним бројевима аутора); на дну странице навести име и презиме, адресу за контакт, број телефона, факса и имејл адресу аутора задуженог за кореспонденцију.

САЖЕТАК. Уз оригинални рад, претходно и кратко саопштење, метаанализу, преглед литературе, приказ случаја (болесника), рад из историје медицине, актуелну тему, рад за рубрику језик медицине и рад за праксу, на другој по реду страници документа треба приложити сажетак рада обима 100–250 речи. За оригиналне радове, претходна и кратка саопштења и метаанализе сажетак треба да има следећу структуру: Увод/Циљ, Методе, Резултати, Закључак; сваки од наведених сегмената писати као посебан пасус који почиње болдованом речи. Навести најважније резултате (нумеричке вредности) статистичке анализе и ниво значајности. Закључак не сме бити уопштен, већ мора бити директно повезан са резултатима рада. За приказе болесника сажетак треба да има следеће делове: Увод (у последњој реченици навести циљ), Приказ болесника, Закључак; сегменте такође писати као посебан пасус који почиње болдованом речи. За остале типове радова сажетак нема посебну структуру.

КЉУЧНЕ РЕЧИ. Испод Сажетка навести од три до шест кључних речи или израза. Не треба да се понављају речи из наслова, а кључне речи треба да буду релевантне или описне. У избору кључних речи користити *Medical Subject Headings – MeSH (http://www.nlm.nih.gov/mesh).*

ПРЕВОД НА СРПСКИ ЈЕЗИК. На трећој по реду страници документа приложити наслов рада на српском језику, пуна имена и презимена аутора (без титула) индексирана бројевима, званичан назив установа у којима аутори раде, место и државу. На следећој – четвртој по реду – страници документа приложити сажетак (100–250 речи) с кључним речима (3–6), и то за радове у којима је обавезан сажетак на енглеском језику. Превод појмова из стране литературе треба да буде у духу српског језика. Све стране речи или синтагме за које постоји одговарајуће име у нашем језику заменити тим називом.

Уколико је рад у целости на српском језику (нпр. рад из историје медицине, језика медицине и др.), потребно је превести називе прилога (табела, графикона, слика, схема) уколико их има, целокупни текст у њима и легенду на енглески језик. Сажетке и радове који су у целости на српском језику аутори из Србије треба да пишу ћирилицом.

СТРУКТУРА РАДА. Сви поднаслови се пишу великим масним словима (болд). Оригинални рад, метаанализа, претходно и кратко саопштење обавезно треба да имају следеће поднаслове: Увод (Циљ рада навести као последњи пасус Увода), Методе рада, Резултати, Дискусија, Закључак, Литература. Преглед литературе чине: Увод, одговарајући поднаслови, Закључак, Литература. Првоименовани аутор метаанализе и прегледног рада мора да наведе бар пет аутоцитата (као аутор или коаутор) радова публикованих у часописима с рецензијом. Коаутори, уколико их има, морају да наведу бар један аутоцитат радова такође публикованих у часописима с рецензијом. Приказ случаја или болесника чине: Увод (Циљ рада навести као последњи пасус Увода), Приказ болесника, Дискусија, Литература. Не треба користити имена болесника, иницијале, нити бројеве историја болести, нарочито у илустрацијама. Прикази болесника не смеју имати више од пет аутора.

Прилоге (табеле, графиконе, слике итд.) поставити на крај рукописа, а у самом телу текста јасно назначити место које се односи на дати прилог. Крајња позиција прилога биће одређена у току припреме рада за публиковање.

СКРАЋЕНИЦЕ. Користити само када је неопходно, и то за веома дугачке називе хемијских једињења, односно називе који су као скраћенице већ препознатљиви (стандардне скраћенице, као нпр. ДНК, сида, ХИВ, АТП). За сваку скраћеницу пун термин треба навести при првом навођењу у тексту, сем ако није стандардна јединица мере. Не користити скраћенице у наслову. Избегавати коришћење скраћеница у сажетку, али ако су неопходне, сваку скраћеницу објаснити при првом навођењу у тексту.

ДЕЦИМАЛНИ БРОЈЕВИ. У тексту рада на енглеском језику, у табелама, на графиконима и другим прилозима децималне бројеве писати са тачком (нпр. 12.5 ± 3.8), а у тексту на српском језику са зарезом (нпр. 12,5 ± 3,8). Кад год је то могуће, број заокружити на једну децималу.

ЈЕДИНИЦЕ МЕРА. Дужину, висину, тежину и запремину изражавати у метричким јединицама (метар – *m*, килограм (грам) – *kg* (*g*), литар – *l*) или њиховим деловима. Температуру изражавати у степенима Целзијуса (°*C*), количину супстанце у молима (*mol*), а притисак крви у милиметрима живиног стуба (*mm Hg*). Све резултате хематолошких, клиничких и биохемијских мерења наводити у метричком систему према Међународном систему јединица (*SI*).

ОБИМ РАДОВА. Целокупни рукопис рада – који чине насловна страна, сажетак, текст рада, списак литературе, сви прилози, односно потписи за њих и легенда (табеле, слике, графикони, схеме, цртежи), насловна страна и сажетак на српском језику – мора износити за оригинални рад, претходно и кратко саопштење, рад из

историје медицине и преглед литературе до 5.000 речи, а за приказ болесника, рад за праксу, едукативни чланак и рад за рубрику „Језик медицине“ до 3.000 речи; радови за остале рубрике могу имати највише 1.500 речи.
Видео-радови могу трајати 5–7 минута и бити у формату *avi, mp4*(*flv*). У првом кадру филма мора се навести: у наднаслову Српски архив за целокупно лекарство, наслов рада, презимена и иницијали имена и средњег слова свих аутора рада (не филма), година израде. У другом кадру мора бити уснимљен текст рада у виду апстракта до 350 речи. У последњем кадру филма могу се навести имена техничког особља (режија, сниматељ, светло, тон, фотографија и сл.). Уз видео-радове доставити: посебно текст у виду апстракта (до 350 речи), једну фотографију као илустрацију приказа, изјаву потписану од свег техничког особља да се одричу ауторских права у корист аутора рада.

ПРИЛОЗИ РАДУ су табеле, слике (фотографије, цртежи, схеме, графикони) и видео-прилози.

ТАБЕЛЕ. Свака табела треба да буде сама по себи лако разумљива. Наслов треба откуцати изнад табеле, а објашњења испод ње. Табеле се означавају арапским бројевима према редоследу навођења у тексту. Табеле цртати искључиво у програму *Word*, кроз мени *Table–Insert–Table*, уз дефинисање тачног броја колона и редова који ће чинити мрежу табеле. Десним кликом на мишу – помоћу опција *Merge Cells* и *Split Cells* – спајати, односно делити ћелије. Куцати фонтом *Times New Roman*, величином слова 12 *pt*, с једноструким проредом и без увлачења текста. Коришћене скраћенице у табели треба објаснити у легенди испод табеле.

Уколико је рукопис на српском језику, приложити називе табела и легенду на оба језика. Такође, у једну табелу, у оквиру исте ћелије, унети и текст на српском и текст на енглеском језику (никако не правити две табеле са два језика!).

СЛИКЕ. Слике су сви облици графичких прилога и као „слике“ у CA се објављују фотографије, цртежи, схеме и графикони. Слике означавају се арапским бројевима према редоследу навођења у тексту. Примају се искључиво дигиталне фотографије (црно-беле или у боји) резолуције најмање 300 *dpi* и формата записа *tiff* или *jpg* (мале, мутне и слике лошег квалитета неће се прихватати за штампање!). Уколико аутори не поседују или нису у могућности да доставе дигиталне фотографије, онда оригиналне слике треба скенирати у резолуцији 300 *dpi* и у оригиналној величини. Уколико је рад неопходно илустровати са више слика, у раду ће их бити објављено неколико, а остале ће бити у е-верзији чланка као *PowerPoint* презентација (свака слика мора бити нумерисана и имати легенду).
Видео-прилози (илустрације рада) могу трајати 1–3 минута и бити у формату *avi, mp4*(*flv*). Уз видео доставити посебно слику која би била илустрација видеоприказа у *e*-издању и објављена у штампаном издању.
Уколико је рукопис на српском језику, приложити називе слика и легенду на оба језика.

Слике се у свесци могу штампати у боји, али додатне трошкове штампе сносе аутори.

ГРАФИКОНИ. Графикони треба да буду урађени и достављени у програму *Excel*, да би се виделе пратеће вредности распоређене по ћелијама. Исте графиконе прекопирати и у *Word*-ов документ, где се графикони означавају арапским бројевима према редоследу навођења у тексту. Сви подаци на графикону куцају се у фонту *Times New Roman*. Коришћене скраћенице на графикону треба објаснити у легенди испод графикона. У штампаној верзији чланка вероватније је да графикон неће бити штампан у боји, те је боље избегавати коришћење боја у графиконима, или их користити различитог интензитета.

Уколико је рукопис на српском језику, приложити називе графикона и легенду на оба језика.

СХЕМЕ (ЦРТЕЖИ). Цртежи и схеме се достављају у *jpg* или *tiff* формату. Схеме се могу цртати и у програму *CorelDraw* или *Adobe Illustrator* (програми за рад са векторима, кривама). Сви подаци на схеми куцају се у фонту *Times New Roman*, величина слова 10 *pt*. Коришћене скраћенице на схеми треба објаснити у легенди испод схеме.

Уколико је рукопис на српском језику, приложити називе схема и легенду на оба језика.

ЗАХВАЛНИЦА. Навести све сараднике који су допринели стварању рада а не испуњавају мерила за ауторство, као што су особе које обезбеђују техничку помоћ, помоћ у писању рада или руководе одељењем које обезбеђује општу подршку. Финансијска и материјална помоћ, у облику спонзорства, стипендија, поклона, опреме, лекова и друго, треба такође да буде наведена.

ЛИТЕРАТУРА. Списак референци је одговорност аутора, а цитирани чланци треба да буду лако приступачни читаоцима часописа. Стога уз сваку референцу обавезно треба навести *DOI* број чланка (јединствену ниску карактера која му је додељена) и *PMID* број уколико је чланак индексиран у бази *PubMed/MEDLINE*.

Референце нумерисати редним арапским бројевима према редоследу навођења у тексту. Број референци не би требало да буде већи од 30, осим у прегледу литературе, у којем је дозвољено да их буде до 50, а у метаанализи до 100. Број цитираних оригиналних радова мора бити најмање 80% од укупног броја референци, односно број цитираних књига, поглавља у књигама и прегледних чланака мањи од 20%. Уколико се домаће монографске публикације и чланци могу уврстити у референце, аутори су дужни да их цитирају. Већина цитираних научних чланака не би требало да

буде старија од пет година. Није дозвољено цитирање апстраката. Уколико је битно коментарисати резултате који су публиковани само у виду апстракта, неопходно је то навести у самом тексту рада. Референце чланака који су прихваћени за штампу, али још нису објављени, треба означити са *in press* и приложити доказ о прихватању рада за објављивање.

Референце се цитирају према Ванкуверском стилу (униформисаним захтевима за рукописе који се предају биомедицинским часописима), који је успоставио Међународни комитет уредника медицинских часописа (*http://www.icmje.org*), чији формат користе *U.S. National Library of Medicine* и базе научних публикација. Примери навођења публикација (чланака, књига и других монографија, електронског, необјављеног и другог објављеног материјала) могу се пронаћи на интернет-страници *http://www.nlm.nih.gov/bsd/uniform_requirements.html*. Приликом навођења литературе веома је важно придржавати се поменутог стандарда, јер је то један од најбитнијих фактора за индексирање приликом класификације научних часописа.

ПРОПРАТНО ПИСМО (*SUBMISSION LETTER*). Уз рукопис обавезно приложити образац који су потписали сви аутори, а који садржи: 1) изјаву да рад претходно није публикован и да није истовремено поднет за објављивање у неком другом часопису, 2) изјаву да су рукопис прочитали и одобрили сви аутори који испуњавају мерила ауторства, и 3) контакт податке свих аутора у раду (адресе, имејл адресе, телефоне итд.). Бланко образац треба преузети са интернет-странице часописа (*http://www.srpskiarhiv.rs*).

Такође је потребно доставити копије свих дозвола за: репродуковање претходно објављеног материјала, употребу илустрација и објављивање информација о познатим људима или именовање људи који су допринели изради рада.

ЧЛАНАРИНА, ПРЕТПЛАТА И НАКНАДА ЗА ОБРАДУ ЧЛАНКА. Да би рад био објављен у часопису *Српски архив за целокупно лекарство*, сви аутори који су лекари или стоматолози из Србије морају бити чланови Српског лекарског друштва (у складу са чланом 6. Статута Друштва) за годину у којој се рад предаје Уредништву. Сви домаћи аутори такође морају бити претплаћени на часопис или измирити накнаду за обраду чланака (*article processing charge*) за годину у којој се рад предаје Уредништву, у износу од 3.000 динара. Аутори и коаутори из иностранства су у обавези да плате накнаду за обраду чланака (*article processing charge*) у износу од 35 евра. Уплата у једној календарској години обухвата и све наредне, евентуалне чланке, послате на разматрање у тој години. Сви аутори који плате ову накнаду могу, уколико то желе, да примају штампано издање часописа. Треба напоменути да ова уплата није гаранција да ће рад бити прихваћен и објављен у *Српском архиву за целокупно лекарство*. Обавеза плаћања накнаде за обраду чланка не односи се на студенте основних студија и на претплатнике на часопис.

Установе (правна лица) не могу преко своје претплате да испуне овај услов аутора (физичког лица). Уз рукопис рада треба доставити копије уплатница за чланарину и претплату / накнаду за обраду чланка, као доказ о уплатама, уколико издавач нема евиденцију о томе. Часопис прихвата донације од спонзора који сносе део трошкова или трошкове у целини оних аутора који нису у могућности да измире накнаду за обраду чланка (у таквим случајевима потребно је часопису ставити на увид оправданост таквог спонзорства).

Додатне информације о чланарини и претплати могу се добити путем имејла (*office@srpskiarhiv.rs*) и на интернет-страници часописа *http://srpskiarhiv.rs/en/subscription/).*

СЛАЊЕ РУКОПИСА. Рукопис рада и сви прилози уз рад могу се доставити имејлом (*office@srpskiarhiv.rs*), електронски преко система за пријављивање на интернет-страници часописа (*http://www.srpskiarhiv.rs*), препорученом пошиљком или лично, доласком у Уредништво. Уколико се рад шаље поштом или доноси у Уредништво, рукопис се доставља одштампан у три примерка и нарезан на *CD* (снимљени материјал треба да је истоветан оном на папиру).

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За све додатне информације, молимо да се обратите на доленаведене адресе и број телефона.

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When writing text in English, linguistic standard American English should be observed. Write short and clear sentences. Generic names should be exclusively used for the names of drugs. Devices (apparatuses, instruments) are termed by trade names, while their name and place of production should be indicated in the brackets. If a letter-number combination is used, the number should be precisely designated in superscript or subscript (i.e., ^{99}Tc, IL-6, O_2, B_{12}, CD8). If something is commonly written in italics, such as genes (e.g. *BRCA1*), it should be written in this manner in the paper as well.

If a paper is a part of a master's or doctoral thesis, or a research project, that should be designated in a separate note at the end of the text. Also, if the article was previously presented at any scientific meeting, the name, venue and time of the meeting should be stated, as well as the manner in which the paper had been published (e.g. changed title or abstract).

CLINICAL TRIALS. Clinical trial is defined as any research related to one or more health related interventions in order to evaluate the effects on health outcomes. The trial registration number should be included as the last line of the Summary.

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The authors should enclose the description of contribution to the article of every co-author individually (within the Submission Letter). Funding, collection of data or general supervision of the research group alone cannot justify authorship. All other individuals having contributed to the preparation of the article should be mentioned in the *Acknowledgment* section, with description of their contribution to the paper, with their written consent.

TITLE PAGE. The first page of the manuscript (cover sheet) should include the following: title of the paper without any abbreviations; suggested running title; each author's full names and family names (no titles), indexed by numbers; official name, place and country of the institu-

tion in which authors work (in order corresponding to the indexed numbers of the authors); at the bottom of the page: name and family name, address, phone and fax number, and e-mail address of a corresponding author.

SUMMARY. Along with the original article, preliminary and short communication, meta-analysis, review article, case report, article on history of medicine, current topic article, article for language of medicine and article for practitioners, the summary not exceeding 100–250 words should be typed on the second page of the manuscript. In original articles, preliminary communications, and meta-analyses, the summary should have the following structure: Introduction/Objective, Methods, Results, Conclusion. Each segment should be typed in a separate paragraph using boldface. The most significant results (numerical values), statistical analysis and level of significance are to be included. The conclusion must not be generalized, it needs to point directly to the results of the study. In case reports, the summary should consist of the following: Introduction (final sentence is to state the objective), Case Outline (Outline of Cases), Conclusion. Each segment should be typed in a separate paragraph using boldface. In other types of papers, the summary has no special outline.

KEYWORDS. Below the summary, 3 to 6 keywords or phrases should be typed. The keywords need not repeat words in the title and should be relevant or descriptive. *Medical Subject Headings – MeSH* (*http://www.nlm.nih.gov/mesh*) are to be used for selection of the keywords.

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LENGTH OF PAPER. The entire text of the manuscript – title page, summary, the whole text, list of references, all enclosures including captions and legends (tables, photographs, graphs, schemes, sketches), title page and summary in Serbian – must not exceed 5,000 words for original articles, preliminary and short communications, review articles and articles on history of medicine, and 3,000 words for case reports, articles for practitioners, educational articles and articles for "Language of medicine"; for any other section maximum is 1,500 words.

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ACKNOWLEDGMENT. List all those individuals having contributed to preparation of the article but having not met the criteria of authorship, such as individuals providing technical assistance, assistance in writing the paper or running the department securing general support. Financial aid and all other support in the form of sponsorship, grants, donations of equipment and medications, etc., should be mentioned too.

REFERENCES. The reference list is the responsibility of the authors. Cited articles should be readily accessible to the journals readership. Therefore, following each reference, its DOI number and PMID number (if the article is indexed for MEDLINE/PubMed) should be typed.

References should be numbered in Arabic numerals in order of citation in the text. The overall number of references should not exceed 30, except in review articles, where maximum of 50 is acceptable, and in meta-analysis, where up to 100 referenceas are allowed. The number of citations of original articles must be at least 80% of the total number of references, and the number of citations of books, chapters and literature reviews less than 20%. If monographs and articles written by Serbian authors could be included

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