58. KONGRES ANTROPOLOŠKOG DRUŠTVA SRBIJE
AKREDITOVAN MEĐUNARODNI KONGRES
29-31. maj 2025. Novi Sad

58th CONGRESS OF ANTHROPOLOGICAL SOCIETY OF SERBIA
ACCREDITED INTERNATIONAL CONGRESS
May 29-31 2025. Novi Sad

ZBORNIK RADOVA

BOOK OF PROCEEDINGS

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A STUDY OF DEGRADED SKELETAL SAMPLES USING FORENSEQ DNA SIGNATURE™ KIT

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Abstract: Recent advances in massively parallel sequencing (MPS) have established it as a promising technology for large-scale genetic sequencing. This study tested the Illumina ForenSeq™ DNA Signature Prep Kit to assess whether MPS provides a more comprehensive evaluation of degraded samples compared to traditional fragment analysis/capillary electrophoresis-based methods. The Illumina® ForenSeq™ DNA Signature MPS Kit incorporates 200 genetic loci. By utilising next-generation sequencing (NGS), analysis time is reduced, and the identification of human remains is enhanced. In this study, we aimed to analyse degraded hard tissue samples using the Illumina® ForenSeq™ DNA Signature MPS Kit. These samples had previously yielded partial profiles with dropout at several loci when using the GlobalFiler™ kit. The MPS kit demonstrated high sensitivity, improving allele recovery for STR loci, and provided valuable information on biogeographic ancestry, identity, and phenotypic traits from a single analysis. The study resulted in the successful amplification and sequencing of 30 degraded bone/teeth samples using the MPS method.

Key words: MPS, ForenSeq™; MiSeq FGX; GlobalFiler™; Autosomal STR; Forensic; Qatar.

INTRODUCTION

Recent advancements in massively parallel sequencing (MPS) offer significant advantages for profiling DNA from skeletal human remains, particularly those that are old, due to its enhanced sensitivity and the ability to analyse a much greater number of loci compared to traditional capillary electrophoresis-based technologies. The ForenSeq™ DNA Signature kit includes autosomal STRs, Y STRs, X STRs, Identity SNPs, and Ancestry SNPs (Børsting et al., 2015; Athey, 2006). In forensic applications, the key advantage of these markers lies in their ability to work effectively with highly degraded DNA, such as in disaster victim identification and forensic samples (Chaitanya et al., 2017; Zeng et al., 2025). A major challenge in the analysis of degraded samples is the risk that any extracted DNA may originate from a non-target source (Fordyce et al., 2025; Morozova et al., 2009). Larger loci (>200bp) are typically the first to be affected by degradation and are often lost in the process (Børsting et al., 2015; Meyer et al. 2014; Morozova et al., 2009). Recent improvements in MPS technology, however, have shown a lot of promise in the analysis of DNA from human remains (Fordyce et al., 2025; Regueiro et al., 2012). One significant advantage of MPS is the ability to multiplex shorter STR markers, which are more suitable for profiling degraded DNA, alongside SNPs. Unlike traditional

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fragment size analysis, MPS provides the actual sequence of the STR, offering higher resolution. The presence of numerous isoalleles allows for the differentiation between individuals who share the same allele call at a locus, enhancing the discriminatory power of the allele and aiding in the deconvolution of mixtures (Rockenbauer et al., 2014; Morozova et al., 2009; Zeng et al., 2015). In this study, we have investigated the performance of the Illumina® ForenSeq™ DNA Signature on a set of degraded skeletal DNA samples from Serbia (Zgonjanin et al., 2017; Zgonjanin et al., 2019).

MATERIALS AND METHODS

Typing success on bone samples

Thirty challenged samples were selected based on their morphological features and the data previously generated using standard capillary electrophoresis (CE) methodology. These samples included DNA from aged bone samples that had produced poor STR results. The majority of these samples yielded only partial profiles when analysed with the GlobalFiler™ kit.

Forenseq DNA Signature Prep kit (beta version)

The beta version of the ForenSeq DNA Signature Prep kit was utilised for MPS analysis. This kit provides PCR primer mixes designed for the targeted amplification of 58 STRs, 94 identity informative SNPs (iSNPs), 56 ancestry informative SNPs (aSNPs), and 22 phenotypic informative SNPs (pSNPs) (Athey, 2006). A primer mix containing tagged oligos for each target sequence was combined with the DNA sample. PCR cycles linked the tags to the copies of each target, forming DNA templates with the regions of interest flanked by universal primer sequences. These tags were then used to attach indexed adapters, which were subsequently amplified by PCR, purified, pooled into a single tube, and sequenced. This process incorporated integrated indexing to facilitate the sequencing of 30 samples in a single run, including both positive and negative controls. The samples were sequenced on the Illumina Miseq FGx platform (Illumina, Inc., 2016).

RESULTS

ForenSeq™ UAS Results

The ForenSeq™ UAS provided quality and coverage data for all samples and was used to determine SßTR and SNP genotypes, as well as to estimate ancestry and phenotypic traits such as eye and hair colour. The analytical threshold was set at 10 reads, while the stochastic threshold was set at 30 reads. The ForenSeq™ UAS calculated Random Match Probability (RMP) from the STR length-based genotypes, STR sequence-based genotypes, and SNP genotypes. Allele frequencies from the ForenSeq™ universal software population datasets (African, admixed American, Asian, and European) were used to generate RMP values.

Table 1. Summary of ForenSeq™ results the bones samples

Marker Type	Average Number of Amplified loci	Total Number of loci in the kit	Percentage of Amplified Loci (%)
Autosomal STRs	24	27	88.8%
X-STRs	5	7	71.4%
Y-STRs	19	24	79.2%
Identity SNPs	80	95	84.3%
Ancestry SNPs	54	56	96.4%
Phenotypic SNPs	24	24	100%

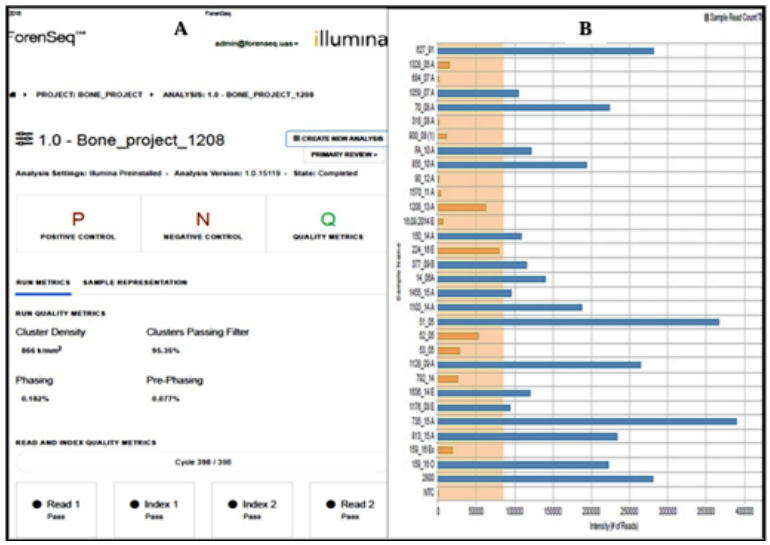

Figure 1. (A & B) Results of 30-bone samples run on FGx-MiSeq showing a cluster density of 866 K/mm2. A total 95 % of clusters generated run passed filters, which showed that the reaction was highly successful. The average intensity of the reads was 200000.

The results obtained using the ForenSeq™ DNA Signature kit were quite robust, given the nature of the samples. 88.8% of all loci amplified, with 84.3% of successful amplifications being less than 150 bp in fragment size. Each sample produced results that were concordant with those from the GlobalFiler™ kit. The MPS results showed a higher number of allele recoveries for autosomal STRs, except for sample No. 22 (1406/12), which had aDNA quantity of 0.018 ng and a Discrimination Index (DI) of 14. This sample had yielded a partial profile using the GlobalFiler™ kit, but the MPS results revealed an almost full autosomal STR profile. For all the bone samples, the autosomal STR (auSTR) allele recovery was considerably higher than with the GlobalFiler™ kit, despite low DNA quantities and the inhibited nature of the samples. Of all the markers amplified, the highest proportions observed were 100% for phenotypic SNPs, 98.6% for autosomal STRs, 98.6% for X-STRs, and 98.6% for identity and ancestry SNPs (Figure 1). The discriminatory power of the MPS results for bone samples was

assessed using random match probabilities (RMPs). The inverse of RMPs (1/RMPs) was reported as the likelihood ratio (LR) of obtaining the same DNA profile from an unrelated individual in the US Caucasian/European population group (Figure 3).

The GlobalFiler™ kit demonstrated high discriminating power, with an average LR of one in 10^{27}. The STR sequence-genotype was more discriminative than the STR length-genotype because the underlying sequence variation in the STRs increased allelic diversity and, consequently, discriminatory power. Additionally, the small number of loci reported, combined with the low discriminating power of bi-allelic SNPs, reduced the 1/RMP values for the MiSeq® iSNP, which had an average 1/RMP value of one in 10^{12}. When examining the unknown bone samples, the results yielded the highest 1/RMP values, with an average of one in 10^{36} for iSNPs alone.

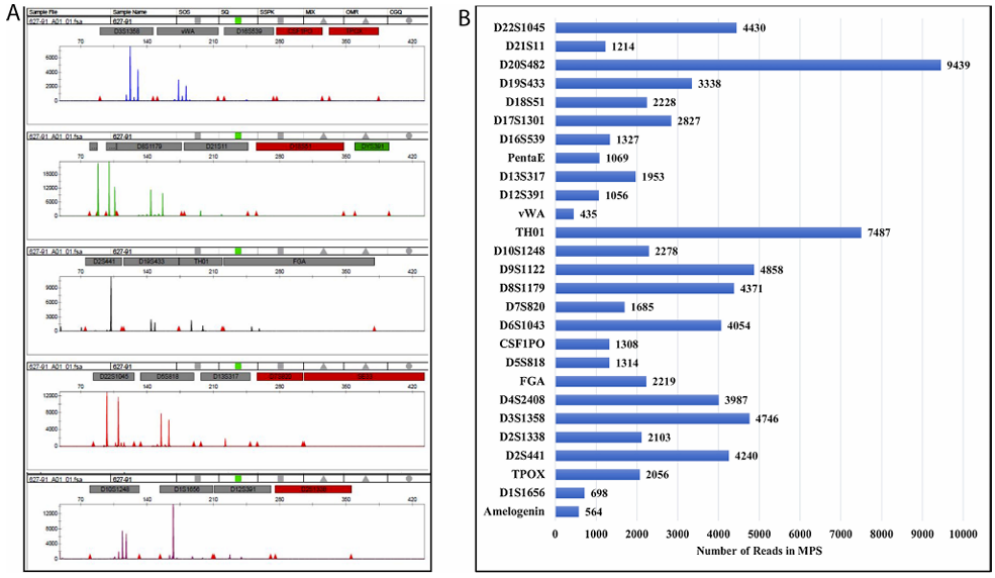

Figure 2. (A & B) DNA Profile of a degraded bone sample (DI = 13) showing a dropout of 7 auSTR loci using GlobalFiler™ kit. MPS results for various markers from the same sample show high efficacy and enhanced discrimination power of Illumina® ForenSeq™ DNA Signature Prep kit.

Biogeographical ancestry of the samples was estimated based on principal component analysis (PCA). The ForenSeq™ UAS created PCA plots that reflected the best-fit population estimate of each sample's biogeographical ancestry, using data from the 1000 Genomes project: Admixed American, African, East Asian, and European. The results indicated that the predominant biogeographical ancestry of the skeletal bones was European, which was consistent with the antemortem records (Figure 3). Furthermore, the MiSeq® FGx™ platform demonstrated the potential to predict hair and eye colour from individual samples using the 22 phenotypic informative (p) SNPs included in the kit (Walsh et al., 2014; Illumina, Inc., 2016). The ForenSeq™ UAS generated individual probabilities for four hair colour categories (black, brown, blonde, and red) and three eye colour categories (brown, blue, and intermediate). To make these estimates, all phenotypic SNP loci had to be detected. In the case of the bone samples, all the samples provided a full profile of the pSNPs (Ekblom et al., 2011).

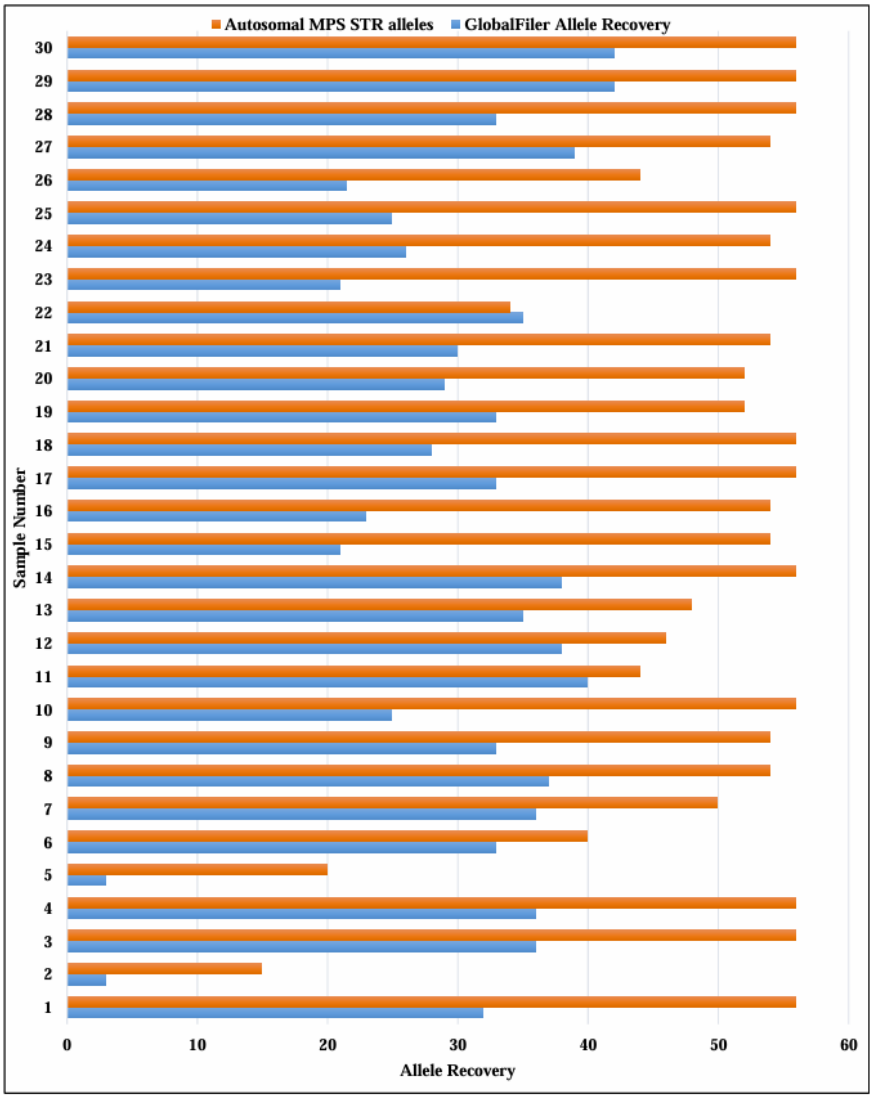

Figure 3. Comparative results of ForenSeq™ DNA Signature kit and GlobalFilerTM kit. The number of alleles recovered for the autosomal STRs included in both kits are shown for 30 bone samples (Figure 2.). The number of markers in the GlobalFiler™ kit were 21 (a maximum of 42 alleles). The number of markers in the ForenSeq™ kit were 27 (a maximum of 54 alleles).

DISCUSSION

In sequencing data, depth of coverage generally refers to the average coverage per base across a sequencing run. However, for forensic applications, targeted sequencing focuses on the amplicon or base count to allow for comparison between alleles or loci and to ensure accurate identification of the present variants. In our study, the depth of coverage for the degraded bone samples was 95.6%, with read depths ranging from 20,000 to 4,000,000 reads, indicating that the MPS-based analyses were highly sensitive. STR allele recovery using MPS was higher than with the GlobalFiler™ kit, for all 30-bone samples in this study. Both positive and negative controls performed as expected. The results were highly informative, displaying the potential of MPS to analyse unidentified human skeletal remains and provide more genetic information from the same initial DNA quantities compared to CE-based analyses. The combined results from all MPS panels included genetic data for 24 Y-STRs, 55 ancestry-informative SNPs, 24 phenotype-informative SNPs, 27 autosomal STRs (including amelogenin), and 8 X-STRs. On average, 2,069 STR loci and 4,546 SNP alleles were detected. Figure 2B illustrates that more loci were detected using the ForenSeq™ DNA Signature kit compared to the GlobalFiler™ kit (Figure 2-3).

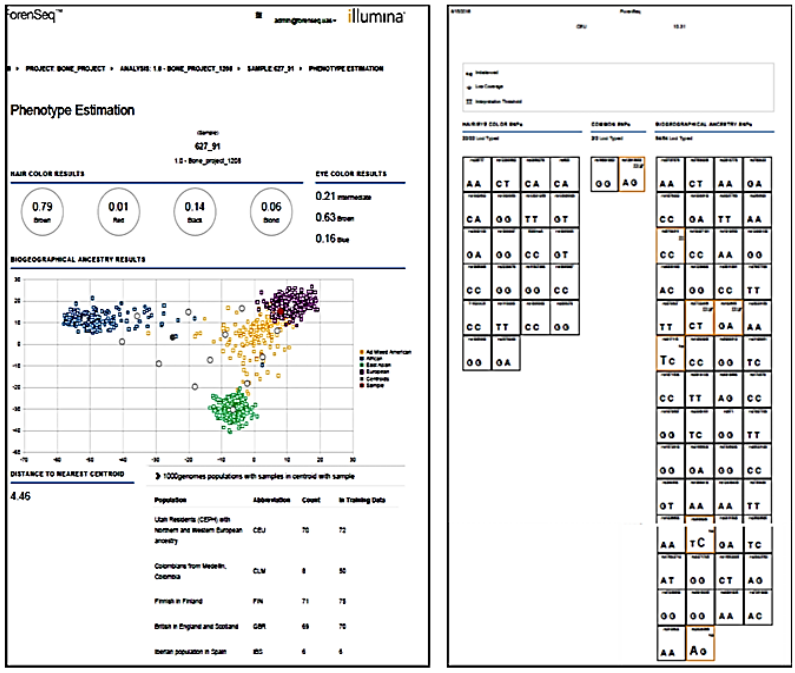

Figure 4. Phenotype estimation of a Serbian bone sample showing a high prediction for blond hair and blue eyes. It was predicted that the sample would have an ancestry of Eastern European population correctly.

An example of an old, highly degraded sample, Case 627-91, which was 28 years old, showed a partial profile with the GlobalFiler™ EPG (Figure 2A-4). However, using the ForenSeq™ DNA Signature kit, a full profile for 27 STRs was obtained. Ancestry-informative SNP results were obtained for all markers in 20 samples, with 10 samples showing a range of 31 to 54 ancestry SNPs. Using this ancestry-informative SNP data, the major biogeographical ancestry of the bone samples was found to be Western European (86% of the samples), followed by Admixed American (7%) and East Asian (7%). Given that the bone cases were from Belgrade, Serbia, this was an expected result.

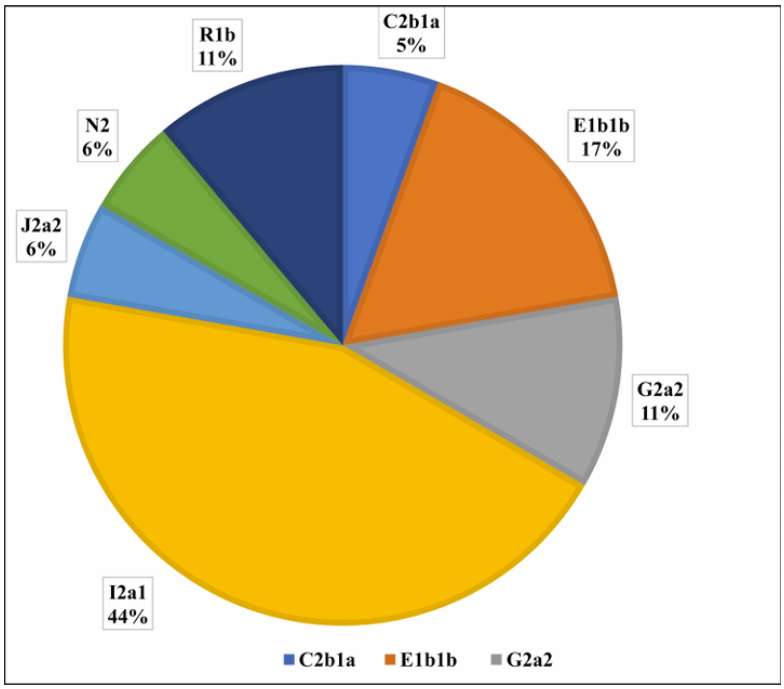

Figure 5. Pie chart showing NevGen Haplogroup Predictor tool results for 18 male bone samples showing the distribution of haplogroups.

The phenotypic SNPs predicted blue eye colour, with the most likely hair colours being blonde or dark blonde/brown for the majority of the samples, in line with the expected phenotypic features (Collins et al., 2003; Walsh et al., 2013). Y-haplogroup predictions were obtained using Nevgen, which indicated that the bone samples were predominantly of Western European ancestry (86%), followed by Northern and Western European (86%), with smaller proportions of Admixed American and European (7%). The data revealed that haplogroup I2a was the most common Y-haplogroup in the European samples studied here, and 44% of the bone samples were predicted to belong to haplogroup I2a (Figure 5), followed by E1b1b (17%), G2a (11%), R1b (11%), J2a (6%), C2b1a (5%), and N2 (6%) (Figure 5). These haplogroups are common in European populations, especially in Serbia. A study by Reguiero et al. (2012) indicated that about 58% of Serbian Y-haplogroups (I1, I2a, and R1a1a) are of ancestries presumed to be pre-Neolithic (Toom et al., 2013). Haplogroup I2a appears to have emerged during the Neolithic period and to be strongly linked to Neolithic cultures in Southeast and North-Western Europe (Toom et al., 2013).

It has been suggested that data should be generated using the limited 150-base single read sequences currently available on the MiSeq platform (Grunenwald et al., 2014; King et al., 2014). In this study, the robustness of the MPS kit was demonstrated for the analysis of partially degraded bone samples, with most loci included in the kit being amplified and providing acceptable results, even with a threshold of 30 reads. If the threshold was lowered to 10 reads, all loci would have been considered as successfully amplified.

In this study, forensic analysis using the ForenSeq™ DNA Signature kit on degraded bone samples demonstrated significantly higher allele recovery for autosomal STRs (auSTRs) compared to the GlobalFiler™ kit. All samples produced concordant results with both the GlobalFiler™ and ForenSeq™ kits. The inclusion of information on visible phenotypic traits, alongside identity and ancestry data, as well as traditional STR markers, greatly enhances the value of MPS analyses in forensic casework. This work highlights the clear advantages of MPS in analysing degraded human remains, providing a greater quantity of relevant information for forensic investigations and great disaster victim identification.

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Original scientific paper *https://doi.org/10.46793/ADS25.10S*

ANALYSIS OF CORRELATIONS BETWEEN BASIC AND DERIVED ANTHROPOMETRIC INDICES AND BODY FAT IN INDIVIDUALS WITH TYPE 2 DIABETES

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Abstract: Anthropometric indices play a key role in medicine, particularly in assessing body composition, obesity, and the risk of metabolic disorders. The aim of this study is to examine the relationship between basic and derived anthropometric indices and body fat percentage in patients with type 2 diabetes. The sample comprised 160 people with an average age of 65.3 years. Body weight, height, waist circumference (WC), and hip circumference (HC) were measured. Body fat percentage was determined using a bioelectrical impedance analysis (BIA) device, Omron BF-511. Derived anthropometric indices were calculated: BMI (body mass index), WHtR (waist-to-height ratio), WHR (waist-to-hip ratio),

BRI (body roundness index), BAI (body adiposity index), CI (conicity index), and AVI (abdominal volume index). Pearson's correlation coefficient was used for statistical analysis. The strongest positive correlation with body fat percentage was shown by BAI ($r=0.553$; $p<0.001$), BMI ($r=0.551$; $p<0.001$), BRI ($r=0.529$; $p<0.001$), and WHtR ($r=0.510$; $p<0.001$), compared to WC ($r=0.362$; $p<0.001$) and HC ($r=0.461$; $p<0.001$), which had weaker correlations than the derived indices, indicating their greater reliability in assessing body composition. On the other hand, WHR ($r=0.052$; $p=0.516$) and CI ($r=0.159$; $p=0.045$) showed the weakest associations, suggesting they are not reliable indicators of body fat. Since certain anthropometric indices exhibited a moderate correlation with the BIA-derived body fat assessment, the findings indicate that additional methods should be utilized to improve the accuracy of body fat estimation.

Key words: anthropometric indices, bioelectrical impedance analysis, type 2 diabetes

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INTRODUCTION

Anthropometric indices are key tools in assessing body composition and identifying the risk of metabolic disorders, including type 2 diabetes, cardiovascular diseases, and other health complications. Type 2 diabetes is the most common form of diabetes, accounting for approximately 90% of all cases, and occurs as a result of insulin resistance, which leads to elevated blood glucose levels (International Diabetes Federation). Obesity is one of the primary risk factors for the development of type 2 diabetes, as excess adipose tissue significantly affects glucose metabolism and further exacerbates insulin resistance (Chandrasekaran & Weiskirchen, 2024).

Although anthropometric indices provide valuable information about body composition and the risk of metabolic diseases, additional methods such as bioelectrical impedance analysis (BIA) allow for more precise assessments of body fat, which is crucial for understanding insulin resistance and metabolic dysfunctions that contribute to type 2 diabetes. Body fat percentage, particularly visceral adipose tissue, plays a crucial role in the development of these disorders, further underscoring the need for precise tools in body composition assessment. Studies have shown that body fat percentages in obese individuals often exceed 25% in men and 30-35% in women (De Lorenzo et al., 2016; Chuan et al., 2022; Salmón-Gómez et al., 2023). Combining BIA with anthropometric indices may further improve body composition assessment and the identification of patients at high risk of complications.

Therefore, the aim of this study was to assess how different anthropometric indices correlate with body fat percentage in patients with type 2 diabetes, which may have important implications for clinical practice.

MATERIALS AND METHODS

Study desing and participans

This cross-sectional study was conducted at the University Clinical Centre of the Republic of Srpska in Banja Luka during 2023. The sample comprised 160 adults diagnosed with type 2 diabetes mellitus, between the ages of 40 and 80. 57.5% were women (N = 92) and 42.5% were men (N = 68). This study was conducted in accordance with the ethical standards established in the Declaration of Helsinki. Ethical approval was obtained from the Ethical Review Committee of the University Clinical Centre of the Republic of Srpska (No. 01-19-420-2/22). Written informed consent was obtained from all the participants prior to data collection.

Anthropometric measurements

All anthropometric measurements were conducted by the same examiner in the morning hours to ensure consistency. Participants were measured wearing light clothing and no shoes. Height was measured using a medical stadiometer (precision 0.1 cm). Weight was measured using the Omron BF-511 digital scale with a precision of 0.1 kg. Waist circumference (WC) and hip circumference (HC) were measured using a non-elastic measuring tape with a precision of 0.1 cm. Body fat percentage (BF, %) was assessed using the Omron BF-511 digital scale (Omron Matsusaka Co, Ltd, Japan), which operates on the bioelectrical impedance analysis principle, emitting a low-intensity electrical current (50 kHz, less than 500 μA) through the participant's body.

Based on basic anthropometric measurements, the following anthropometric indices were calculated: abdominal volume index (AVI); body adiposity index (BAI); body mass index (BMI); body roundness index (BRI); conicity index (CI); waist to height ratio (WHtR) and waist to hip ratio (WHR) (Figure 1.) (Xie et al., 2021).

$$AVI=\frac{2\times WC^2(cm)+0.7\times(WC-HC)^2(cm)}{1000}$$

$$BAI=\frac{HC(m)}{height^{\frac{3}{2}}(m)}-18BMI$$

$$BMI=\frac{weight(kg)}{height^2(m)}$$

$$BRI=364.2-365.5\times\sqrt{1-\frac{\Pi-2\times WC^2(m)\times height^{-2}(m)}{}}$$

$$CI=0.109^{-1}\times WC(m)\times\sqrt{\frac{weight(kg)}{height(m)}}$$

$$WHtR=\frac{WC(cm)}{height(cm)}$$

$$WHR=\frac{WC(cm)}{HC(cm)}$$

Figure 1. Equations of derived anthropometric indices

Slika 1. Formule za izvedne antropometrijske indekse

Statistical analysis

Statistical analysis was performed using SPSS software (version 25). Descriptive statistics were used to summarize data, including mean (M), standard deviation (SD), and 95% confidence intervals (CI) for continuous variables. Independent samples t-test was used to assess sex differences. For correlation analysis, the Pearson correlation coefficient was used to examine the relationship between body fat percentage and anthropometric indices. Statistical significance was set at $p < 0.05$.

RESULTS

Descriptive statistics for all the participants are presented in Table 1. The mean age of participants was 65.3 years, while the average height and body weight were 169.38 cm and 88.45 kg, respectively. The mean BMI was 30.78 kg/m², with an average WC of 102.74 cm, WHR of 0.99, and WHtR of 0.61.

Table 1. Descriptive statistics of the total sample (N = 160)

Tabela 1. Deskriptivna statistika ukupnog uzorka (N = 160)

Variable	N	Mean	SD	Confidence interval 95%	
				Lower CI	Upper CI
Age (years)	160	65.3	8.343	63.99734767	66.60265233
Height (cm)	160	169.384	8.713	168.0235769	170.7444231
Weight (kg)	160	88.448	14.92	86.11843357	90.77756643
BMI (kg/m²)	160	30.784	4.472	30.08575462	31.48224538
WC (cm)	160	102.743	11.354	100.9702187	104.5157813
HC (cm)	160	103.494	7.781	102.2790968	104.7089032
WHR	160	0.994	0.096	0.979010833	1.008989167
WHtR	160	0.608	0.069	0.597226536	0.618773464
BF (%)	160	35.074	7.673	33.87595957	36.27204043
BRI	160	5.693	1.611	5.441463035	5.944536965
BAI	160	29.205	5.315	28.37513099	30.03486901
CI	160	1.308	0.085	1.294728341	1.321271659
AVI	160	21.439	4.72	20.7020326	22.1759674

The values of anthropometric characteristics presented according to sex are shown in Table 2. No statistically significant difference in age was observed between men and women. However, men exhibited significantly higher mean values for height and body weight compared to women ($p < 0.001$ and $p = 0.001$, respectively). Men also demonstrated significantly higher WHR values than women ($p < 0.001$). In contrast, women had significantly higher mean values for HC ($p < 0.001$), WHtR ($p = 0.003$), BF ($p < 0.001$), BRI ($p = 0.002$), and BAI ($p < 0.001$). Additionally, the mean BMI was significantly higher in women compared to men ($p = 0.018$). No significant differences between sexes were found for WC, CI or AVI. These findings highlight the presence of notable sex-related differences across most of the analyzed anthropometric parameters.

Table 2. Sex-related differences in anthropometric parameters

Tabela 2. Polne razlike u antropometrisjkim karakteristikama

Variable	Male		Female		t	p
	M	SD	M	SD		
Age (years)	66.261	8.516	64.571	8.182	1.271	0.206
Height (cm)	176.47	5.855	164.009	6.363	12.696	**<0.001**
Weight (kg)	92.872	14.459	85.092	14.454	3.371	**0.001**
WC (cm)	103.84	10.828	101.909	11.728	1.067	0.288
HC (cm)	100.18	6.034	106.004	8.042	-5.231	**<0.001**
WHR	1.036	0.079	0.962	0.096	5.304	**<0.001**
WHtR	0.589	0.063	0.622	0.071	-3.053	**0.003**
BF (%)	29.886	6.564	39.009	5.929	-9.203	**<0.001**
BRI	5.255	1.447	6.025	1.656	-3.075	**0.002**
BAI	24.801	3.088	32.544	4.07	-13.68	**<0.001**
CI	1.317	0.084	1.301	0.086	1.158	0.249
AVI	21.852	4.711	21.126	4.728	0.963	0.337
BMI (kg/m²)	29.821	4.05	31.500	4.65	2.391	**0.018**

Legend: M - mean; SD – standard deviation; t – t test value; p – value statistically significant at $p < 0.05$

A statistically significant positive correlation was identified between body fat percentage and body weight ($r = 0.216$; $p = 0.006$), WC ($r = 0.362$; $p < 0.001$), HC ($r = 0.461$; $p < 0.001$), WHtR ($r = 0.510$; $p < 0.001$), BMI ($r = 0.551$; $p < 0.001$), BRI ($r = 0.529$; $p < 0.001$), BAI ($r = 0.553$; $p < 0.001$), CI ($r = 0.159$; $p = 0.045$), and AVI ($r = 0.354$; $p < 0.001$).

Conversely, a significant negative correlation was found between BF and height ($r = -0.372$; $p < 0.001$), while age and WHR showed no statistically significant association with body fat ($p > 0.05$) (Table 3).

Table 3. Pearson correlation coefficients between body fat and anthropometric characteristics

Tabela 3. Pirsonovi koeficijenti korelacije između procenta tjelesne masti i antropometrijskih karakteristika

Variable	Body fat (%)	
	r	p - value
Age (years)	- 0.009	0.915
Height (cm)	- 0.372**	0.000
Weight (kg)	0.216**	0.006
WC (cm)	0.362**	0.000
HC (cm)	0.461**	0.000
WHR	0.052	0.516
WHtR	0.510**	0.000
BMI (kg/m²)	0.551**	0.000
BRI	0.529**	0.000
BAI	0.553**	0.000
CI	0.159*	0.045
AVI	0.354**	0.000

Legend: r - Pearson correlation coefficient; * statistically significant at the $p < 0.05$; ** statistically significant at the $p < 0.01$

DISCUSSION

According to data from the World Health Organization (WHO), obesity and diabetes are increasingly significant public health concerns due to their contribution to the global burden of disease. In the 2022 report, it was stated that over 2.5 billion adults had excessive body weight, including 890 million individuals with obesity. This alarming rise in obesity is closely linked to the growing prevalence of T2D, a condition that frequently coexists with obesity and other metabolic disorders.

Anthropometric indices such as BMI, WC, HC, WHR and WHtR have proven useful in assessing the risk of insulin resistance, prediabetes, metabolic syndrome, and T2D development (Liu et al., 2024; Vera-Ponce et al., 2024; Ceolin et al., 2019; Jayedi et al., 2022; Sadeghi et al., 2024). Although these indicators are widely used in population studies for identifying individuals at risk, fewer studies have focused on the anthropometric characteristics of individuals already diagnosed with T2D.

The results of this study suggest that certain anthropometric indices may be reliable indicators of body composition in patients with T2D, emphasizing the need for a comprehensive approach in risk assessment and intervention planning.

In our sample, the average age of participants was 65.3 years, with a mean body weight of 88.4 kg and a BMI of 30.8 kg/m², which classifies the participants as obese according to WHO criteria. These demographic characteristics were expected since older population is more likely

to exhibit higher body weight and BMI values. Amiri et al. (2021) reported similar findings in their study of the morphological characteristics of individuals with T2D. BMI is widely used in population studies due to its simplicity and is most often employed as an indicator of nutritional status and a risk factor for various health conditions, including T2D and cardiovascular diseases. However, the primary limitation of BMI is its inability to provide information about fat distribution within the body (Nuttal, 2015).

This similarity in results reinforces the frequent association between T2D and elevated BMI values, which is expected given that obesity is one of the key risk factors for the development of this condition.

Fat distribution has been shown to be crucial in assessing the risk of metabolic complications, with WC, HC, and their ratios (WHR and WHtR) identified as significant indicators. In our study, WC values were elevated above the established risk thresholds (Ness-Abramof & Apovian, 2008), confirming an increased risk of metabolic complications. The mean WHtR value exceeded the critical threshold of 0.6, which according to previous research (Amiri et al., 2021) indicates a significantly elevated risk of metabolic disorders. WHtR has proven to be a superior predictor of T2D compared to BMI and WC, as it accounts for both central fat distribution and individual height differences (Skogberg et al., 2018). Additionally, WHR values in our study were above the recommended thresholds established by the WHO, further confirming an increased risk in both sexes. These results highlight the importance of parameters assessing central obesity in identifying individuals at increased risk of metabolic complications.

The BIA method used to assess body fat percentage in our population revealed values of 29.886% in men and 39.009% in women, which according to established literature criteria indicates the presence of obesity (Chuan et al., 2022; Salmón-Gómez et al., 2023). It is well known that older women naturally have higher body fat percentages compared to men, due to hormonal changes, muscle mass reduction, and fat redistribution with age. This process is further intensified in individuals with T2D, given that diabetes is often accompanied by increased body weight and unfavorable changes in body composition. Sex-based differences in body fat distribution are well documented, with men typically exhibiting higher levels of visceral fat, whereas women tend to accumulate more subcutaneous fat, particularly in the gluteofemoral region (Stevens, Katz, & Huxley, 2010; Gavin & Bessesen, 2020).

In addition to traditional indices, advanced anthropometric indices such as BAI, BRI, CI, and AVI have demonstrated greater reliability in identifying individuals at increased risk of metabolic disorders. Studies conducted in various populations, including the United Kingdom and Jordan, have highlighted the superior predictive ability of WHtR and BRI compared to traditional measures in assessing T2D risk (Boonpor et al., 2023; Khader et al., 2019). Similar findings were observed in a South African study among adult women, where both traditional and newer indices, including WC, WHtR, AVI, BRI, and CUN-BAE, showed comparable predictive ability for identifying T2D (Sekgala et al., 2024).

Abdominal volume index was developed as a tool for assessing abdominal fat volume and has shown a strong association with glucose intolerance and T2D (Guerrero-Romero & Rodríguez-Morán, 2003). In our study, AVI values were higher compared to previous reports (Liu et al., 2021), which may indicate a higher prevalence of abdominal obesity or differences in sample demographics. Similar sex-based differences were not statistically significant, consistent with findings by Khan et al., (2019).

Body roundness index designed to estimate body roundness using height and WC, has demonstrated associations with metabolic syndrome and cardiovascular risk (Xu et al., 2021). In our study, BRI values were higher than those reported by Liu et al. (2019) but remained above the threshold for elevated metabolic risk (Motamed et al., 2016), confirming its value

as a risk indicator. Women exhibited higher BRI values than men, which is consistent with findings by Sadeghi et al., (2024).

Conicity index a marker of abdominal obesity and cardiometabolic risk (Valdez, 1991), showed values similar to those reported by Liu et al. (2021), confirming its consistency in assessing abdominal obesity. No significant sex differences were observed, aligning with the findings of Khan et al. (2019).

Body adiposity index developed as an alternative method for assessing body fat (Bergman et al., 2011), showed values similar to those reported by Liu et al. (2021), reinforcing its reliability. Consistent with previous studies, women exhibited higher BAI values than men (Sadeghi et al., 2024).

According to the available literature and the authors' knowledge, this is the first study to analyze correlations between basic and derived anthropometric indices and body fat percentage in T2D patients in the Republic of Srpska, conducted within the University Clinical Center of the Republic of Srpska (UKC RS).

Pearson's correlation analysis revealed the strongest positive correlations for BAI, BMI, BRI and WHtR, indicating a moderate association between these indices and body fat percentage. Moderate correlations were found for WC, HC, and AVI. These correlations were weaker compared to indices such as BAI and BMI. The weakest correlations with body fat percentage were observed for CI and WHR while age was not significantly correlated with body fat percentage ($p > 0.05$). The negative correlation between height and body fat may be attributed to differences in body composition, metabolic efficiency, and fat distribution patterns in taller individuals.

Ehrampoush et al., (2016) conducted a study involving 1,360 healthy participants in Iran to examine the relationship between various anthropometric indices and body fat percentage. This study analyzed multiple indices, including BMI, WHtR, AVI, BAI, and ABSI. The results indicated that BMI, WHtR, and AVI showed the strongest correlation with body fat percentage, demonstrating the highest accuracy in body fat assessment of both sexes. This study is particularly significant as it was conducted on a healthy population, emphasizing the role of these indices in preventive medicine and metabolic risk assessment. Compared to our study, which focused on T2D patients, these findings highlight the consistency of certain indices (such as BMI and WHtR) as reliable indicators of body composition across different populations.

CONCLUSION

Our findings suggest that BMI, BAI, BRI, and WHtR are the most reliable indicators of body composition in T2D patients. Given the significant sex differences observed in index values, future research should further explore these differences and identify the most effective methods for assessing body composition in various populations.

However, certain limitations should be acknowledged. The relatively small sample size may limit the generalizability of the results, warranting larger-scale studies. Additionally, BIA, although practical, has limitations in precision. Therefore, combining BIA with advanced techniques such as DEXA scanning may provide a more accurate assessment of body composition. Despite these limitations, our findings provide a valuable basis for future studies aimed at identifying reliable indicators of body composition in individuals with T2D.

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ANALIZA KORELACIJA IZMEĐU OSNOVNIH I IZVEDENIH ANTROPOMETRIJSKIH INDEKSA I TJELESNE MASTI KOD OSOBA S DIJABETESOM TIP 2

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Apstrakt: Antropometrijski indeksi imaju ključnu primjenu u medicini posebno u procjeni tjelesne kompozicije, gojaznosti i rizika od metaboličkih oboljenja. Cilj ovog istraživanja je da se ispita povezanost osnovnih i izvedenih antropometrijskih indeksa sa procentom tjelesne masti kod pacijenata sa dijabetesom tipa 2. Istraživanje je sprovedeno na uzorku od 160 ispitanika, prosječne dobi 65,3 godina. Izmjereni su tjelesna masa, visina, obim struka (OS) i obim kukova (OK). Procenat tjelesne masti je određen aparatom za bioimpedansnu analizu (BIA) Omron BF-511. Izračunati su izvedeni antropometrijski indeksi: BMI (body mass index), WHtR (waist to height ratio), WHR (waist to hip ratio) BRI (body roundness index), BAI (body adiposity index), CI (conicity index), AVI (abdominal volumen index). Za statističku analizu korišten je Pirsonov koeficijent korelacije. Najjaču pozitivnu korelaciju sa procentom tjelesne masti pokazali su BAI (r=0.553; p<0,001), BMI (r=0.551; p<0,001), BRI (r=0.529; p<0,001) i WHtR (r=0.510; p<0,001), u odnosu na OS (r=0.362; p<0,001) i OK (r=0.461; p<0,001) koji su imali slabiju korelaciju u odnosu na izvedene indekse, što ukazuje na njihovu veću pouzdanost u procjeni tjelesne kompozicije. S druge strane, WHR (r=0.052; p=0,516), CI (r=0.159; p=0,045) imali su najslabiju povezanost, sugerišući da nisu pouzdani pokazatelji tjelesne masti. S obzirom na to da su određeni antropometrijski indeksi pokazali srednje jaku korelaciju s BIA procjenom masnog tkiva, rezultati sugerišu da za precizniju procjenu masnog tkiva treba koristi i druge metode.

Ključne riječi: antropomterijski indkesi, bioimpendansna analiza, dijabetes tip 2

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KULTURNA USLOVLJENOST RAZVOJA I UČESTALOSTI POJEDINIH MODALITETA NESIGURNOG AFEKTIVNOG VEZIVANJA

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Apstrakt: Teorija afektivne vezanosti pruža osnovu za razumevanje duboke emocionalne povezanosti između deteta i njegovog primarnog negovatelja, koja značajno utiče na dalji socio-emocionalni razvoj tokom života. Jednim od najvećih nedostataka ove teorije dugo se smatrala njena zapadnoevropska orijentacija, budući da se temelji na istraživanjima rađenim u Americi i Engleskoj. Početak istraživanja na drugim podnebljima značio je početak ispitivanja varijacija u obrascima afektivne vezanosti u različitim kulturama.

Cilj ovog rada jeste prikaz i diskusija o rezultatima nekih dosadašnjih istraživanja u oblasti kros-kulturalnog pristupa teoriji afektivne vezanosti, odnosno onih rezultata koji sugerišu da su određeni nesigurni obrasci tipični za neku kulutru.

Takva istraživanja došla su do zanimljivih otkrića, koja se pre svega tiču kulturne uslovljenosti razvoja i učestalosti pojedinih modaliteta nesigurnog afektivnog vezivanja.

Osim toga, navedeni nalazi otvaraju novu, veoma važnu diskusiju: da li su ljudi kod kojih je dominantan neki od nesigurnih obrazaca nužno socijalno „neuspešni“, ukoliko su rođeni i odrastali unutar kulturnog konteksta koji podstiče razvoj nekog od nesigurnih obrazaca? Postoje osnove za pretpostavku da će ti ljudi, iako su nesigurno vezani, živeti produktivan socijalni život, bez emocionalnih problema, baš zato što su usvojili vrednosti i norme sopstvene sredine.

Navedeni nalazi su posebno značajni jer doprinose prevazilaženju etnocentrizma, podstičući kulturno osetljivu primenu teorije afektivne vezanosti u naučnim i kliničkim praksama. U antropološkom smislu, oni pozivaju na takav pristup koji otvara put prilagođavanju jedne univerzalne psihološke teorije specifičnostima svake kulture, i razumevanju njenog uticaja na socio-emocionalni razvoj čoveka.

Ključne reči: afektivna vezanost, obrasci vezanosti, kultura, norma, etnocentrizam.

UVOD

Afektivna vezanost se odnosi na „specifičan odnos koji se u najranijem detnjstvu formira između majke i deteta i traje kroz čitav život, kao trajna psihološka veza uspostavljena između dvoje ljudi“ (Holmes, 2004; nav. prema Stefanović-Stanojević, 2015: 17). Osnivač ove teorije, Džon Bolbi, smatrao je da je majka primarna figura afektivne vezanosti deteta, pre svega zbog primarne brige koju mu pruža nakon rođenja. U vreme nastanka ove teorije, majka se smatrala primarnim negovateljem deteta, a samim tim i primarnom figurnom afektivne vezanosti. Međutim, usled velikih društvenih promena u savremenom svetu, a pre svega transformacije

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porodice i porodičnih odnosa, primarna figura afektivne vezanosti suštinski može postati bilo ko ko u najranijem razvojnom periodu preuzme brigu o detetu.

Kvalitet afektivne vezanosti zavisi od vremena koje primarni negovatelj provede sa detetom, pažnje koju ulaže u reagovanje na potrebe deteta, kvaliteta emocionalnog odgovora i posvećenosti detetu i dugoročne dostupnosti detetu tokom života (Colin, 1996; Cassidy, 1999; nav. prema Zastrow & Kirst-Ashman, 2007: 111).

Definisana su četiri obrasca afektivne vezanosti (Lamb, M. et al., 1984):

1. Nesigurni/izbegavajući obrazac (tip A) karakteriše dosledna emocionalna nedostupnost primarnog negovatelja, na osnovu čega dete dolazi do zaključka da drugi nisu osobe od poverenja i nastoji da podigne zaštitnu „barijeru“ oko sebe.
2. Sigurni obrazac (tip B) podrazumeva postojanje modela primarnog negovatelja kao dosledno dostupnog i responzivnog na detetove signale, što kod deteta dovodi do formiranja pozitivnih slika o sebi i drugima.
3. Nesigurni/preokupirani obrazac (tip C) odlikuje nedosledna dostupnost primarnog negovatelja. Dete pronalazi strategije kojima može da pridobije negovateljevu pažnju, dok je njegova slika o sebi „obojena“ upravo tom borbom i preteranom emocionalnom vezanošću za negovatelja. To se kasnije preslikava i na druge emocionalne odnose, gde osoba postaje kontrolišuća i preterano fokusirana na buduće figure afektivne vezanosti, a malo na sebe ili sredinu.
4. Nesigurni/dezorganizovani obrazac (tip D) nije bio uključen u prvobitnu klasifikaciju, budući da su ovde svrstana deca koja su pokazala neobična ponašanja. Kod ove dece narušena je pozitivna slika o sebi i drugima, ali ne postoji jasna strategija za prevazilaženje stresa, a najčešće se razvija kao posledica traumatskog iskustva.

Teorija afektivne vezanosti pruža značajan doprinos u razumevanju uticaja ranih odnosa sa primarnim negovateljima na emocionalni razvoj i socijalno funkcionisanje dece, čime u velikoj meri doprinosi kvalitetu savetodavnog, psihoterapijskog, pedagoškog i socijalnog rada. Istraživanja su pokazala da deca koja razviju siguran obrazac imaju više pozitivnih socijalnih interakcija s vršnjacima u poređenju sa nesigurno vezanima, a kako odrastaju, lakše formiraju bliska prijateljstva (Schneider, Atkinson & Tardif, 2001; nav. prema Zastrow & Kirst-Ashman, 2007).

Upravo zbog široke mogućnosti primene Bolbijevih i saznanja Ejnsvortove unutar psihološke prakse, a zatim i prakse drugih pomagačkih profesija poput socijalnog rada, istraživači su nastojali da formulišu čitav spektar instrumenata i tehnika kojima može biti procenjivana afektivna vezanost. Neki od njih su: strana situacija, AQS tehnika, tehnika AEED, Affect task, tehnika CAI, tehnika AAI i dr. (opširnije: Stefanović-Stanojević, 2015)

Nedugo nakon nastanka prvih instrumenata za procenu afektivne vezanosti, pred istraživačima se javila značajna dilema, koja je podstakla veliki broj budućih istraživanja: da li su razvoj i učestalost pojedinih obrazaca afektivne vezanosti na bilo koji način kulturno uslovljeni? Jedno od takvih istraživanja sproveli su Ajzendorn i Sagi (IJzendoorn & Sagi) 1999. godine, kada su otkrili da afektivno vezivanje ima karakter univerzalnosti, te da sigurni obrazac jeste norma (što je postao široko prihvaćen stav), ali da ostali (nesigurni) obrasci značajno odstupaju od norme i da je njihov razvoj kulturno uslovljen (nav. prema Stefanović-Stanojević, 2015).

METOD

U skladu sa navedenim, predmet ovog rada jeste kulturna uslovljenost razvoja i učestalosti pojedinih modaliteta nesigurnog afektivnog vezivanja, dok se problem istraživanja tiče upravo uticaja kulture na to kako će se ovi obrasci formirati i koliko će biti zastupljeni u pojedinim kulturnim sredinama. Cilj rada jeste prikaz i diskusija rezultata nekih dosadašnjih istraživanja u oblasti kros-kulturalnog pristupa teoriji afektivne vezanosti, odnosno onih rezultata koji sugerišu da su određeni nesigurni obrasci tipični za neku kulutru ili da čak pogoduju toj kulturi.

REZULTATI

Dosadašnja istraživanja pokazala su da je unutar ispitivane populacije dominantan obrazac afektivne vezanosti najčešće sigurni obrazac, zatim izbegavajući, preokupirani i na kraju dezorganizovani (van Ijzendoorn & Kroonenberg, 1988). Ono što je bio glavni nedostatak ovih nalaza jeste pre svega područje gde su istraživanja rađena – u pitanju su Evropa i Amerika. Iako se rani radovi Ejnsvortove vezuju za Ugandu, najveći deo svog naučnoistraživačkog rada ona je obavila u Americi, dok je Bolbi svoju teoriju razvio u Engleskoj. Teorija je, dakle, nastala na ovim područjima i to jeste doprinelo njenoj „obojenosti“ zapadnoevropskom kulturom. Bolbi je, ipak, na samom početku naglasio da formiranje obrazaca afektivne vezanosti u izvesnoj meri zavisi od kulture majke, dok je Ejnsvortova postulirala da na formiranje obrasca afektivne vezanosti utiču ne samo genetski, već i socijalni i sredinski faktori (Bowlby, 1969; Ainswort, 1967; nav. prema Agishtein & Brumbaugh, 2013).

Početak istraživanja afektivne vezanosti na drugim kontinentima podstakao je pre svega modifikaciju instrumenata za procenu, a zatim je doveo u pitanje normativnost obrazaca afektivne vezanosti. Važni nalazi u skladu sa time jesu oni koji su pokazali da sigurna afektivna vezanost jeste norma, ali da to ne važi i za ostala tri nesigurna obrasca (Van IJzendoorn & Kroonenberg, 1988; Agishtein & Brumbaugh, 2013. i dr.).

Naime, metaanalizom oko 2.000 istraživanja koja su koristila klasifikaciju na osnovu „Strane situacije“ (nije bio uključen dezorganizovani obrazac)[1] iz osam različitih zemalja, istraživači su došli do rezultata koji ukazuju na to da je „tip A“ (nesigurna/izbegavajuća vezanost), nakon sigurne, dominantna vezanost u zapadnoevropskim zemljama, dok je u Aziji zastupljeniji „tip C” (nesigurna/ preokupirana vezanost) (Van IJzendoorn & Kroonenberg, 1988). Ajzendorn je 1992. godine nadgradio rezultate ovog istraživanja analizirajući 21 američku studiju i potvrdio veću prisutnost izgavajućeg (21%) u odnosu na preokupirani (12%) obrazac afektivne vezanosti (nav. prema Stefanović-Stanojević, 2015). Osim toga, istraživanje koje su radili Grosman i saradnici (Grossmann et al.) 1985. godine u Severnoj Nemačkoj na uzorku od 46 parova majki i dece otkrili su izuzetno visok procenat dece izbegavajuće vezanosti, čak 52%, dok je preokupiranih bilo svega 13% (nav. prema Keller, 2012).

Sa druge strane, Takašijeva studija iz Pariza (Takahashi, 1986) na uzorku od 60 parova japanskih majki i dece pokazala je da je među decom dominantan sigurni obrazac afektivne

[1] Ova tehnika podrazumeva odigravanje mini-drame, gde su u jednoj prostoriji prisutni majka, dete, stranac i posmatrač. Majka povremeno izlazi iz prostorije i vraća se, što znači da dete u nekim trenucima ostaje samo sa strancem. Posmatrač beleži reakcije deteta na separaciju, te se na osnovu tih reakcija procenjuje afektivna vezanost deteta (Stefanović-Stanojević, 2015: 32-35).

vezanosti (68%). Međutim, unutar grupe nesigurno vezanih nije bilo izbegavajućeg, već isključivo preokupiranog obrasca. Slični rezultati dobijeni su i u okviru Sagijeve studije (Sagi et al, 1985) u Izraelu (nav. prema Keller, 2012).

Kada je reč o Africi, istraživanje rađeno od strane Trua (True) 1994. godine unutar plemena Dogon (država Mali) pokazalo je veliki udeo preokupirano vezane dece (25%), dok izbegavajuće vezane dece nije bilo (nav. prema Stefanović-Stanojević, 2015).

Konačno, australijska studija rađena na uzorku od 134 majke i bebe u Sidneju pokazala je da je 70% beba sigurno vezano za svoje majke, dok je 30% nesigurno vezano (16% je izbegavajuće, 14% preokupirano) (Barnett et al., 1987).

Veoma interesantni podaci tiču se upravo rezultata istraživanja rađenih u Nišu, Skoplju i Banjoj Luci. Naime, istraživanje Stefanović–Stanojević rađeno 2007. godine na uzorku od ukupno 100 ispitanika iz ovih gradova pokazalo je da je najveći udeo sigurno vezanih (39%), zatim preokupirano (26%), dezorganizovano (20%) i izbegavajuće (15%). Autorka navodi da je najviše preokupirano vezanih u tom trenutku bilo u Nišu, ali upozorava na činjenicu da je njeno kasnije istraživanje, sprovedeno 2011. godine, ukazalo na porast učestalosti dezorganizovanog obrasca afektivne vezanosti u ovom gradu (Stefanović-Stanojević, 2011).

DISKUSIJA

Rezultati prikazanih istraživanja pokazali su da postoje značajne kulturne razlike kada je reč o učestalosti pojedinih modaliteta nesigurnog afektivnog vezivanja. Kako navodi Stefanović-Stanojević „moglo bi se reći da sve distribucije van Zapadne Evrope i Amerike imaju naglašeniji preokupirani od izbegavajućeg obrasca“ (Stefanović-Stanojević, 2015: 46).

Postoji više mogućih kulturno determinisanih objašnjenja takvih nalaza. Neki istraživači smatraju da su etnička pripadnost, religija i duh individualizma ili kolektivizma ključni faktori koji određuju relacione modele i način „socijalizacije“ emocija, a samim tim i formiranje obrazaca afektivne vezanosti kod dece (Agishtein & Brumbaugh, 2013). Domaći autori dodaju da siromaštvo, takođe, može dovesti do povećane zastupljenosti preokupiranog obrasca afektivne vezanosti, zbog izražene potrebe za udruživanjem i ostajanjem u zajednici radi opstanka. Sa druge strane, ratne okolnosti u kojima deca odrastaju i kolektivno sećanje obojeno njima mogu proizvesti dezorganizovani obrazac afektivne vezanosti. Osvrtom na istorijski poznate geopolitičke činjenice, na ovaj način se možda mogu objasniti rezultati dobijeni na teritoriji naše zemlje.

Deo kulturnih razlika jeste i drugačiji sistem vrednosti, te ono što se u jednoj kulturi smatra nepoželjnim ili čak patološkim oblikom ponašanja, u drugoj kulturi može se smatrati poželjnim i visoko vrednovati. Na primer, izrazito bliski odnosi majke i dece u zapadno orjentisanoj kulturi spadaju u red nepoželjnih obrazaca ponašanja, dok se u drugim nezapadnim sredinama smatraju veoma cenjenom praksom (Keller, 2012). Slična situacija se primećuje kada je reč o vrednosnim sistemom u Nemačkoj u poređenju sa, na primer, japanskom kulturom. Naime, u Nemačkoj se neguje duh individualizma, ranog osamostaljivanja dece i sticanja nezavisnosti, dok japanska filozofija odgoja naglašava značaj bliskih porodičnih veza i lojalnosti, a posebno kada je reč o odnosu majke i deteta. Osim toga, praksa koju su istraživači opazili u Japanu, ali i u Izraelu, jeste retko izlaganje dece strancima, koje se verovatno javlja kao vid zaštite, zbog čega deca pokazuju visok stepen anksioznosti prilikom susreta sa nepoznatim ljudima. Budući da je to jedna od odlika preokupiranog obrasca, može se zaključiti da roditeljski postupci oblikovani kulturnim normama svakako utiču na prevagu preokupiranog nad izbegavajućim obrascem afektivne vezanosti u nezapadnim sredinama (Keller, 2012).

Kada je reč o Africi, važno je ukazati na jednu specifičnost prakse u odgoju dece. Naime, decu u pomenutom plemenu Dogon odgaja u proseku oko četrnaest hraniteljica, što znači da deca nemaju prilike da se vežu isključivo za jednu negovateljsku figuru. Sa druge strane, deca imaju kontakt sa nekom od figura afektivne vezanosti skoro dvadeset sati dnevno, što bi, zajedno sa kolektivističkim duhom unutar ove plemenske zajednice, moglo objasniti prevagu preokupiranog obrasca afektivne vezanosti nad izbegavajućim (Stefanović-Stanojević, 2011).

Konačno, rezultati dobijeni istraživanjem u Australiji mogli bi se, takođe, tumačiti u kros-kulturnom kontekstu. Primećeno je da, kao i svuda u svetu, u Australiji dominira sigurni obrazac afektivne vezanosti kod dece. Međutim, primećeno je i to da je učestalost preokupiranog i izbegavajućeg obrasca skoro identična. Australija jeste kontinent naseljen pretežno engleskim, škotskim i irskim stanovništvom, ali se 2005. godine susrela sa velikim migratornim kretanjima, tokom kojih se u zemlju doselio veliki broj ljudi iz Afrike, Azije, Južne Amerike i Južne Evrope. To bi značilo da je Australija danas izrazito multikulturalno područje. Navedene osobenosti ovog kontinenta bi mogle obajsniti blago povećanu zastupljenost izbegavajuće vezanih, ali i nedostatak ubedljive prevage bilo kog modaliteta nesigurnog obrasca afektivne vezanosti.

Nalazi prikazani u ovom radu otvaraju pitanje da li pojedinci, koji su razvili neki modalitet nesigurnog obrasca afektivne vezanosti u skladu sa načinom društvenog funkcionisanja u njihovoj kulturi, mogu produktivno da žive i rade. Osim toga, ovi nalazi objašnjavaju prevagu preokupiranog nad izbegavajućim obrascem afektivne vezanosti kod dece koja žive van Zapadne Evrope i Amerike. Konačno, oni podstiču uzdržavanje od proglašavanja pojedinih oblika ponašanja kao univerzalno patoloških, pre svega u sferi brige o potomstvu i načina organizacije društvenog života.

Zanemarivanje ovog područja, odnosno tumačenja teorije afektivne vezanosti u kros-kulturalnom kontekstu, nesumnjivo može voditi etnocentrizmu. Ovaj termin odnosi se na postojanje uverenja da je sopstvena kultura superiorna u odnosu na druge i da predstavlja standard za procenu drugih kultura, što često dovodi do predrasuda i diskriminacije (Zastrow & Kirst-Ashman, 2007). Postoje autori koji upozoravaju na to da psiholozi i drugi stručnjaci koji se bave pomagačkim profesijama često nisu u dovoljnoj meri svesni etnocentrične pristrasnosti unutar onoga što pojedine teorije predstavljaju kao „normalno“ ili „univerzalno“ (Fracasso, Lamb, Scholmerich, & Leyendecker, 1997; nav. prema Agishtein & Brumbaugh, 2013). Ovakvo stanje pogoduje razvoju stigmatizacije, koja za sobom povlači predrasude, stereotipe, kršenje ljudskih prava, diskriminaciju, nasilje, marginalizaciju i socijalno isključivanje pojedinaca ili čitavih društvenih grupa.

Jedan od važnih oblika prevencije navedenih socijalnih problema jeste upravo razvoj kulturno kompetentne prakse. Budući da se usled naglih i nepredvidivih društvenih promena učestalost interkulturne interakcije povećava, posebno je važno osloniti se na multikulturalnu perspektivu kada je reč o razumevanju svakog aspekta čovekovog života, pa i razvoja njegove emocionalnosti.

Obrazac afektivne vezanosti temelji se na formiranju slike o sebi i drugima, što zavisi od interakcija sa primarnim negovateljima. Budući da ponašanje pripadnika jedne kulture, pa i roditelja kao prenosioca tekovina te kulture na dete, zavise od dominantnog vrednosnog sistema, može se očekivati da će formiranje obrasca afektivne vezanosti zavisiti od kulture u kojoj dete odrasta (Agishtein & Brumbaugh, 2013).

Brojna istraživanja su potvrdila ovu postavku, pokazavši da način života i vrednosti koje se neguju unutar jednog kulturnog konteksta u značajnoj meri determinišu nastojanje roditelja da te iste vrednosti prenesu na svoju decu od najranijih dana, čime spontano utiču i na fomiranje obrasca afektivne vezanosti kod dece.

Bavljenje kulturnim aspektom formiranja i učestalosti pojedinih modaliteta nesigurnih obrazaca afektivne vezanosti doprinelo bi razrešenju važne dileme: da li su ljudi kod kojih je dominantan neki od nesigurnih obrasca nužno socijalno „neuspešni“, ukoliko su rođeni i odrastali unutar kulturnog konteksta koji podstiče (ili čak vrednuje) razvoj nekog od nesigurnih obrazaca? Postoje osnove za pretpostavku da će ti ljudi, iako su nesigurno vezani, živeti produktivan socijalni život unutar sredine gde su odrasli. Oni verovatno mogu biti socijalno adaptirani i živeti bez emocionalnih problema baš zato što su usvojili vrednosti i norme te sredine.

Stručnjacima u oblasti pomagačkih profesija bi sagledavanje i primena teorije afektivne vezanosti u kros-kulturanom kontekstu pružalo mogućnost za usklađivanje pristupa i intervencija sa osobenostima kulture u kojoj su korisnici rođeni i odrasli. Pristupi poput ovog, samim tim, predstavljaju osnov za kreiranje inkluzivnih i pravednih uslova u okviru stručnog i savetodavnog rada.

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APPLICATION OF THE NEW MULTIPLEX STR SYSTEMS IN FORENSIC DNA ANALYSIS OF SKELETAL REMAINS IN HUMAN IDENTIFICATION CASES

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Abstract: Various forensic techniques are used to identify a human corpse, depending on the circumstances and the state of remains. Unfortunately, the standard forensic identification methods were not sufficient in 30 to 35 % of all victims, therefore DNA identification was necessary. Since the beginning of our work in 2003, our laboratory has focused exclusively on STR DNA from bone, a powerful tool in missing person cases. 92% of skeletal remains analyzed were samples submitted for body identifications by law enforcement and only 8% were samples submitted to answer family identity or historical questions. In order to expand DNA typing capabilities on challenging samples such as bones, teeth, poorly preserved corpses, such as those found in water, buried or burned; modifications were introduced in protocols Phenol Chloroform Isoamyl Alcohol (PCIA) Extraction methods - organic extraction method and extraction PrepFiler® BTA Forensic DNA Extraction Kit (Applied Biosystems) enabled obtaining profiles in 98% cases. We have followed the standardization process used in Europe and the recommendations of the European Network of Forensic Science Institutes (ENFSI) for adoption of new genetic markers through the application of new STR multiplex systems in the DNA analysis. In this paper we have presented 30 bone samples of skeletal remains from routine casework submitted for body identifications by law enforcement, which were analyzed through the application of new STR multiplex system using Investigator® 24plex QS kit and GlobalFiler™ PCR Amplification kit, previously analyzed AmpFLSTR® Identifiler® Plus kit. Advanced extraction and purification techniques, together with more sensitive and robust new amplification kits allowed us to overcome the challenges associated with processing compromised skeletal remains and ultimately obtain full STR DNA profiles in 99% of the bones.

Keywords: Skeletal remains; DNA typing; Human identification; Forensic identification; DNA extraction

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INTRODUCTION

Typically, with missing person's cases, only skeletal remains are available as an evidentiary source of DNA (Hagelberg et al., 1989; Hagelberg et al., 1991; Gill et al., 1994; Holland et al., 1993). When conventional identification methods fail, DNA may provide the only link to identity. The ability to analyze trace amounts of human DNA isolated from old teeth and bone samples (Hagelberg et al., 1989; Hagelberg et al., 1991; Gill et al., 1994) using methods based on polymerase chain reaction (PCR), offers the opportunity to identify unknown skeletal remains by a comparative genetic analysis with their presumed relatives. The first successful DNA tests obtained from several months to 10 year old bones were carried out by Lee et al., (1991), Hochmeister et al., (1991) and Hagelberg et al. (1989 and 1991).

The identification of human remains belonging to missing persons is one of the main challenges for forensic genetics. Human remains are exposed to a myriad of environmental challenges, as occurs with all forensic biological evidence. The result is that samples can be limited in quantity, degraded and contain contaminants that affect the ability to type DNA samples. An efficient extraction protocol is required to maximize DNA recovery from bone samples while minimizing the coextraction of the PCR inhibitors naturally present in bone.

At the beginning of our work on the identification of skeletal human remains since 2003, we used phenol chloroform isoamyl alcohol (PCIA) organic extraction method for DNA extraction from bone samples. In order to improve our laboratory success rate with skeletal remains analysis we have been applying magnetic particle-based method for DNA extraction of bones using PrepFiler® BTA Forensic DNA Extraction Kit (Applied Biosystems) since 2013. Modifications by traditional phenolchloroform isoamyl alcohol (PCIA) organic extraction method have been introduced by our laboratory in order to assess its success with skeletal remains and provide a benchmark for the forensic community involved in identification of these remains (Zgonjanin et al., 2015). Additionally, modifications of PrepFiler® BTA Forensic DNA Extraction kit have also been introduced, which enabled extraction of complete and reliable multilocus STR profiles from difficult, challenging and highly degraded samples from the skeletal remain bones during the human identification cases (Zgonjanin et al., 2017). The DNA analysis results have shown that both optimized protocols for the DNA extraction from bones, which were exposed to a longstanding influence of the environmental factors, as well as those that were found in water, buried or burnt, can produce a quality sample for STR DNA analysis, Y-STR and mtDNA analysis (Zgonjanin et al., 2015; Zgonjanin et al., 2017; Almohammed et al., 2017).

This study evaluated the sensitivity and performance of the GlobalFiler™ PCR Amplification and Investigator® 24plex QS kits with 30 bone samples of skeletal remains from routine casework in our laboratory, each with different age and environmental exposure (Zgonjanin et al., 2015; Zgonjanin et al., 2017). The two kits being tested are both 6-dye multiplex kits each with 24 markers, including the mini-STR loci with amplicon size falling below 220 bps, which was designed to maximize performance on degraded samples and robust PCR buffer can tolerate certain levels of PCR inhibitors that may be present in DNA extracts. However, GlobalFiler™ and Investigator® 24plex QS could potentially be the optimum amplification choice for the limited amounts of DNA obtained from challenged bone samples.

MATERIAL AND METHODS

Samples and DNA Extraction

This study analyzed 30 bones, 24 femurs, 3 tooth samples, 2 skulls and 1 rib, submitted for routine casework body identifications by law enforcement. Samples were processed individually and extracted in parallel with a reagent blank control that accompanied the sample throughout testing. First, all bones were cleaned from the remnant soft tissue and all soil traces. The cut bone fragments were washed and air-dried (Holland et al., 1993). The resulting sample was pulverized into fine powder in mill MM 301 (Retsch).

Total DNA was isolated from bone samples following two different protocols: phenol chloroform isoamyl alcohol (PCIA) organic extraction method (Zgonjanin et al., 2015) and PrepFiler® BTA Forensic DNA Extraction Kit (Applied Biosystems) (Zgonjanin et al., 2017). Extraction of DNA using PrepFiler® BTA Forensic DNA Extraction Kit (Applied Biosystems) was performed using 50 mg of powdered bone following the protocol recommended by the manufacturer. Attempts were made to improve the DNA recovery in some challenging bone samples by overnight incubation, up to 18 h, of bone samples in lysis buffer solution component during lysis step. Moreover, another modification was made by adding 3 µl 1 M DTT in addition to 300 µl PrepFiler® Lysis Buffer with incubation at 56 °C and 900 rpm for 45 min. Modifications to both extraction methods have been introduced in order to improve our laboratory success rate with identification of these skeletal remains (Zgonjanin et al., 2015, Zgonjanin et al., 2017).

Family reference samples accompanying the skeletal remains are usually obtained from buccal swabs or dried blood samples using the QIAamp DNA Micro Kit (QIAGEN).

PCR Amplification and typing

DNA was quantified with an ABI Prism® 7500 Sequence Detection System (Applied Biosystems) using Quantifiler™ Human DNA Quantification kit. Amplifications were performed on the GeneAmp PCR System 9700 Gold Plate (Applied Biosystems) using the AmpFLSTR® Identifiler® Plus kit (Applied Biosystems), GlobalFiler™ PCR Amplification kit (Thermo Fisher Scientific) and Investigator® 24plex QS kit (QIAGEN) following the manufacturers' protocols. Amplified products are separated and detected on ABI 3500 Genetic Analyzer (Applied Biosystems).

RESULTS

Thirty DNA extractions were completed on skeletal remains from routine casework and the results are presented in Table 1. Three different groups of bones were selected based on exposure of skeletal remains to different environmental influences, which may have led to degradation of DNA: burned bodies (8 samples), remains recovered from water (8 samples) and remains recovered from fields (14 samples) (Table 1). Twenty-seven samples, from each amplification kits produced full STR profiles beside on extraction method are used (Table 1).

Table 1

Bone sample (Q)/casework	Environmental exposure	Extraction method	Quantity (ng/µl)	Efficiency of STR typing		
				Identifiler® Plus	GlobalFiler™	Investigator® 24plex QS
Femur/Case #1	Buried	PrepFiler® BTA[a]	13.337	16/16	24/24	24/24
Femur/Case #2	Burned	PrepFiler® BTA[a]	1.089	16/16	24/24	24/24
Femur/Case #3	Water	PrepFiler® BTA[a]	2.981	16/16	24/24	24/24
Femur/Case #4	Buried	PrepFiler® BTA[a]	0.163	16/16	24/24	24/24
Femur/Case #5	Burned	Organic[b]	2.989	16/16	24/24	24/24
Skull /Case# 6	Buried	PrepFiler® BTA[a]	11.262	16/16	24/24	24/24
Femur/Case #7	Buried	Organic[b]	0.127	13/16	24/24	24/24
Femur/Case #8	Water	PrepFiler® BTA[a]	0.382	16/16	24/24	24/24
Femur /Case #9	Buried	PrepFiler® BTA[a]	0.121	12/16	24/24	23/24
Skull /Case #10	Buried	PrepFiler® BTA[a]	0.302	16/16	24/24	24/24
Femur/Case #11	Buried	PrepFiler® BTA[a]	0.188	16/16	24/24	24/24
Femur /Case#12	Water	PrepFiler® BTA[a]	0.281	16/16	24/24	24/24
Femur/Case #13	Buried	PrepFiler® BTA[a]	2.132	16/16	24/24	24/24
Femur/Case #14	Water	Organic[b]	25.647	16/16	24/24	24/24
Femur/Case #15	Water	PrepFiler® BTA[a]	0.258	16/16	24/24	24/24
Femur/Case #16	Buried	PrepFiler® BTA[a]	0.180	16/16	24/24	24/24
Femur/Case #17	Water	Organic[b]	0.321	16/16	24/24	24/24
Femur/Case#18	Burned	Organic[b]	0.009	9/16	22/24	22/24
Femur/Case #19	Buried	Organic[b]	0.282	16/16	24/24	24/24
Femur/Case #20	Water	Organic[b]	0.116	16/16	24/24	24/24
Rib/Case #21	Burned	Organic[b]	1.781	16/16	24/24	24/24
Femur/Case #22	Burned	Organic[b]	12.111	16/16	24/24	24/24
Femur/Case #23	Burned	Organic[b]	1.282	16/16	24/24	24/24
Femur/Case #24	Burned	Organic[b]	1.752	16/16	24/24	24/24
Femur/Case #25	Burned	Organic[b]	1.141	16/16	24/24	24/24
Tooth/Case #26	Water	PrepFiler® BTA[a]	10.263	16/16	24/24	24/24
Femur/Case #27	Buried	PrepFiler® BTA[a]	1.161	16/16	24/24	24/24
Tooth/Case #28	Buried	PrepFiler® BTA[a]	8.731	16/16	24/24	24/24
Tooth/Case #29	Buried	PrepFiler® BTA[a]	0.755	16/16	24/24	24/24
Femur/Case #30	Buried	PrepFiler® BTA[a]	0.987	16/16	24/24	24/24

Nuclear DNA quantity; the efficiency of autosomal STR typing (AmpFℓSTR® Identifiler® Plus Amplifiction Kit) expressed as the number of successfully typed STRs; efficiency of GlobalFiler™ expressed as the number of successfully typed number of successfully typed STRs Y-indel, and a DYS391; Investigator® 24plex QS, expressed as the number of

successfully typed number of successfully typed STRs, DYS391and a Quality Sensor in bones from 30 human identification cases.

[a]PrepFiler® BTA Forensic DNA Extraction Kit (Zgonjanin et al., 2017)
[b]PCIA organic extraction method (Zgonjanin et al., 2015)

The results showed that in 3 samples which were partial with Identifiler® Plus, with GlobalFiler™ and Investigator® 24plex QS gave a greater number of loci producing better STR profiles (Table 1). In two of the three samples, both amplification kits gave a complete, 24-locus profile (Case #7 and Case #9) regardless detection PCR inhibition by Case #7 and confirm DNA degradation by Case #9. Sample Case #18 was still considered a partial profile, but the amount of reportable loci increased using GlobalFiler™ and Investigator® 24plex QS on 22 out of 24 loci available in those multiplex, while applying AmpFℓSTR® Identifiler® Plus kit obtained only 9 out of 16 loci. For sample Case #18 amplified with GlobalFiler®, the loci that were not amplified were primarily the longest loci CSF1PO and SE33, while with Investigator® 24plex QS loci that were not amplified were D2S1338 and SE33. Regardless of the DNA input, dropped out the same loci also observed by other authors (Pionzio et al., 2014).

Our work on the identification of skeletal remains from several cases of criminal burning was of particular interest (Case #21, Table 1), where the intent was to destroy the body (Zgonjanin et al., 2015) which actually helped to identify the victim of the murder that shook the public. In this lab, nine extraction attempts of skeletal remains from nine cases of criminal burning, where the intent was to destroy the body, were successful. Cases of remains recovered from water resulted in full profiles. When comparing genetic profiles, we matched 29 of the 30 skeletal remains analyzed to accompanying reference sample with high confidence of correct identification for all 29 victims (probability from 99.9% to 99.999999%).

The environment where the bone is found potentially provides another source of PCR inhibition. As for the exposure of examined samples of skeletal remains to different environmental influences which may have led to degradation of DNA, samples can be categorized into three larger groups: burned bodies (21.9%), remains recovered from the water (28.1%) and remains recovered from the fields or buried (50.0%) (Zgonjanin et al., 2015; Zgonjanin et al., 2017). Skeletal remains submitted to this laboratory can generally be divided into two categories: (1) samples for body identification submitted by law enforcement (92%) and (2) samples submitted to answer historical or family identity questions (8%). Overall, body identification cases were more likely to yield a full profile whereas historical cases were more likely to result in partial profiles. The average age of samples in historical cases is far greater than that of body identification cases. Most samples in historical cases were more than 50 years old, whereas most of the samples in body identification cases were less than 15 years old.

DISCUSSION

DNA extraction is a critical step for the efficient recovery of highly degraded and damaged DNA from burned skeletal remains, and for forensic DNA profiling of missing person remains. Burned remains are typically brittle, blackened, and friable, therefore the failure to read DNA-type is not surprising (Cattaneo et al., 1999). However, appearances may be deceiving which means that practitioners should not generalize their expectation about potential success of sample analysis based on their appearance, as noted by other authors (Edson et al., 2005). Our experience has shown, in accordance with the experiences of others (Tucker et al., 2011; Albinsson et al., 2011), that the new multiplex kits with the ESS loci is more tolerant to

common inhibitors, which enabled us to overcome the challenges associated with processing compromised skeletal remains.

In our earlier publications, we presented our approach and the results obtained during identification of unknown skeletal remains. We have found that a forensic autopsy does not always give reliable answers to important questions. In fact, it could often cause an erroneous identification strategy choice. In cases of wrong or partial estimate, as well as the cases where time elapsed between the moment of death and the skeletal remains discovery, process of human identification has relied on the DNA typing to acquire additional genetic information from bone samples of skeletal remains. The results suggest that the identification of skeletal remains should rely on the DNA analysis because it leads to the identification of more missing individuals (Zgonjanin et al., 2019).

The results of this research suggest that the GlobalFiler® kit is slightly more sensitive than the Investigator® 24plex QS kit, producing more complete and balanced STR profiles with peak heights at least 2-fold greater, supporting the results of previous work (Lin et al.2005; Almohammed et al., 2019). The two kits produced concordant STR profiles for all samples.

Kits that can analyze 24 loci provide a greater power of discrimination and provide DNA analysts with greater confidence in associations formed between skeletal remains and family reference samples. With this ability to obtain increased genetic information from the skeletal remains with Investigator® 24plex kit, we could detect PCR inhibition or confirm DNA degradation and amplification success in general.

This paper describes the forensic application of current DNA technology to solve a missing person's case. The data indicated that the GlobalFiler™ and Investigator® 24plex QS are extremely sensitive multiplex STR amplification systems and can produce greater quality DNA profiles from skeletal remains compared to the Identifiler® Plus kit. They have been successfully used to obtain multilocus STR profiles from bone samples with minimal amounts (pg) of human DNA, highly inhibited, and degraded challenging samples.

Our results have shown that simple modifications to extraction techniques, together with more sensitive and robust new amplification kits with the mini-STR loci that are more tolerant to common inhibitors, allowed us to overcome the challenges associated with processing compromised skeletal remains and lead to the identification of more missing individuals.

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PRIMENA NOVIH MULTIPLEKSNIH STR SISTEMA U FORENZIČKOJ DNK ANALIZI SKELETNIH OSTATAKA U SLUČAJEVIMA HUMANIH IDENTIFIKACIJA

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Apstrakt: U zavisnosti od okolnosti i stanja ostataka tela, danas se koriste različite forenzičke tehnike za identifikaciju ljudskih ostataka. Nažalost, u oko 30 – 35% slučajeva standardne forenzičke identifikacije nisu bile dovoljne, pa je zahtevana i DNK identifikacija. Od početka našeg rada 2003. godine naša laboratorija je fokusirana isključivo na STR DNK iz kostiju, koje je moćno oruđe za rešavanje slučajeva nestalih osoba. 92% analiziranih skeletnih ostataka bili su uzorci dostavljeni radi identifikacije tela od strane organa za sprovođenje zakona, a samo 8% su uzorci dostavljeni da bi se odgovorilo na porodični identitet ili istorijska ispitivanja. U cilju proširenja mogućnosti DNK profilisanja sa izazovnim/teškim uzorcima kao što su kosti, zubi, slabo očuvana tela/leševi, pronađeni u vodi, zakopani ili spaljeni; modifikacije su uvedene u protokole fenol hloroform izoamilalkohol (PCIA) ekstrakcije - metoda organske ekstrakcije i ekstrakcije PrepFiler® BTA Forensic DNK ekstrakcionim priborom (Applied Biosistems) što je omogućilo dobijanje profila u 98% slučajeva. U našem radu sledili smo proces standardizacije koji se koristi u Evropi i preporuke Evropske mreže instituta za forenzičkih nauka (ENFSI) za usvajanje novih genetskih markera kroz primenu novih STR multipleks sistemera u DNK analizi. U ovom radu predstavili smo analizu 30 uzoraka kostiju skeletnih ostataka iz rutinske obrade slučajeva dostavljenih na identifikaciju tela od strane organa za sprovođenje zakona, koji su analizirani primenom novih STR multipleks sistema, korišćenjem Investigator® 24plek KS pribora i GlobalFiler™ PCR Amplification pribora, prethodno analiziranog AmpFLSTR® Identifiler® Plus setom. Napredne tehnike ekstrakcije i prečišćavanja, zajedno sa osetljivijim i robusnijim novim kompletima za amplifikaciju, omogućili su nam da prevaziđemo izazove povezane sa obradom kompromitovanih ostataka skeletnih ostataka i na kraju dobijemo pune STR DNK profile u 99% uzoraka kostiju.

Ključne reči: Skeletni ostaci; DNK profilisanje; Humana idetifikacija; Forenzička identifikacija; Ekstrakcija DNK

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ETHNOPHARMACOLOGY OF DIABETES: FROM TRADITIONAL RECIPES TO MODERN MEDICINE

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Abstract: Diabetes is one of the most prevalent chronic diseases of the 21st century. Current pharmacological interventions used in the management of this condition regulate hyperglycemia; however, they do not fully prevent the onset or progression of its secondary complications, including diabetic nephropathy, hepatic dysfunction, osteoporosis, neuropathy, retinopathy, cardiovascular diseases, increased risk of dementia, etc. Consequently, a growing number of individuals with diabetes, in addition to conventional pharmacotherapy, are incorporating herbal alternatives derived from traditional medicine, particularly in the form of herbal mixtures, which are believed to enhance therapeutic outcomes in the management of multifactorial diseases such as diabetes. Given that medicinal plants, and especially herbal mixtures, consist of a diverse array of bioactive compounds that may exert synergistic, additive, or antagonistic effects, it is plausible that their concomitant use with standard pharmaceutical agents could offer benefits in terms of glycemic control, attenuation of secondary complications, and mitigation of pharmacological side effects. However, this practice may also pose risks, including the potential for hypoglycemia, hepatotoxicity, nephrotoxicity, and other adverse effects. Therefore, comprehensive investigations into biological activities and their mechanisms of actions of both individual herbs and their combinations, particularly when used as adjuncts to conventional therapies, are imperative for ensuring their safety and efficacy in the treatment of diabetes and its associated complications.

Key words: Traditional medicine; Pharmacology; Diabetes.

INTRODUCTION

Diabetes mellitus is a complex metabolic disorder and one of the major social and economic burdens globally. Namely, according to the International Diabetes Federation, in 2021, diabetes affected over 1.2 million children and adolescents, 537 million adults, and contributed to 6.7 million deaths. The total global health expenditure related to diabetes was estimated at 930 billion EUR. These figures may be underestimated, particularly in regions like Africa, South-East Asia, and the Western Pacific, where more than half of individuals with diabetes remain undiagnosed (IDF, 2021). The situation in Serbia reflects a similarly concerning trend, with 770,000 individuals diagnosed with diabetes, making it the fourth leading cause of death in the country (Institute of Public Health 'Dr. Milan Jovanovic Batut', 2021).

Chronic low-grade inflammation and increased oxidative stress represent the hallmarks of diabetes. They are major contributors to the number of its secondary complications (Bastaki, 2005; Mirmiran et al., 2014), such as hyperlipidaemia, non-alcoholic fatty liver disease (NAFLD), nephropathy (Evans et al., 2002; Gross et al., 2005; Guven et al., 2006; Savage et

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al., 2007; Manna et al., 2010, Volpe et al, 2018), neuropathy (Feldman et al., 2019), retinopathy (Engerman, 1989), eating disorders (Dziewa et al., 2023), even dementia and the risk of Alzheimer's disease (Ott et al., 1999; Arvanitakis et al., 2004; Inzucchi et al., 2015). Thus, diabetes is well known as one of the most significant silent killers in the 21st century (Campbell, 2001).

Even though standard pharmacotherapies manage to regulate hyperglycaemia, they cause growing concerns regarding its potential adverse effects, such as glomerular hyperfiltration, metabolic dysfunctions, alterations in arterial blood pressure, genitourinary infections, dehydration, and ketoacidosis caused by SGLT2 inhibitors (FDA, 2015), weight gain caused by insulin (Russell-Jones and Khan, 2007), gastrointestinal problems caused by metformin (Kirpichnikov et al., 2002) and increased risk of myocardial infarction and weight gain caused by sulfonylureas (Panten et al., 1996; Schloot et al., 2016).

At the same time, the use of medicinal herbs in conjunction with standard pharmacotherapy among individuals with diabetes is increasing (Damnjanović et al.,2015; Madić et al. 2018), driven in part by traditional heritage and in part by the perceived ability of these remedies to address certain limitations of conventional pharmacological treatments. Simultaneously, the widespread dissemination of misleading advertisements for so-called "miracle" anti-diabetic remedies, coupled with insufficient health literacy and inadequate quality and safety regulatory frameworks, poses significant public health risks. This misinformation can not only exacerbate adverse health outcomes but may also contribute to the progression from type 2 diabetes to type 1 diabetes, via overstimulation of beta-cells, through their accelerated aging, loss of functionality, and eventual inability to produce insulin (Heinrich, 2015; Madić et al., 2018; Madić et al., 2023). Additionally, certain herbal remedies may act antagonistically, diminishing the effectiveness of pharmaceutical treatments or amplifying adverse interactions, further complicating diabetes management (Uchida et al., 2006). Thus, comprehensive investigations into the biological activities and mechanisms of action of both individual herbs and their combinations, particularly when used as adjuncts to conventional therapies, are imperative for ensuring their safety and efficacy in the treatment of diabetes and its associated complications. The purpose of this review is to outline the necessary steps involved in testing new therapeutic strategies derived from the ethnopharmacologically used recipes.

MEDICINAL PLANTS VERSUS POLYHERBAL MIXTURES

In the quest for the discovery of ideal supplementary therapies and new pharmaceuticals, there are two schools of thought. The first one posits that isolating and modifying a single compound from a specific medicinal species is more advantageous to developing a functional drug, such as aspirin (Sneader, 2000) or metformin (Bailey, 2017). The second one advocates for a holistic approach, emphasizing the use of polyherbal mixtures, which are considered far more beneficial in treating multifactorial diseases such as diabetes (Umamaheswari et al., 2009; Bera et al., 2010; Mahajan et al., 2018). Namely, it is believed that synergistic effect of medicinal herbs used in polyherbal preparations leads to a better therapeutic outcome by decreasing their toxicity and increasing their protective effects (Parasuraman et al., 2014; Han et al., 2019; Madić et al., 2019).

However, in both cases, full scientific validation of the ethnopharmacological value of herbal remedies must include both pre-clinical studies - comprising compound characterization, *in vitro* studies, and in *vivo* evaluations - as well as clinical studies, which, in the case of polyherbal mixtures, need to be more extensive (Madić et al., 2019; Madić et al. 2021; Petrović et al., 2024).

IDENTIFICATION OF HIGH-POTENTIAL CANDIDATES

Numerous herbal remedies as well as polyherbal formulations are described in traditional medical practices. Therefore, an anthropological point of view comprising comprehensive literature research, direct discussions with traditional medicine practitioners as well as with final consumers, usually done via cross-sectional studies, are essential to identify the most promising formulations. Thus, our previous research showed that polyherbal mixtures are used more prevalently than single medicinal plants in the context of anti-diabetic remedies. Furthermore, while a substantial number of polyherbal formulations are documented in the field of ethnopharmacology, certain recipes have been employed with greater frequency than others (Madić et al., 2018; Madić et al., 2021; Madić et al., 2023; Petrović et al., 2024).

PHYTOCHEMICAL PROFILING

After identifying the most promising candidates, the next step is the preparation of standardized extracts to minimize the intrinsic complexity and variability of herbal blends, which can fluctuate due to geographic, environmental, and other influencing factors. To enhance yield and quality, a range of methods may be used, such as decoction, maceration, ultrasound-assisted extraction, etc. The quality of the resulting extracts is then assessed through phytochemical profiling using high-performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC–MS), or gas chromatography/mass spectrometry (GC/MS), among others. Finally, to determine the optimal storage requirements for preserving bioactive integrity over time, assessing chemical composition under various storage conditions such as temperature, light, and humidity, i.e., stability studies should be conducted (Madić et al., 2021; Petrović et al., 2024).

IN VITRO STUDIES

Following the compound profiling of the obtained extracts, it is necessary to clarify whether their chemical profiles support the traditional medicinal claims attributed to them. The first step of this process encompasses an *in vitro* appraisal of a diverse array of biological activities, including but not limited to antioxidant, antidiabetic, and cytoprotective potentials, alongside an assessment of their toxicity profiles. When analyzing polyherbal formulations, the depth of *in vitro* evaluations is considerably greater. Notably, the predominant objective of this rigorous examination is to elucidate the potential benefits of utilizing polyherbal compositions over solitary medicinal plants, thus rendering the entire methodological process more intricate and time-consuming. Additionally, our previous research demonstrated that, even under *in vivo* conditions, certain traditionally used 'anti-diabetic' polyherbal remedies exhibit lower potency compared to other polyherbal formulations (Madić et al., 2019; Petrović et al., 2022; Petrović et al., 2024).

The *in vitro* research can be conducted using various biochemical and biological assay systems. However, to obtain a comprehensive understanding, a multifaceted approach is essential. For example, when evaluating antioxidant activity, chemical *in vitro* assays such as the DPPH assay directly correlate antioxidant activity with the concentration of antioxidant molecules. In contrast, biological *in vitro* antioxidant assays, such as anti-hemolytic tests on red blood cells (RBCs), are more sensitive and offer a more realistic insight into antioxidant potential under physiological conditions, underscoring the importance of employing both chemical and biological assays in studies of this nature (Madić et al., 2022). Furthermore,

common *in vitro* approaches for evaluating the molecular mechanisms of potential antidiabetic agents include methods such as the α-amylase inhibition assay (Petrović et al., 2024) and beta-cell culture models (Đorđević et al., 2019).

The final step in this part of pre-clinical studies is the assessment of cytotoxicity and genotoxicity. This is usually done via cell culture approach, and preferably confirmed by an *in vivo* plant system, such as the *A. cepa* test. Our previous studies showed that this method provides excellent precision when studying the cytotoxicity and genotoxicity of medicinal herbs. When combined with tests on 'rat cells,' particularly RBC hemolytic assays, it can specifically demonstrate cytotoxicity at the cell membrane level and allows for a precise distinction between cytotoxic and genotoxic effects. Furthermore, analysis using this model can help explain some of the molecular mechanisms underlying the physiological of antidiabetic treatments evaluated using *in vivo* animal models (Madić et al., 2019; Madić et al., 2021). This ensures that only the most promising treatments progress to the next stage, *in vivo* animal testing, while upholding the principles of the Three Rs.

IN VIVO STUDIES

The final step in pre-clinical testing of potential anti-diabetic therapeutics involves *in vivo* studies using chemically induced rodent diabetic models, such as those induced by alloxan or streptozotocin. Although this type of diabetes does not completely mimic human forms of diabetes, such as type 1, type 2, gestational diabetes, or latent autoimmune diabetes in adults, our previous research has shown that the use of insulin glargine as a control can help distinguish the degree of similarity between chemically induced diabetes and type 1 or type 2 diabetes. Thereby providing detailed insight into the potential of the tested therapies (Madić et al., 2022). Furthermore, although the male rodent model is more frequently used, our previous research demonstrated a more severe secondary diabetic complications phenotype in a chemically induced female diabetic model compared to its male counterpart (Madić et al., 2021). Consequently, this model suggests a more pronounced therapeutic efficacy of the evaluated potential treatments.

In brief, the first step of any *in vivo* experiment is obtaining ethical approval for the proposed study. Next, *in vivo* toxicological studies are prerequisites for further testing. Namely, high doses of medicinal plants or polyherbal remedies may cause hepatotoxicity and nephrotoxicity (Madić et al., 2021) or even lead to the development of osteopetrosis (Petrović et al., 2021). After confirming that the proposed doses have no toxic effects, testing of their 'anti-diabetic' properties can begin. First, diabetes is chemically induced in acclimated animals. Once stable diabetes is confirmed, the animals are randomly divided and treated with the tested herbal extracts, while control groups include untreated healthy and diabetic animals, animals co-treated with both the herbal mixture and standard pharmaceuticals, and animals treated with standard pharmaceuticals alone. At the end of the experiment, harvested tissues from the experimental animals are analyzed using biochemical, histochemical, immunohistochemical, and molecular techniques. Since many herbal mixtures act holistically, addressing both primary diabetic issues such as hyperglycemia and preventing secondary diabetic complications such as hyperlipidemia, NAFLD, diabetic kidney disease, neurodegenerative problems, etc., multiple tissues must be evaluated. The treatment effect is then assessed by statistical comparison of treated groups and controls (Madić et al., 2021; Petrović et al., 2021; Petrović et al., 2024).

CLINICAL STUDIES

Given the physiological differences between laboratory animals (Kararli, 1995) and the fact that humans do not live in controlled environments like laboratory animals, clinical trials are essential to confirm the safety and efficacy of new therapeutics, including herbal remedies. These clinical studies represent the final step in this research process. While numerous bioactive compounds isolated from medicinal plants have been validated for safety and efficacy in treating various diseases, such as diabetes (Luo et al., 2023), it is crucial to acknowledge that some bioactive substances may interfere with pharmacotherapy (Auten et al., 2013), exhibit additive effects (Damnjanovic et al., 2015), or even possess co-carcinogenic potential in certain populations or lifestyles (Paolini et al., 1999).

Initially, the first step in clinical trials involves obtaining ethical approval and securing written informed consent from the participants. The study cohorts should include both healthy individuals and those with pre-existing medical conditions. Half of the participants will receive experimental treatment, while the other half will be administered a placebo. Unlike *in vivo* animal studies, where multiple tissue types are analyzed, the evaluation of treatment efficacy in clinical trials is typically performed by analyzing serum samples. Finally, the statistical analysis must be rigorous and comprehensive, accounting for variables such as age, sex, ethnicity, lifestyle, comorbidities, and concurrent pharmaceutical treatments (Piantadosi, 2024).

Thus, large cohort studies are the crown of the research continuum - from ethnopharmacology to modern medicine.

CONCLUSION

The path toward identifying safe and effective herbal therapeutics is complex and multifaceted. It requires a collaborative approach involving interdisciplinary teams of experts, including anthropologists, botanists, pharmacologists, cell and molecular biologists, physiologists, histopathologists, endocrinologists, and clinicians, among others. Thus, increasing scientific and health literacy among the general population is essential to empower individuals to critically assess and navigate the growing body of information, helping to mitigate the spread of misinformation.

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ETNOFARMAKOLOGIJA DIJABETESA: OD TRADICIONALNE RECEPTURE DO SAVREMENE MEDICINE

Apstrakt: Dijabetes je jedna od najčešćih bolesti 21. veka. Savremeni lekovi korišćeni u farmakoterapiji ovog oboljenja regulišu hiperglikemiju, ali ne sprečavaju progresiju sekundarnih komplikacija poput dijabetesne nefropatije, oštećenja jetre, osteoporoze, neuropatije, retinopatije, bolesti kardiovaskularnog sistema, povećanog rizika od demencije, itd. Zbog toga, sve veći broj ljudi sa dijabetesom uz propisane lekove koristi i biljne supstituente iz narodne medicine, naročito u vidu biljnih mešavina za koje se veruje da pri terapiji složenih bolesti poput dijabetesa dovode do boljeg terapeutskog ishoda. Imajući na umu da lekovito bilje, a naročito biljne mešavine, predstavljaju kompleksnu smešu brojnih bioaktivnih susptanci koje mogu delovati sinergistički, aditivno ili, pak, antagonistički, nije teško pretpostaviti da istovremena primena standardnih lekova i biljnih suplemenata može biti blagotvorna u normalizaciji nivoa glikemije, usporavanju razvoja sekundarnih komplikacija dijabetesa, kao i smanjenju nus-pojava farmakoterapije, ali da može dovesti i do pojave niza neželjenih efekata poput hipoglikemije, oštećenja jetre i bubrega, itd. Zbog toga, detaljno ispitivanje bioloških aktivnosti i mehanizama delovanja pojedinačnih biljaka i biljnih mešavina, naročito prilikom njihove upotrebe u vidu ko-tretmana sa standardnom terapijom, od ključne je važnosti za procenu bezbednosti primene u terapiji dijabetesa i njegovih sekundarnih komplikacija.

Ključne reči: Narodna medicina; farmakoterapija; dijabetes.

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RELATIONSHIP OF EARLY AND LATE MENARCHE WITH BODY HEIGHT AND BODY MASS INDEX IN YOUNG FEMALES

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Introduction: A number of endogenic and exogenic factors tend to affect the body height and body mass index (BMI) of adults. The current study aims at exploring the relationship between age at menarche with body height and BMI in young females. **Material and methods:** A transversal anthropometric survey was conducted in the period between 2021 and 2024 on 209 female university students between the ages of 21 and 25. The surveyed traits included body height and weight, BMI was calculated based on these values. The age at menarche was obtained by applying the retrospective method. **Results:** Early menarche (<12 years) was detected in 23% of women, while late menarche (>14 godina) was present in 16.7% of women. The average age at menarche was 12.78±1.19 years. The prevalence of short stature (Z-score<-1) and overweight (BMI≥25) was higher in early menarche cases in comparison to those with late menarcheal age (39.4% and 61% v.s. 0% and 2.4%, respectively). The prevalence of tall stature (Z-score>1) and underweight (BMI<18.50) was higher in women with late menarche in comparison with their early menarcheal peers (43.6% and 47.4% v.s. 2.4% and 15.8%, respectively). The odds ratio (OR) equaled 0.393 for short stature, 1.862 for tall stature, 1.789 for underweight and 0.327 for overweight (P<0.05). The categories of age at menarche significantly affected the body height (BETA=0.432) and BMI (BETA=-0.450). **Conclusion:** Early menarche is associated with short stature and overweight, while late menarche relates to tall stature and a higher risk of being underweight.

Key words: early menarche, late menarche, body height, body mass index, young females

Introduction

Menarche occurs in late puberty and is preceded by intense physical growth and significant metabolic changes (Bubach et al. 2016). According to the literature (Żurawiecka and Wronka, 2021), the categorization of menarche includes: early menarche (<12 years), average (12–14 years) and late menarche (>14 years). The timing of menarche is influenced by various factors such as genetics (Carty, 2024), socioeconomic status (Karim et al. 2021), body composition (Gemelli et al. 2020) and body fat distribution (Xue et al. 2024). It can also be an indicator of health status and body constitution in later life. Numerous studies have demonstrate that obesity and increased BMI values in premenarcheal girls can lead to earlier onset of menarche (Abou et al. 2020; Adami et al. 2020).

It is known that adult height is influenced by many factors and one of them is the time of menarche in women. Chan and Soong (1976) demonstrated that the girls with early menarche had a greater growth in stature and earlier and faster deceleration of growth and earlier completion of growth than the girls with later menarche. Also, the girls who had early menarche had relatively shorter lower limb length and relatively longer sitting height than the girls with late menarche. On the other hand, girls with later menarcheal age grow taller compared with girls who reach menarche at an earlier age (Onland-Moret et al. 2005).

Recent research shows (Żurawiecka and Wronka, 2021; Rakić et al. 2024) that early menarche can be associated with elevated risk of overweight and obesity (BMI≥25) and abdominal obesity (WC > 80 or WHtR≥0.5), with metabolic cardiovascular risk factors (Bubach et al. 2021), higher BMI values (Asrullah et al. 2022; Amiri et al. 2023), and increased risk for breast cancer (Goldberg et al. 2020).

Over the decades, age at menarche is constantly decreasing, however the magnitude and direction of the secular trend of age at menarche varies in different regions of the world. In African and Southeast Asian countries, (Arafa et al., 2019) as well as in USA, Canada, Australia and the UK (Biro et al., 2018; Hickey et al., 2018), the age at menarche is still decreasing. In Japan (Hosokawa et al., 2012), Spain, Denmark, Belgium, Norway and Sweden, data have shown lower rates of decline (Bjelland et al., 2018), while in some other European countries (Papadimitriou et al. 2008; Gohlke and Woelfle, 2009; Talma et al. 2013; Lalys and Pineau, 2014; Piras et al. 2020) the secular trend appears to have been halted. In Serbia, recent research on the occurrence of menarche also shows a declining trend. In three time periods Rakić et al. (2020) showed that at the beginning of the 21st century, the average menarcheal age in Vojvodina (Serbia) was 12.60 (2001-2004), 12.38 (2011-2014) and 12.33 years (2019).

The aim of this study was to determine the relationship between early and late menarche with body height and body mass index in young females.

Material and methods

The study was conducted at the Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, from 2021 to 2024. The sample comprised 209 female students between the ages of 20.50 and 25.49. All students gave their consent to participate in the research. A decimal age was calculated for each individual, based on the date of survey and the date of birth.

Height and weight were measured with standard anthropometric instruments (Sieber Hegner Maschinen AG Zürich, Switzerland). These measures were used for assessing body mass index (BMI kg/m^2). The categorization of nutritional status was done according to the criterion by WHO (1997). The subjects with BMI<18.50 kg/m^2 were classified as underweight, those with BMI range 18.50-24.99 kg/m^2 with normal weight, those with BMI ranging 25-29.99 kg/m^2 were grouped as overweight, while those with BMI≥30 kg/m^2 were in obese category.

It is generally accepted that short stature is defined when adult height is more than two standard deviations below the mean for age and sex (Pedicelli et al. 2009). Accordingly, the categorization for body height was determined based on the following z-score values:

Very short height (z-score<-2)

Low or below average height (-2≤z-score<-1)

Average height (-1≤z-score<1)

High or above average (1<z-score≤2)

Very tall height (z-score>2)

The retrospective method was used to determine the time of menarche. The age at menarche was assessed by subtracting the date of birth from the date of the menarche onset. The age at menarche categorization included early age (<12 years), average (12-14 years) and late age (>14 years), complying with the literature reports (Werneck and Oyeyemi, 2018; Żurawiecka and Wronka, 2021).

The results are presented as means ± standard deviation (SD) or median (standard error) for numerical variables and as absolute frequencies with the appropriate percentage values for the category variables. The association between age at menarche with body height and BMI

was determined using Pearson's correlation analysis and a linear regression model. The association of age at menarche with body height categories and BMI was determined using logistic regression analysis. The influence of menarche categories on body height and BMI values was assessed using linear regression analysis. The difference between the two groups was determined using the t-test or the Mann-Whitney U test depending on the fulfillment of the test conditions.

Data processing was done using IBM Statistics SPSS, version 23.0. Statistically significant difference was set as $p<0.05$.

Results

The sample comprised 209 female students average age of 22.21±0.87. Their body height ranged from 155 to 185.5 cm and average 167.98±6.60 cm. The average body weight was 63.22±9.33 kg and ranged widely from 45.5 kg to 105 kg. The BMI was on average 22.48 kg/m^2 and ranged from 16.65 to 36.33 kg/m^2 (Table 1).

Table 1. Demographic and anthropometric characteristics

	Mean	SD	Minimum	Maximum
Age (years)	22.21	0.87	20.93	25.40
Height (cm)	167.98	6.60	155.00	185.50
Weight (kg)	63.22	9.33	45.50	105.00
BMI (kg/m^2)	22.48	3.47	16.65	36.33

SD: standard deviation; BMI: body mass index

Early menarche females showed significantly lower height (t_{207}=4.638; P=0.000) and higher BMI values (t_{207}=-6.555; P=0.000) than those with the age at menarche of ≥12 years. On the other hand, late menarche females exhibited significantly higher height values (Z=-5.253; P=0.000) and lower BMI (Z=-4.368; P=0.000) when compared to those with the menarche ≤14 years. The difference in height between early menarche females and those with the ≥12 years menarche equaled 4.8 cm, while late menarche women exhibited height values greater by 6.78cm than those with ≤14 years menarche. As for BMI, the value was greater by 3.41 kg/m^2 in early menarcheal age females in comparison with other subjects, and lower by 1.50kg in late menarche females than in those with ≤14 years of menarcheal age. The difference in height values recorded in late and early menarche females was 9.31cm, and in case of BMI, the difference was 4.82 kg/m^2 (Table 2).

Table 2. Average height and BMI in relation to early and late menarche

	Height (cm)	BMI (kg/m^2)
<12 years (n=52)$^{\$}$	164.29±5.41	25.11±4.82^a
≥12 years (n=76) $^{\$}$	169.09±6.53^a	21.70±2.47
Difference	4.8 cm	3.41 kg/m^2
≤14 years (n=80) $^{\&}$	166.82±6.09	22.94±3.49^a
>14 years (n=22) $^{\&}$	173.60±6.10^a	20.29±2.35
Difference	6.78 cm	1.50 kg/m^2
Total Difference	9.31 cm	4.82 kg/m^2

$ t-test between early menarche and other subjects; &Mann-Whitney U test between late menarche and other subjects

a: P<0.001; BMI: body mass index

The females with height values below the average (z-score<-1) reported early menarche in 39.4% of cases and none of the cases of late menarche. As for the average height females, almost a quarter (24.1%) had early menarche and 13.1% reported late menarcheal age. Two thirds (66.7%) of females with very tall stature were with late menarche and there were no cases of early menarche onset. Out of 39 females with above-average height (z-score>2), 17 of them (43.6%) had late menarche and only 2 (5.1%) early menarche. There were no very short stature (z-score<-2) females in the sample. The overweight prevalence (BMI≥25) was higher in female students with early menarche 61% (25/41) in comparison with those with late menarche 2.4% (1/41). However, the underweight prevalence (BMI<18.50) was greater in late menarcheal age subjects 47.4% (9/19) when compared with those with early menarche 15.8% (3/19). The average age at menarche equaled 12.78±1.19 years, and the median was 12.71 (0.08) years (Table 3).

Table 3. Age at menarche in relation to height and BMI

	Age at menarche ((n (%))			
	Early	Late	Mean (SD)	Median (SE)
Height (-2≤z-skor<-1) (n=33)	13 (39.4)	-	12.09 (1.07)	12.23 (0.19)
Height (-1≤z-skor<1) (n=137)	33 (24.1)	18 (13.1)	12.73 (1.12)	12.67 (0.10)
Height (1<z-skor≤2) (n=33)	2 (6.1)	13 (39.4)	13.44 (1.14)	13.36 (0.20)
Height (z-skor>2) (n=6)	-	4 (66.7)	14.23 (0.97)	14.46 (0.40)
BMI<18.50 kg/m^2 (n=19)	3 (15.8)	9 (47.4)	13.12 (1.35)	13.20 (0.29)
BMI (18.50-24.99 kg/m^2) (n=149)	20 (13.4)	25 (16.8)	13.06 (1.41)	12.93 (0.12)
BMI (25-29.99 kg/m^2) (n=32)	16 (50)	1 (3.1)	12.02 (1.29)	12.12 (0.23)
BMI≥30 kg/m^2 (n=9)	9 (100)	-	11.34 (0.96)	11.78 (0.32)
Total (N=209)	48 (23)	35 (16.7)	12.78 (1.19)	12.71(0.08)

Z: score; SD: standard deviation; SE: standard error; BMI: body mass index;

Each year of later menarcheal age decreased the possibility of having short stature (z-score<1) by 60.7% and increased the probability of being with above-average height (z-score>1) by 86.2%, when compared with early menarche subjects. Moreover, later menarche age increased the probability of being underweight (BMI<18.50 kg/m^2) by 78.9%, and decreased the possibility of having overweight or obesity status (BMI (≥25kg/m^2) by 67.3%, all these compared with the subjects with early menarche (Table 4).

Table 4. The effect of age at menarche on body height and BMI

	B	Wald	P-value	OR	95% C.I.for OR	
					Lower	Upper
Height (z-score<-1)	-0.933	5.778	0.016	0.393	0.184	0.842
Height (z-score>1)	0.621	10.121	0.001	1.862	1.269	2.730
BMI<18.50 kg/m^2)	0.582	8.267	0.004	1,789	1.203	2.659
BMI (≥25kg/m^2)	-1.118	26.020	0.000	0.327	0.213	0.503

B: coefficient; OR: odds ratio; P: significance level; 95% CI: 95% confidence interval; BMI: body mass index

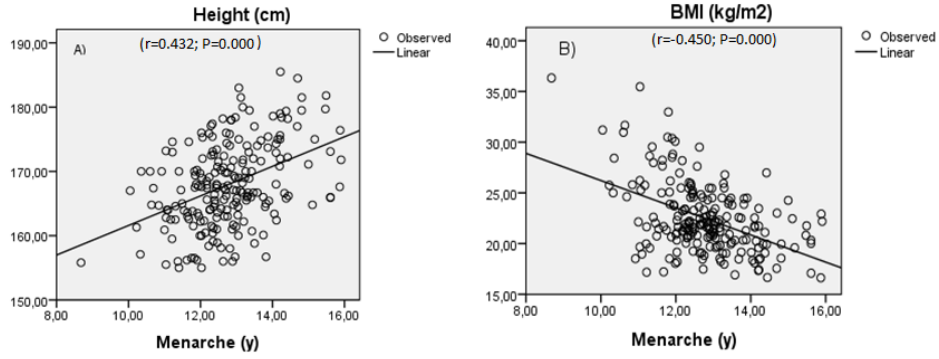
The linear regression results suggested that the model explained only 18.7% and 20.3% of the variability of height and underwight respectively, thus pointing to the presence of other factors that had effect on the subjects' height and BMI. The subjects' height showed significantly positive correlation with the age at menarche categories (BETA=0.432; P=0.000), while BMI showed negative correlation (BETA=-0.450; P=0.000). With each year of menarcheal age lowering, the height value was by 4.54 cm lower than the average, whereas BMI was greater by 2.48 kg/m^2 (Table 5).

Table 5. The effect of age at menarche categories on body height and BMI

						95% CI	
	B	R^2	BETA	t	P-value	Lower	Upper
Height (cm)	4.539	0.187	0.432	6.901	0.000	3.242	5.836
BMI (kg/m^2)	-2.483	0.203	-0.450	-7.251	0.000	-3.158	-1.808

B: regression coefficient; R^2 correlation coefficient; BETA: regression coefficients; P: significance level; 95% CI: 95% confidence interval; BMI: body mass index

A significantly positive correlation was detected between the age at menarche and the body height (r=0.432; P=0.000), whereas the correlation with BMI proved to be significantly negative (r=-0.450; P=0.000). This implies that as the age at menarche increases so does the body height, while the BMI values decrease (Graph 1).



Graph 1 Pearson's correlation between age at menarche and A) body height and B) BMI

Discussion

In this study we found that 18.7% variability of adult height depended on the age at menarche. There was a significant positive correlation between the age at menarche and body height of young females, and with each year of delay in menarche, body height was 4.54 cm lower than the average. Also, each year of later menarche reduced the probability of being short by 60.7% (z-score<1) and increased the probability of being above average height (z-score>1) by 86.2%, compared to the subjects with earlier menarche.

These results are consistent with studies of young women in Korea (Kang et al. 2019), where it was found that for each year earlier that menarche occurred the adult height was 0.445 cm lower. The same study also found that females with early menarche had a 10.5% chance of having short adult stature, which is similar with the results obtained in other countries in the world (Shangold et al. 1989; Hauspie et al. 1997; Onland-Moret et al. 2005). Also, an extensive study conducted in 9 European countries (Onland-Moret et al. 2005) showed that women grew approximately 0.35 cm taller when menarche occurred one year later. This could be explained by mechanisms of growth plate maturation and epiphyseal fusion. Women with earlier onset of menarche would experience earlier closure of epiphyseal growth plates due to an increase in ovarian estrogens (Emons et al. 2011; Börjesson et al. 2013). A low dose of estrogen would induce pubertal growth spurt in early puberty and early epiphyseal fusion by advancing growth plate senescence. Late menarche allows continued growth of long bones before epiphyseal fusion, leading to an increase in adult height. Pai et al. (2022) report that early menarche affects the adult height in the sense that menarche does not occur until peak height velocity has been reached, which has been observed in other studie (Sinclair, 1989; Kacerosky, 2011). Girls who reach menarche early lose pre-pubertal growth and hit peak height velocity at a younger age and early stoppage of long bone growth. On the other hand, girls with late menarche experience extra pre-pubertal growth and a delayed age of peak height velocity, leading to an extended time of long bone growth.

The results of BMI showed that 20.3% variability in adult BMI depended on the age at menarche. The subjects belonging to the early menarche category had a higher BMI by 3.41 kg/m^2 than the others, while the subjects from the late menarche group had a lower BMI by 1.50 kg than the subjects from the group ≤14 years. The total difference between late and early menarche was 4.82 kg/m^2. Later menarche increases the probability of being underweight by 78.9%, and decreases the probability of having exceeded weight (overweight/obese) by 67.3% compared to those with earlier menarche. With each year of decreasing menarche, BMI was 2.48 kg/m^2 higher.

These data are in line with previous results (Rakic et al. 2024) where it was found that age at menarche was in negative correlation with general and abdominal obesity. Young women with early age at menarche show statistically higher values of BMI, WC and WHtR, while those with late menarcheal age show greater susceptibility of becoming underweight. Another study showed (Sumi et al. 2018) that each consecutive year drop of age at menarche is associated with an increase in BMI by 0.25 kg/m^2 and 0.75 kg/m^2 in each later menarcheal age (Prentice and Viner, 2013). These results point to a high relationship between age at menarche and BMI and support the hypothesis that menarche can lead to obesity in young females (Rafique et al. 2019; Kim et al. 2021). A possible explanation is that a higher incidence of obesity in early menarche women is due to continuous fat accumulation induced by prolonged exposure to estrogen and adrenal steroids (Cho and Han, 2023). However, other studies (Gopalakrishna et al. 2016) found no relationship between BMI and age at menarche. The current study results suggested that females with late age at menarche had significantly lower

BMI in comparison with their peers with early age at menarche, and thus stand congruent with other reports (Das and Dasgupta, 2018; Arcoverde et al. 2020).

Conclusion

The present results point to a significant positive relationship between body height but negative association between BMI with age at menarche. The results are in line with other similar investigations and indicate that early menarche could be a risk factor for lower height and increased BMI in young females. However, it should be emphasized that the present data are the results of a cross-sectional survey and therefore should be extrapolated with caution. Another limitation of the study is self-reported age at menarche. Nevertheless, given that in Serbia there is still a negative secular trend in the age at menarche, the present results are important from a public health perspective, more precisely, in identifying females at risk from exceeded weight.

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POVEZANOST RANE I KASNE MENARHE SA VISINOM TELA I INDEKSOM TELESNE MASE KOD MLADIH ŽENA

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Uvod: Više endogenih i egzogenih faktora utiče na visinu tela i indeks telesne mase (ITM) kod odraslih osoba. Cilj ove studije bio je da se utvrdi povezanost godina menarhe sa visinom tela i indeksom telesne mase kod mladih žena. **Materijal i metode:** Transferzalno antropometrijsko istraživanje izvršeno je 2021-2024. godine a obuhvatilo je 209 studentkinja starosti 21-25 godina. Izmerene su visina tela i masa tela iz kojih je izračunat ITM. Pojava menarhe utvrđena je retrospektivnom metodom. **Rezultati:** Rana menarha (<12 godina) uočena je kod 23% studentkinja, a kasna (>14 godina) kod 16,7%. Prosečna godina menarhe iznosila je 12,78±1,19 godina. Prevalenca niskih (Z-skor<-1) i sa prekomernom masom tela (ITM≥25) je veća kod studentkinja sa ranom menarhom u odnosu na one sa kasnom (39,4% i 61% v.s. 0% i 2,4% respektivno). Prevalenca visokih (Z-skor >1) i pothranjenih (ITM<18,50) veća je kod ispitanica sa kasnom menarhom u odnosu na one sa ranom (43,6% i 47,4% v.s. 2,4% i 15,8% respektivno). Odnos šansi (OR) iznosio je za nizak rast 0,393, visok rast 1,862, pothranjenost 1,789 i gojaznost 0,327 (P<0,05). Kategorije godina menarhe su značajno uticale na visinu tela (BETA=0,432) i ITM (BETA=-0,450). **Zaključak:** Rana menarha je povezana sa niskim rastom i gojaznošću, a kasna sa visokim rastom i većim rizikom od pothranjenosti.

Ključne reči: rana menarha, kasna menarha, visina tela, indeks telesne mase,odrasle mlade žene

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PREVALENCE AND IVF OUTCOMES OF DIFFERENT INFERTILITY CAUSES IN WOMEN OF ADVANCED AGE

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Abstract **Background/ Aim.** Infertility is one of the posible causes for a decrease in fertility rates. It is a significant social and medical problem affecting one in six couples worldwide with the current prevalence of infertility being 8- 12% among women aged 20-44. The most significant and influental factor of infertility is women's age. This study aims to evaluate clinical outcomes among different infertility causes in women aged ≥35, as well as to investigate the prevalence of infertility causes. **Methods:** medical records were obtained from 873 women subjected to an IVF (in vitro fertilization) treatment cycle. Incidence and clinical outcomes of four infertility causes and factors leading to female cause of infertility were investigated. **Results:** The highest proportion of couples who had taken part in an assisted reproduction program, did so due to female cause of infertility, particullary due to ovulatory problems. Statistically significant differences between the four causes of infertility were found in terms of the number of retrieved and mature oocytes, fertilization rates. Differences in pregnancy and live birth rates were found between factors leading to female causes of infertility. **Conclusions:** Female factors, especially ovulatory problems are, as expected, the most common cause of infertility in the investigated age group and are associated with an increased risk of adverse clinical outcomes. This is useful information for the management of infertility but further investigation of infertility causes and prevalence is necessary.

Key words infertility, advanced age, fertility, in vitro fertilization, ovarian reserve

Introduction

Due to negative natural growth, Serbia is facing population reduction and modification of the population age structure [1]. A decrease in the population amounts to about 76,000 people per year, which is equal to a medium sized city, while the greatest negative natural growth thus far was recorded in 2020, when less than 62,000 babies were born and around 116,000 people had died [2]. The fertility rate in 2019. was 1.52 [3] and 28% below the European average of 1.6 children per woman [4]. Reasons for the fertility rate decline are various and should be thoroughly investigated.

Infertility is one of the possible causes for the decrease in fertility rate [5]. According to the World Health Organization (WHO), infertility is defined as the impossibility of achieving pregnancy after "a reasonable time" of sexual intercourse with no applied contraceptive

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measures [6]. About 80% of couples will achieve pregnancy in the first 6 months of attempting with the probability of concieving per month being the greatest during the first 3 months [7]. Hence, the general opinion on the "reasonable time" is questionable. The WHO and the European Society for Human Reproduction and Embryology (ESHRE) recommends that a couple should be considered infertile if pregnancy has not occured after 12 months or more of regular unprotected sexual intercourse [8,9]. Thus, most clinitians propose an analysis of infertile couple after one year of inefficient pregnancy attempts. In cases when the woman is >35 years old, the investigation should be recommended after six months of failed pregnancy attempts, taking into account age related infertility [10].

Infertility is a significant social and medical problem affecting one in six couples worldwide and it is estimated that the current prevalence of infertility is 8- 12% among women aged 20-44 [9]. In Serbia, calculations of infertility prevalence with this assessment show that the number of infertile couples of reproductive age is about 84031 [1]. The causes of infertility are different and can be divided into four groups: male, female, combined (both male and female) and unknown (unexplained, idiopathic) cause [11]. According to ESHRE, the current prevalence of each cause of infertility is as follows: female 20-35%, male 20-35%, combined 20-35% and unknown cause 10-20% [9].

Researches have shown that certain risk factors can influence and lead to infertility occurrence in men and women. The most significant and influential factor is women's age [12]. During the course of the last few decades, developed countries have recorded an increasing trend in delayed childbearing until advanced age which can result in reduced ovarian reserve [13]. A decrease in the number and quality of available oocytes over time and a rise in the genetic errors in the oocytes, negatively affect the ability of women in advanced age to conceive [14]. The average age of a mother delivering her first child in Serbia was 26,7 years in 2001 but in 2018 increases to 30 years [15]. Advanced maternal age (AMA) refers to the turning point at which pregnancy rates significantly decline [16]. There is no accepted consensus on the particular maternal age at which an adverse pregnancy outcome increases significantly [17]. Some researches consider AMA when birthing woman is ≥35 years [18,19] while others find ≥40 years [20, 21] as the cut-off for advanced age. Despite the continuous progress of assisted reproductive technologies (ART), live birth rates after IVF remain low in advanced maternal age, especially in women over 40 [22].

In Serbia, there is no national evaluation of biomedicaly assisted procedures, so the incidence and causes of infertility are still unspecified. At this moment, an awareness of this growing problem can only be achieved through clinical studies or detailed surveys of patients and infertility professionals. Thus, this study aimed to evaluate clinical outcomes among different infertility causes in women aged ≥35 as well as to investigate the prevalence of infertility causes.

Materials and methods:

Between January 2020 and December 2021, this retrospective study was conducted at Ferona Fertility Clinic with the approval of the Ferona Fertility Clinic's Ethics Board (approval No 1125-1/1-19) in accordance with the Declaration of Helsinki. The clinic's medical records were obtained as pertains to 873 women aged ≥35 who had been subjected to an IVF treatment cycle with autologous oocytes. The exclusion criteria were patients who had frozen all embryos, preimplantation genetic testing (PGT), donated oocytes, as well as patients without mature (metaphase II) oocytes or developed embryos.

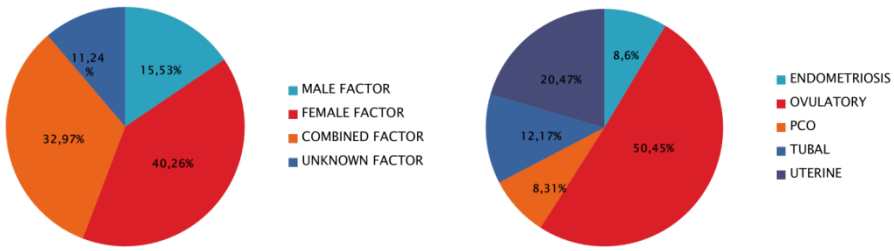
The IVF treatment of selected patients was previously described [23]. Briefly, the process of ovarian stimulation was carried out using the flexible GnRH antagonist protocol and 36 hours after ovulation trigger, oocyte pick-up was done. Fertilization method was ICSI (intracytoplasmic sperm injection) followed by ET (embryo transfer) on Day 3 or Day 5 after oocyte retrieval, and vitrification of surplus embryos, while successful implantation was confirmed by increased serum-βhCG level and fetal heartbeat at the 6th gestational week. The decision about the day of ET, number of transferred embryos, vitrification of surplus embryos as well as embryo grading was made according to the formerly published protocol [23].

The patients were categorized into four groups according to the cause of infertility: female, male, combined and unexplained. The female infetility cause group was divided into five subgroups: endometriosys, tubal factor, uterine factor, ovarian factor factor and PCOS (polycistic ovarian syndrome).The main IVF outcomes for these groups were the following: the number of retrieved and mature (MII) oocytes, fertilization rates, the number of cleaved and transferred embryos, the rate of A quality embryos, positive βhCG rates, clinical pregnancy rates, live birth and miscarriage rates, cumulative positive βhCG, clinical pregnancy, live birth and miscarriage rates. In order to investigate the main IVF outcomes in the female cause group as the most frequent infertility cause, additional analysis was applied.

Statistical interpretation was done using SSPS software version 25.0. while analysis of variance, chi-square test and post-hoc contrast analysis were used for testing differences between infertility causes groups and age groups.

Results

Eight hundred and sevety-three women subjected to IVF were investigated and the percentage of four main infertility causes have been given in Figure 1a while the percentage of five causes of female infertility have been given in Figure 1b.



a) b)

Figure 1. Prevalence of the main infertility causes (a) and female causes of infertility (b)

Table 1. Clinical outcoms for various infertility causes

Variables/causes of infertility	Female cause	Male cause	Combined cause	Unknown cause	F/χ^2	*p*
No of participants	337	130	276	94		
No of retrieved oocytes (Mean±SD)	5.48±4.09	7.16±3.87	5.87±4.23	6.43±3.55	5.85	<0.01
No of MII oocytes (Mean±SD)	3.71±2.71	4.92±2.73	4.08±2.99	4.33±2.48	6.11	<0.01
Fertilization rate (%)	89.13±17.32	82.42±20.36	85.22±19.25	82.00±19.52	6.32	<0.01
No of developed embryos (Mean±SD)	3.06±2.20	3.65±2.28	3.21±2.36	3.24±1.98	2.22	NS
No of A quality embryos (Mean±SD)	1.91±1.80	2.12±2.08	1.97±1.88	1.88±1.65	1.69	NS
No of transfered embryos (Mean±SD)	1.96±0.78	2.01±0.71	1.92±0.78	2.09±0.77	1.19	NS
Blastocyst rate (%)	58.51±22.42	42.11±20.79	48.69±26.46	60.44±30.92	2.46	NS
βhCG positive rate (%)	28.48	30.01	29.71	32.97	0.73	NS
Clinical pregnancy rate (%)	22.85	23.84	23.55	21.28	0.26	NS
Misscariage rate (%)	3.86	4.61	5.07	4.26	0.55	NS
Live birth rate (%)	18.69	13.23	18.48	17.02	0.19	NS
Cumulative clinical pregnancy rate (%)	25.22	27.69	26.09	24.46	0.40	NS
Cumulative live birth rate (%)	21.66	20.77	20.29	21.27	0.18	NS

*Abreviations:NS-not significant

Series analysis of variance and chi- square tests established statistically significant differences between the four causes of infertility (Table 1) on the variables of the total number of participants, the average number of retrieved oocytes, the average number of MII oocytes and the fertilization rates. There are significantly more respondents in the female cause and combined cause groups ($\chi^2 = 192.78$, $p < 0.01$). In terms of the average number of retrieved oocytes, there was a significant difference in the male cause in relation to the female cause ($\Delta M=1.68$, $p<0.01$) and the combined cause ($\Delta M=1.29$, $p<0.05$). The same significant difference between male cause and female/combined cause ($\Delta M=1.21$, $p=0.01/\Delta M=0.84$, $p<0.05$) was recorded in the average number of mature oocytes. Fertilization rates also showed a significant difference between female and male cause ($\Delta M=6.71$, $p<0.05$), as well as between female and combined cause ($\Delta M=7.13$, $p<0.01$) suggesting a higher percentage of fertilized oocytes with the female cause of infertility. No statistically significant differences were found in pregnancy, live birth, misscariage rates or cumulative rates.

Table 2. Clinical outcomes in various causes of the female infertility factor

Variables/infertility causes	Endometriosis	Ovarian cause	PCOS	Tubal cause	Uterine cause	F/χ^2	*p*
No of participants	29	170	28	41	69		
No of retrieved oocytes (Mean±SD)	4.82±3.61	3.91±2.92	11.18±5.40	6.53±3.88	6.65±3.68	29.75	<0.01
No of MII oocytes (Mean±SD)	3.70±1.95	2.79±1.96	7.64±3.81	4.14±2.64	4.39±2.46	28.16	<0.01
Fertilization rate (%)	89.02±18.02	92.17±16.28	79.57±16.05	88.86±17.39	85.80±18.37	4.23	<0.2
No of developed embryos (Mean±SD)	2.55±1.57	2.39±1.49	6.00±3.40	3.39±2.15	3.51±2.23	22.17	<0.01
No of transfered embryos (Mean±SD)	1.90±0.62	1.86±0.79	2.07±0.66	2.10±0.77	2.12±.83	1.86	NS
Clinical pregnancy rate (%)	31.03	14.70	46.42	31.71	24.63%	18.28	<0.1
Misscariage rate (%)	6.90	2.94	7.14	4.87	2.90%	2.21	NS
Live birth rate (%)	24.14	11.18	39.28	26.82	21.74%	16.90	<0.01
Cumulative clinical pregnancy rate (%)	31.03	15.8	50.00	34.14	30.43%	20.22	<0.01
Cumulative live birth rate (%)	27.59	12.94%	42.86	29.27	27.54%	18.43	<0.01

*Abreviations: NS-not significant

Analysis of variances and chi- square tests applied to investigate clinical outcomes among causes leading to female infertility (Table 2) demonstrated differences in almost all variables. The largest number of participants were in the group ovulatory cause group (χ^2 =211.47, p<0.01). There were significant differences in the average number of retrieved oocytes between the PCOS group compared to the ovulatory group (ΔM= 7.27, p<0.01), tubal cause (ΔM=2.62, p<0.01) and uterine cause groups (ΔM=2.74, p<0.01) in the direction that women in the ovulatory group had a significantly lower average number of retrieved oocytes. In case of mature oocytes, differences were found in the PCOS group compared to the endometriosis (ΔM=3.94, p<0.01), tubal cause (ΔM=3.50, p<0.01) and uterine cause groups (ΔM=2.74, p<0.01), as well as the ovulatory group compared to the tubal (ΔM=2.74, p<0.01) and uterine cause groups (ΔM=3.25, p<0.01). With respect to fertilization rates, the only difference was found between the ovulatory group and the PCOS group (ΔM=7.33, p<0.01) where women with ovulatory causes had a significantly higher number of fertilized oocytes. The PCOS group had a significantly higher number of developed embryos compared to all other groups (ΔM=3.45, p<0.01 endometriosis group, ΔM=3.61,p<0.01 ovulatory group, ΔM=2.61, p<0.01 tubal group and ΔM=2.49, p<0.01 uterine group). At the same time, statistically significant fewer embryos were obtained between the ovulatory group and the tubal (ΔM=1.00, p<0,01) and uterine groups (ΔM=1.12, p<0.01). In the ovulatory group, a significant decrease was observed in clinical pregnancy rates (χ^2 = 18.28, p < .01), live birth rates (χ^2 = 16.90, p < .01),

cumulative pregnancy rates ($\chi^2 = 20.22$, $p < .01$) and cumulative live birth rates ($\chi^2 = 18.43$, $p < .01$) compared to all other groups.

Among different age groups, the most prevalent factor for infertility among all three groups was female cause, followed by combined, male and unkown causes in age groups 35-38 years and 9-41 years, while in the +42 age group unknown cause was more present than male cause (Figure 2). An analysis of the female infertility group (Figure 3) indicates that the highest prevalence is due to the ovulatory cause among all three age groups, followed by uterine cause. In the 35-38 age group, more so than in the other two age groups, PCOS was of greater prevalence compared to tubal cause. In contrast to younger groups, endometriosis was more present in relation to PCOS and tubal causes in the oldest group.

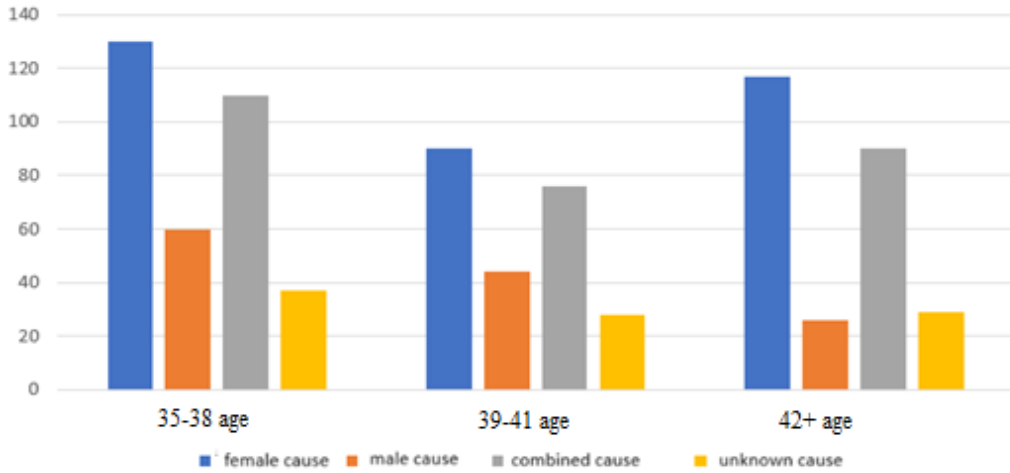


Figure 2. Distribution of infertility causes among various age groups

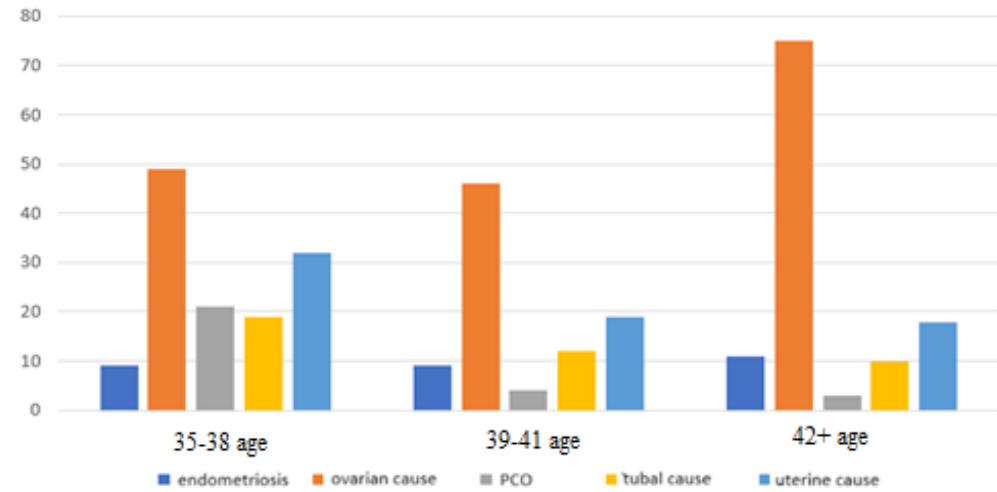


Figure 3. Distribution of female infertility causes among various age groups

Discussion

The results of the presented study have shown that the highest proportion of couples who have taken part in assisted reproduction program did so due to a female cause of infertility (40, 26%), in particular due to ovulatory problems such as reduced ovarian reserve and anovulation, which is in correlation with advanced women's age. Our study supports all previous findings as reduced ovarian reserve was the commonest cause, accounting for 58.9% of infertile advanced aged women [24, 25,26]. The male factor of infertility was present in a lower frequency (15,53%) than worldwide data [9] , but when associated with the female cause indicates a higher frequency, which corresponds to increasing tendency of male infertility worldwide [27] . It was observed that in 11.4% of cases of infertility remains unknown, which is in correlation with the global data [9] . The incidence of other investigated infertility causes in the subgroup of female infertility, such as endometriosis, tubal causes and PCOS was below other study findings [28]. The prevalence of the uterine cause (20,47%) was higher compared to a general and infertile population [29], in accordance with the participant's age considering evidence of a higher probability of uterine fibroids and polyps in woman of advanced age [30] .

An analysis of the frequency of the infertility causes, by age groups, has revealed that female and combined causes are the most common cause among all age groups, and the ovulatory cause was present in an all age groups with the highest percentage being in the oldest age group. This result indicates a significant decrease in the ovarian reserve. Unlike the other groups, uterine cause was less present in the oldest group while endometriosis was present to the greatest extent, contrary to recent research [31, 32] . We assume that this is most likely connected to the different management of endometriosis and uterine abnormalities.

An evaluation of clinical outcomes has revealed that patients with female and particularly ovulatory causes of infertility had a significantly lower number of retrieved and mature oocytes, which corresponds to other reports [33, 34]. These findings are related mostly to the women's age, hypothalamus and the pituitary gland [35],where women with a depleted pool of ovarian follicles, are faced with a decreasing number of available oocytes or even cycle cancelation after longer stimulation duration and higher doses of gonadotropins [36]. It has been well recorded that during the last few decades, the average childbearing age has increased and has become a key infertility factor [13]. With aging, the inevitable process of ovarian capacity reduction leads to less ovarian efficacy and an increase in the possibility of chromosomal abnormalities and miscarriages [37]. Furthermore, results have demonstrated that the oocyte number decrease among the endometriosis group, as does fertilization rate. It has been well established that patients with endometriosis have significantly fewer oocytes, mature oocytes and embryos compared to patients without endometriosis [38]. Endometriosis also lowers the cumulative live birth rate by decreasing the number of available embryos as well as by reduced implantation capacity [39].

The highest number of retrieved and mature oocyte was detected in male infertility group, which is expected given that this group mostly consists of normal and high responders. Decreased fertilization rate was observed in the male cause group which correlates with other studies suggesting decreased fertilization rates due to abnormal sperm morphology and DNA fragmentation [40, 41]. An analysis of female infertility has shown that the PCOS group had the highest number of retrieved oocytes,mature oocytes and developed embryos which corresponds to a high AMH (Anti Müllerian hormone) level in PCOS patients [42] . Fertilization rate was also significantly higher compared to other subgroups due to a higher number of MII oocytes.

In terms of pregnancy, live birth and miscarriage rates, our results have revealed no significant difference among the four infertility causes groups, while the most significant

finding was made in a subgroup of ovulatory cause where pregnancy and live birth rates, as well as cumulative rates, were significantly decreased compared to other groups.

Conclusion

Infertility is a health problem affecting one sixth of couples of reproductive age and requires an appropriate treatment strategy. Female factors specifically ovulatory problems are the commonest cause of infertility as expected in the investigated age group and were associated with an increased risk of adverse clinical outcomes. This is useful information for the management of infertility but further investigation of infertility causes and prevalence is necessary.

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PREVALENCA I ISHOD IVF-A KOD RAZLIČITIH UZROKA NEPLODNOSTI MEĐU ŽENAMA STARIJE REPRODUKTIVNE DOBI

Apstrakt Uvod/ Cilj. Neplodnost je jedan od mogućih uzroka smanjenja stope fertiliteta. To je značajan socijalni i medicinski problem koji pogađa jedan od šest parova u svetu uz globalnu prevalencu neplodnosti od 8-12% među ženama 20-44 godina starosti. Najznačajniji faktor sa najvećim uticajem na neplodnost su godine žene. Cilj ove studije je evaluacija kliničkih ishoda različitih uzroka neplodnosti kod žena ≥35 godina, kao i ispitivanje prevalence različitih uzroka neplodnosti. Metode: analizirani su medicinski podaci 873 žene podvrgnute IVF (in vitro fertilizacija) tretmanu. Incidenca i klinički ishodi četiri uzroka neplodnosti kao i faktora koji dovode do ženskog uzroka neplodnosti su ispitivani. Rezultati: Najzastupljeniji uzrok neplodnosti parova koji su podvrgnuti asistiranoj reprodukciji je ženski, posebno ovulatorni faktor. Statistički značajna razlika između četiri uzroka neplodnosti utvrđena je u pogledu ukupnog i broja zrelih jajnih ćelija, kao i stopa uspešne fertilizacije. Značajna razlika u stopama kliničkih trudnoća i rađanja je utvrđena između faktora koji dovode do ženskog uzroka neplodnosti. Zaključak: Ženski uzrok neplodnosti, posebno ovulatorni faktor je očekivano, najučestaliji uzrok neplodnosti u ispitivanoj starosnoj grupi i povezan je sa povećanim rizikom od nepovoljnog kliničkog ishoda. Ovo može biti korisna informacija u menadžmentu neplodnosti, ali su dalja kontinuirana istraživanja uzroka neplodnosti i njihove učestalosti neophodna.

Ključne reči: neplodnost, starije reproduktivno doba, fertilitet, in vitro fertilizacija, ovarijalna rezerva

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STEPEN UHRANJENOSTI I ŽIVOTNI STILOVI DJECE NIŽIH RAZREDA OSNOVNE ŠKOLE NA PODRUČJU ŠAMCA

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Sažetak Istraživanja stepena uhranjenosti djece nižih razreda osnovne škole (od prvog do petog razreda) na području opštine Šamac, kao i zastupljenost određenih životnih stilova (prehrambene navike i fizička aktivnost) provedena su 2021. godine. Uzorak je obuhvatio 80 ispitanika, 43 dječaka i 37 djevojčica, uzrasta od 6 do 11 godina. Izmjereni su osnovni antropometrijski parametri, visina tijela i masa tijela, na osnovu kojih je određen indeks tjelesne mase (ITM). Podaci o prehrambenim navikama i nivou fizičke aktivnosti prikupljeni su putem anonimnog anketnog upitnika. Prosječna vrijednost ITM za dječake iznosila je 18,29 kg/m^2 a za djevojčice 18,40 kg/m^2. Prosječne vrijednosti ovog indeksa, u odnosu na uzrast bile su: 17,43 kg/m^2 kod ispitanika iz prvog razreda, 17,90 kg/m^2 kod ispitanika iz drugog razreda, 18,26 kg/m^2 kod ispitanika iz trećeg razreda, 19,07 kg/m^2 kod ispitanika iz četvrtog razreda i 18,99 kg/m^2 kod ispitanika iz petog razreda. Mada najveći broj ispitanika pripada kategoriji normalno uhranjenih, zabrinjavajući je značajan udio prekomjerno uhranjene i gojazne djece. Analizom anketnog upitnika ustanovljeno je da prehrambene navike u manjoj ili većoj mjeri odstupaju od zdrave ishrane, dok je stepen fizičke aktivnosti na zadovoljavajućem nivou.

Ključne riječi: ITM, djeca, prehrambene navike, fizička aktivnost

UVOD

Broj ljudi s povećanim stepenom uhranjenosti u svijetu svakodnevno raste. Svjetska zdravstvena organizacija izvještava da se njihov broj popeo s 1,7 milijardi u 2010. godini na gotovo 2 milijarde u 2014. godini, a među njima je čak 13% gojaznih (WHO, 2014). Ovaj globalni zdravstveni problem nije zaobišao ni djecu pa se navodi da je prevalencija gojazne ili djece koja su prekomjerno uhranjena uzrasta ispod pet godina oko 20% (Ogden i sar., 2014). Prevalencija prekomjerne tjelesne mase i gojaznosti kod djece uzrasta preko pet godina u periodu između 1975. i 2016. godine povećana je sa 4% na preko 18%. U 2016. godini prevalencija prekomjerne mase tijela iznosila je 18% za djevojčice i 19% za dječake (WHO, 2022). S tim u vezi, analize stepena uhranjenosti mogu poslužiti kao polazna tačka za izradu prehrambene politike i ciljanih programa intervencije, te analizu prehrambenog i zdravstvenog stanja, kao i za unapređenje prehrane, kako na lokalnom tako i na nacionalnom nivou (Antonić-Degač i sar., 2004).

Redovno praćenje tjelesnog rasta i razvoja, doprinosi detekciji abnormalnosti rasta i razvoja, kao i ranoj identifikaciji gojaznosti i pothranjenosti (Smajić i sar., 2017). U ekspanziji su nepovoljni socijalni uslovi kao posljedica masovne urbanizacije i uticaja moderne kulture u svim oblastima života, a naročito među školskom djecom. Ovi pomenuti trendovi su integralni dio "nutritivne tranzicije" koja, udružena sa sedentarnim načinom života (deficit

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fizičke aktivnosti), nosi multiple rizike po zdravlje (Gligorijević, 2008). Načela pravilne prehrane predstavljaju važan dio zdravog stila života, te ih je zbog njihovog pozitivnog uticaja na cjelokupno zdravlje stanovništva neophodno usvojiti već u najranijoj životnoj dobi (Larson i Neumark-Sztainer, 2009).

Na uhranjenost utiče niz faktora od genetskih do sredinskih (socio-ekonomski, demografski, prehrambene navike, geografsko podneblje, fizička aktivnost), te različite bolesti (Lančić i Zelić, 2007). Prema Centru za kontrolu i prevenciju bolesti (CDC, 2023) danas se kao najjednostavniji pokazatelj prekomjerne tjelesne mase i gojaznosti, ali i rizika od razvoja bolesti povezanih s gojaznošću, najčešće koristi indeks tjelesne mase, skraćeno ITM (eng. *Body Mass Index, BMI*). Vrijednosti preporučenog ITM iste su za oba pola i za odraslu populaciju iznose od 18,50 kg/m^2 do 24,99 kg/m^2, za evropsko stanovništvo (WHO, 2006). Indeks tjelesne mase kod djece zavisi od pola i uzrasta, pa apsolutan ITM kod djece i mladih nije dobar parametar za klasifikaciju prekomjerne tjelesne mase i gojaznosti. Kod djece se masno tkivo mijenja sa uzrastom, a djevojčice i dječaci imaju različite udjele masnog tkiva tokom odrastanja (Grgurić, 2001). Većina djece po uhranjenosti ulazi u kategoriju normalno uhranjenih koja obuhvata širok raspon visine tijela i mase tijela (Gavin i sar., 2007), dok se povećane vrijednosti ITM povezuju sa povećanim rizikom od razvoja hroničnih i metaboličkih bolesti (Prebeg i sar., 1999). Neadekvatan energentski unos može imati za rezultat prekomjernu tjelesnu masu, a s druge strane, pothranjenost djeteta (Antonić-Degač i sar., 2004).

Igra i fizičke aktivnosti su osnovne i najvažnije djelatnosti djece predškolskog uzrasta i zato je njihovo uključivanje u različite tjelesne i sportske aktivnosti od posebnog interesa. Fizički aktivnija djeca imaju jače mišiće i kosti, kao i vitkije tijelo jer se vježbanjem redukuje količina masnog tkiva, zatim imaju niži krvni pritisak i niži nivo holesterola u krvi (Gavin i sar., 2007). Piramida fizičke aktivnosti djece se sastoji od četiri sprata. Prvi sprat su fizičke, sportske i nesportske aktivnosti koje se dešavaju svakodnevno, to su, na primjer, šetnja sa psom, hodanje uz stepenice, pomaganje u kući i dr. Drugi sprat piramide kretanja podrazumijeva aerobne vežbe i rekreativne sportove. Aerobne vježbe uključuju, na primjer, trčanje, brzo hodanje, biciklizam, plivanje, rolanje. Rekreativni sportovi su fudbal, košarka, odbojka, ples. Treći sprat piramide je slobodno vrijeme i agilnost. Slobodne aktivnosti su npr. pješačka grupa, kuglanje, joga, preskakanje konopca i sl. Četvrti sprat piramide čine aktivnosti koje treba da se svedu na minimum. Aktivnosti kao što su: gledanje TV-a, video zapisa, DVD-a, igranje igara, kompjuterske igre i dugo sjedenje bez svrhe ili cilja (Gajdošová i Košťálová, 2006).

Zbog nedostatka podataka o stanju uhranjenosti djece osnovnoškolskog uzrasta na području opštine Šamac, ali i na području Republike Srpske, cilj ovog rada bio je da se pomoću ITM utvrdi stanje uhranjenosti djece koja pohađaju niže razrede osnovne škole. Takođe, cilj je bio i da se ustanovi kakvi životni stilovi (prehrambene navike i fizička aktivnost) su zastupljeni kod ovog dijela populacije na području šamačke opštine.

MATERIJAL I METODE

Istraživanje je provedeno u nižim razredima Osnovne škole „Srbija“, u aprilu 2021. godine, na području Šamca. Djeca su dobrovoljno i uz saglasnost roditelja i direktora škole, učestvovali u svim aktivnostima koje su vršene u svrhu izrade ovog rada.

Uzorak je obuhvatio 80 ispitanika, djece iz nižih razreda osnovne škole (od 1. do 5. razreda), uzrasta od 6 do 11 godina. Za svakog ispitanika izračunat je decimalni uzrast iz datuma ispitivanja i datuma rođenja. Uzrasne kategorije formirane su u odnosu na razred koji su ispitanici pohađali: 1. razred (između 6 i 7 godina), 2. razred (između 7 i 8 godina), 3. razred

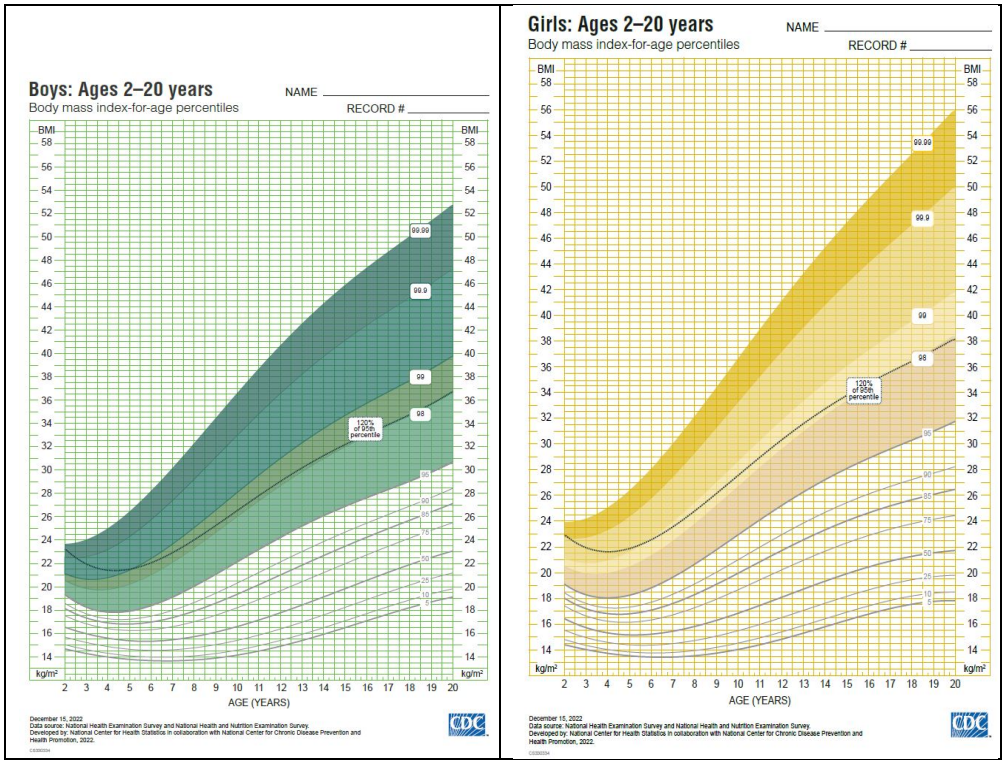
(između 8 i 9 godina), 4. razred (između 9 i 10 godina) i 5. razred (između 10 i 11 godina), pri čemu je određen i prosječan uzrast. Najbrojniji su ispitanici drugog razreda (25,00%), potom petog razreda (22,50%) i trećeg razreda (21,25%), nešto manja brojnost je bila u četvrtom razredu (16,25%), i najmanji broj ispitanika bio je iz prvog razreda (15,00%). Od ukupnog broja ispitanika 53,75% su bili dječaci, a 46,25% djevojčice (Tabela 1).

Tabela 1. Distribucija ispitanika s obzirom na uzrast i pol

Table 1. Distribution of participans by age and sex

Razred	Ispitanici				Ukupno	
	Dječaci		Djevojčice			
	N	%	N	%	N	%
1	8	18,60	4	10,81	**12**	**15,00**
2	7	16,28	13	35,14	**20**	**25,00**
3	11	25,58	6	16,22	**17**	**21,25**
4	6	13,95	7	18,92	**13**	**16,25**
5	11	25,58	7	18,92	**18**	**22,50**
Ukupno	**43**	**53,75**	**37**	**46,25**	**80**	**100,00**

Antropometrijski parametri korišteni za procjenu opšte uhranjenosti djece su visina tijela i tjelesna masa. Tjelesna masa je mjerena digitalnom vagom (preciznosti 0,1 kg), pri čemu su ispitanici stajali bosi na njoj, mirno, u uspravnom stavu. Tjelesna visina je mjerena pomoću samostojećeg visinomjera, mjernog područja od 0 do 205 cm. Ispitanici su stajali bosi na ravnoj podlozi, u uspravnom stavu, relaksiranih ramena i sastavljenih peta, težine raspoređene na obje noge, ruku opuštenih uz tijelo, a glava u vodoravnom položaju u ravnini s tijelom. Izmjerene vrijednosti tjelesne visine i tjelesne mase ispitanika poslužile su za određivanje indeksa tjelesne mase prema formuli: ITM=masa/visina^2 (kg/m^2) (Freedman i sar., 2013). Vrijednosti su očitane na osnovu percentila ITM pa se na taj način odredilo kojoj grupi pripada ispitanik (Slika 1). Djeca do 5. percentila svrstani su u grupu pothranjene djece, od 5. do 85. percentila u grupu normalno uhranjene djece, od 85. do 95. percentila u grupu prekomjerno teške djece, a oni iznad 95. percentila pripadali su grupi gojaznih (CDC, 2023).



Slika 1. Percentilne krivulje BMI prema uzrastu – lijevo za dječake, desno za djevojčice
Figure 1. BMI percentile curves by age – left: boys, right: girls

(https://www.cdc.gov/growthcharts/data/extended-bmi/BMI-Age-percentiles-BOYS.pdf; https://www.cdc.gov/growthcharts/data/extended-bmi/BMI-Age-percentiles-GIRLS.pdf)

Putem anonimnog anketnog upitnika prikupljeni su podaci o životnom stilu, odnosno prehrambenim navikama i nivou fizičke aktivnosti kod ispitanika. Ispitanici su samostalno i/ili uz pomoć učitelja/učiteljice ispunjavali anketni upitnik. Anketni upitnik je sadržavao 13 pitanja pri čemu se 10 pitanja odnosilo na prehrambene navike, a tri na nivo fizičke aktivnosti.

Deskriptivnim statističkim metodama određeni su srednja vrijednost, minimalne i maksimalne vrijednosti, standardna devijacija za kvantitativne varijable kao i broj i učestalost određenih stanja koja se opserviraju za kvalitativne varijable. Zbog malog broja ispitanika u razredima polna razlika visine tijela, mase tijele i ITM izračunata je na ukupnom uzroku ispitanika od 1. do 5. razreda pomoću t-testa nezavisnosti ($p<0,05$), a za statističku obradu podataka korišten je Microsoft Office Excel 2016.

REZULTATI

Prosječna visina dječaka je 141,05 cm, a masa tijela 36,91 kg. Prosjek tjelesne visine djevojčica je 139,53 cm, a težine 36,29 kg. Dječaci su u prosjeku 1,52 cm viši od djevojčica, dok je tjelesna težina relativno ujednačena. Prosječne vrijednosti ITM kod dječaka (18,29 kg/m^2) i djevojčica (18,40 kg/m^2) (Tabela 2). Statistički značajnih razlika među polovima u

vrijednostima antropometrijskih karakteristika i ITM nije bilo (tjelesna visina p=0,7797, tjelesna masa p=0,7877, ITM p=0,8946).

Tabela 2. Deskriptivna statistika antropometrijskih karakteristika i ITM u odnosu na pol

Table 2. Descriptive statistics of anthropometric characteristics and BMI by sex

Dječaci			
	Min	**Max**	**SV±SD**
Visina (cm)	121,00	162,10	141,05±11,26
Masa (kg)	21,60	72,10	36,91±10,42
ITM	13,04	29,80	18,29±3,60
Djevojčice			
	Min	**Max**	**SV±SD**
Visina (cm)	119,00	156,00	139,53±8,43
Masa (kg)	20,90	59,80	36,29±9,57
ITM	12,50	26,80	18,40±3,44

(Legenda: Min-minimalna vrijednost; Max-maksimalna vrijednost; SV-srednja vrijednost; SD-standardna devijacija)

U poduzorku dječaka 53,49% bilo je normalno uhranjenih, dok je kod djevojčica normalna uhranjenost zabilježena kod 48,65% ispitanica. Kategoriji prekomjerno uhranjenih pripadalo je 20,93% dječaka i 24,32% djevojčica, dok je u kategoriji gojaznih bilo 20,93% dječaka i 21,62% djevojčica (Tabela 3).

Tabela 3. Frekvencija ITM prema kategorijama uhranjenosti u odnosu na pol

Table 3. BMI frequency by nutritional status categories and sex

Katgorija ITM	**Dječaci**		**Djevojčice**	
	N	%	N	%
Pothranjenost	2	4,65	2	5,41
Normalna uhranjenost	23	53,49	18	48,65
Prekomjerna uhranjenost	9	20,93	9	24,32
Gojaznost	9	20,93	8	21,62
Ukupno	43	100,00	37	100,00

U tabeli 4 prikazani su osnovni deskriptivni statistički podaci antropometrijskih karakteristika i ITM u odnosu na uzrast, odnosno za ispitanike prvog, drugog, trećeg, četvrtog i petog razreda. Kada je u pitanju visina tijela, prosječne vrijednosti kretale su se od 126,38 cm kod ispitanika prvog razreda pa do 154,83 cm kod ispitanika petog razreda. Tjelesna masa, odnosno njene prosječne vrijednosti kretale su se od 28,09 kg kod ispitanika prvog razreda pa do 43,89 kg kod ispitanika petog razreda. Prosječne vrijednosti ITM kretale su se od 17,43 kg/m^2 kod ispitanika prvog razreda do 18,99 kg/m^2 kod ispitanika petog razreda (Tabela 4).

Tabela 4. Deskriptivna statistika antropometrijskih karakteristika i ITM u odnosu na uzrast

Table 4. Descriptive statistics of anthropometric characteristics and BMI by age

1. razred (6,25 godina)			
	Min	**Max**	**SV± SD**
Visina (cm)	119,00	135,00	126,38±4,89
Masa (kg)	21,60	43,30	28,09±7,10
ITM	13,90	26,02	17,43±3,55
2. razred (7,25 godina)			
	Min	**Max**	**SV± SD**
Visina (cm)	125,00	143,00	133,25±5,90
Masa (kg)	21,70	46,80	32,17±6,63
ITM	13,45	25,06	17,90±3,32
3. razred (8,65 godina)			
	Min	**Max**	**SV± SD**
Visina (cm)	137,00	157,00	144,25±7,05
Masa (kg)	20,90	59,80	37,34±10,04
ITM	12,50	26,80	18,26±3,89
4. razred (9,23 godina)			
	Min	**Max**	**SV± SD**
Visina (cm)	135,00	157,00	145,67±7,69
Masa (kg)	27,50	72,10	40,37±11,52
ITM	14,00	29,80	19,07±4,01
5. razred (10,11 godina)			
	Min	**Max**	**SV± SD**
Visina (cm)	149,00	162,00	154,83±6,08
Masa (kg)	31,50	55,50	43,89±7,07
ITM	14,00	24,50	18,99±3,07

(Legenda: Min-minimalna vrijednost; Max-maksimalna vrijednost; SV-srednja vrijednost; SD-standardna devijacija)

Kada su u pitanju kategorije uhranjenosti, rezultati naših istraživanja pokazuju da je najviše bilo normalno uhranjene djece i to 58,30% u prvom, 40,00% u drugom, 41,20% u trećem, 69,20% u četvrtom i 55,55% u petom razredu. Svakako se ne smiju zanemariti rezultati koje ukazuju na značajnu zastupljenost djece sa prekomjernom tjelesnom težinom, kao i gojazne djece. Djece sa prekomjernom tjelesnom težinom bilo je 16,70% u prvom, 30,00% u drugom, 23,50% u trećem, 7,70% u četvrtom i 27,80% u petom razredu. Zabilježen je i izuzetno visok udio gojazne djece u svim uzrastima, i to po 25,00% u prvom i drugom razredu, 23,50% u trećem, 23,10% u četvrtom i 11,10% u petom razredu. Što se tiče pothranjene djece, u prvom i četvrtom razredu nisu zabilježeni, ali su konstatovani u trećem, (11,80%) i u petom razredu (5,55%) (Tabela 5).

Tabela 5. Frekvencija ITM prema kategorijama uhranjenosti u odnosu na uzrast

Table 5. BMI frequency by nutritional status categories and age

ITM katgorija	1. razred		2. razred		3. razred		4. razred		5. razred	
	N	%	N	%	N	%	N	%	N	%
Pothranjenost	0	0,00	1	5,00	2	11,80	0	0,00	1	5,55
Normalna uhranjenost	7	58,30	8	40,00	7	41,20	9	69,20	10	55,55
Prekomjerna uhranjenost	2	16,70	6	30,00	4	23,50	1	7,70	5	27,80
Gojaznost	3	25,00	5	25,00	4	23,50	3	23,10	2	11,10
Ukupno	12	100,00	20	100,00	17	100,00	13	100,00	18	100,00

Rezultati anketnog upitnika koji se odnose na prehrambene navike i životni stil ispitanika prikazani su tabelarno, po pitanjima (Tabela 6).

Tabela 6. Prehrambene navike i stepen fizičke aktivnosti ispitanika (%)

Table 6. Dietary habits and physical activity level of participants (%)

1. Imaš li naviku doručkovanja u toku školske sedmice?								
	Ukupno	Dječaci	Djevojčice	1. razred	2. razred	3. razred	4. razred	5. razred
Svaki dan	43,75	37,22	51,35	8,33	40,00	47,06	30,77	77,78
Ponekad	33,75	32,55	35,14	33,33	25,00	41,18	53,85	22,22
Nikada	22,50	30,23	13,51	58,34	35,00	11,76	15,38	0,00
2. Imaš li naviku doručkovanja vikendom?								
	Ukupno	Dječaci	Djevojčice	1. razred	2. razred	3. razred	4. razred	5. razred
Oba dana vikenda	82,50	88,37	78,37	58,33	5,00	88,24	61,54	94,44
Jedan dan vikenda	12,50	9,30	16,22	16,67	95,0	5,88	38,46	5,56
Nikada	5,00	2,33	5,41	25,00	0,00	5,88	0,00	0,00
3. Koliko često jedeš voće?								
	Ukupno	Dječaci	Djevojčice	1. razred	2. razred	3. razred	4. razred	5. razred
Svaki dan	40,00	25,58	56,76	33,33	40,00	23,53	38,46	61,11
Ponekad	58,75	72,09	43,24	66,67	60,00	70,59	61,54	38,89
Nikada	1,25	2,33	0,00	0,00	0,00	5,88	0,00	0,00
4. Koliko često jedeš povrće?								
	Ukupno	Dječaci	Djevojčice	1. razred	2. razred	3. razred	4. razred	5. razred
Svaki dan	36,25	32,56	40,50	58,33	30,00	41,18	30,77	27,78
Ponekad	62,50	65,12	59,59	41,67	70,00	58,82	69,23	66,67
Nikada	1,25	2,32	0,00	0,00	0,00	0,00	0,00	5,55
5. Koliko često jedeš kolače, kekse ili druge slatkiše?								
	Ukupno	Dječaci	Djevojčice	1. razred	2. razred	3. razred	4. razred	5. razred
Svaki dan	26,25	27,91	24,32	16,67	35,00	35,29	30,77	11,11
Ponekad	71,25	67,44	75,68	83,33	60,00	64,71	61,54	88,89
Nikada	2,50	4,65	0,00	0,00	5,00	0,00	7,69	0,00

6. Piješ li većinom vodu ili sokove?								
	Ukupno	Dječaci	Djevojčice	1. razred	2. razred	3. razred	4. razred	5. razred
Uglavnom voda	37,50	30,23	45,95	75,00	25,00	29,41	30,77	38,89
Uglavnom sok	1,25	2,33	0,00	0,00	0,00	5,88	0,00	0,00
Podjednako voda i sok	61,25	67,44	54,05	25,00	75,00	64,71	69,23	61,11
7. Koliko često piješ gazirane sokove (*Pepsi, Coca Cola, Sprite*...)?								
	Ukupno	Dječaci	Djevojčice	1. razred	2. razred	3. razred	4. razred	5. razred
Svaki dan	6,25	9,30	2,70	8,33	0,00	5,88	0,00	16,67
Ponekad	73,75	79,07	67,57	91,67	65,00	64,71	92,31	66,66
Nikada	20,00	11,63	29,73	0,00	35,00	29,41	7,69	16,67
8. Koliko često jedeš brzu hranu (npr. pica, burek, hamburger, hotdog...)?								
	Ukupno	Dječaci	Djevojčice	1. razred	2. razred	3. razred	4. razred	5. razred
Svaki dan	3,75	4,65	2,70	8,33	0,00	11,76	0,00	0,00
Ponekad	85,00	83,72	86,49	83,34	90,00	82,35	84,62	83,33
Nikada	11,25	11,63	10,81	8,33	10,00	5,89	15,38	16,67
9. Koliko često jedeš mesne prerađevine (npr. salame, hrenovke, paštete...)?								
	Ukupno	Dječaci	Djevojčice	1. razred	2. razred	3. razred	4. razred	5. razred
Svaki dan	10,00	16,28	2,70	8,33	0,00	29,41	7,69	5,56
Ponekad	82,50	74,42	91,89	91,67	85,00	70,59	84,62	83,33
Nikada	7,50	9,30	5,41	0,00	15,00	0,00	7,69	11,11
10. Kako se hraniš u školi?								
	Ukupno	Dječaci	Djevojčice	1. razred	2. razred	3. razred	4. razred	5. razred
Nosi hranu od kuće	57,50	58,13	56,76	83,33	70,00	70,59	23,08	38,89
Kupi hranu	32,50	32,56	32,43	0,00	0,00	23,53	69,23	55,56
Ne jede u školi	10,00	9,31	10,81	16,67	30,00	5,88	7,69	5,55
11. Baviš li se nekom fizičkom aktivnosti i kojom?								
	Ukupno	Dječaci	Djevojčice	1. razred	2. razred	3. razred	4. razred	5. razred
Bavim	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00
Ne bavim	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
12. Koliko puta sedmično se baviš nekom fizičkom aktivnošću?[1]								
	Ukupno	Dječaci	Djevojčice	1. razred	2. razred	3. razred	4. razred	5. razred
Svaki dan	65,00	55,81	75,68	41,67	100,00	29,41	53,85	83,33
2-3 puta sedmično	31,25	37,22	24,32	50,00	0,00	70,59	46,15	5,56
1 sedmično	3,75	6,97	0,00	8,33	0,00	0,00	0,00	11,11

[1] Fizičke aktivnosti kojima se bave ispitanici najčešće su bile: fudbal, igranje kod kuće, škola sporta, vožnja bicikla, rolera i/ili skejtborda, folklor, šetnja (sa kućnim ljubimcem), ples, gimnastika, tenis, karate, odbojka, košarka i druge.

13. Šta uglavnom radiš za vrijeme velikog odmora?								
	Ukupno	Dječaci	Djevojčice	1. razred	2. razred	3. razred	4. razred	5. razred
Sjedi	43,75	30,23	59,50	58,33	40,00	23,53	61,54	44,44
Šeta	38,75	48,84	27,02	16,67	50,00	58,82	15,38	38,89
Stoji	3,75	2,32	5,41	0,00	0,00	5,88	7,69	5,56
Trči	13,75	18,61	8,10	25,00	10,00	11,77	15,39	11,11

Kada je u pitanju učestalost doručkovanja tokom radne sedmice, 43,75% ispitanika doručkuje svaki dan, dok 22,50% uopšte nema naviku doručkovanja. Veći procenat djevojčica (51,35%) u poređenju s dječacima (37,22%) ima naviku svakodnevnog doručkovanja tokom radne sedmice, a dječaci češće izostavljaju doručak, što potvrđuje podatak da 30,23% njih nema naviku doručkovanja, u odnosu na 13,51% djevojčica. Ispitanici prvog razreda pokazuju najlošije prehrambene navike, sa samo 8,33% onih koji doručkuju svaki dan, dok ispitanici petog razreda pokazuju najbolje prehrambene navike, pri čemu 77,78% doručkuje svakodnevno, a nijedan ispitanik ne izostavlja doručak.

Navike doručkovanja vikendom pokazuju da 82,50% ispitanika ima naviku doručkovanja oba dana vikenda, pri čemu dječaci češće doručkuju oba dana vikenda (88,37%) u poređenju s djevojčicama (77,37%). Analiza po razredima pokazuje značajne razlike: ispitanici prvog razreda imaju najniži procenat doručkovanja oba dana vikenda (58,33%), pri čemu čak 25% ne doručkuje ni jedan dan vikenda. S druge strane, čak 94,44% ispitanika petog razreda doručkuje oba dana vikenda, a niko nije izjavio da izostavlja doručak.

Navike konzumacije voća pokazuju da 58,75% ispitanika jede voće ponekad, 40,00% svaki dan, a samo 1,25% ne jede voće nikada. Djevojčice češće jedu voće svaki dan (56,76%) u poređenju s dječacima (25,58%). Ispitanici prvog razreda imaju najniži procenat svakodnevne konzumacije voća (33,33%), dok peti razred opet pokazuje najpoželjnije prehrambene navike, pri čemu 61,11% jede voće svaki dan.

Navike konzumacije povrća pokazuju da 62,50% ispitanika jede povrće ponekad, 36,25% konzumira povrće svaki dan, dok samo 1,25% ne jede povrće nikada. Analiza prema polu pokazala je da djevojčice češće jedu voće svaki dan (40,50%) u poređenju s dječacima (32,56%). Ono što je zanimljivo je da ispitanici prvog razreda imaju najviši procenat svakodnevne konzumacije povrća (58,33%), dok ispitanici petog razreda najrjeđe svakodnevno konzumiraju povrće (27,78%).

Analiza navike konzumacije slatkiša među ispitanicima pokazuje da 71,25% jede slatkiše ponekad, 26,25% svakodnevno, dok 2,50% ne jede slatkiše nikada. Analiza prema polu ukazuje da dječaci nešto češće jedu slatkiše svakodnevno (27,91%) u poređenju s djevojčicama (24,32%). Analiza po razredima ukazuje da ispitanici svih razreda najčešće konzumiraju slatkiše ponekad, pri čemu je najviše ispitanika trećeg razreda koji svakodnevno konzumiraju slatkiše.

Navike konzumacije pića među ispitanicima pokazuju da najviše njih (61,25%) pije podjednako i vodu i sokove, 37,50% preferira vodu, a samo 1,25% pije uglavnom sokove. Analiza prema polu otkriva da djevojčice češće biraju vodu (45,95%) u poređenju s dječacima (330,23%). Analiza po razredima pokazuje da ispitanici prvog razreda najčešće piju vodu (75,00%), dok kod ispitanika drugog razreda dominira konzumacije podjednako i vode i soka (75,00%), sa najmanjih učešćem onih koji piju uglavnom vodu (25,00%)

Kada je u pitanju konzumacija gaziranih sokova, konstatovano je da 73,75% ispitanika pije gazirane sokove ponekad, 6,25% svakodnevno, dok 20,00% ne konzumira gazirane sokove nikada. Analiza prema polu pokazuje da dječaci u većem broju piju gazirane sokove i svakodnevno i ponekad (9,30%, odnosno 79,07%) u poređenju s djevojčicama (2,70%,

odnosno 67,57%). Ispitanici svih razreda najčešće konzumiraju gazirane sokove ponekad, dok se navika svakodnevnog konzumiranja gaziranih sokova značajnije javlja u petom razredu (16,67%).

Analize konzumacije brze hrane pokazuju da 85,00% ispitanika jede brzu hranu ponekad, 3,75% svakodnevno, dok 11,25% ispitanika ne jede brzu hranu nikada. Analiza prema polu ukazuje na to da dječaci češće konzumiraju brzu hranu svakodnevno u odnosu na djevojčice (4,65% naspram 2,70%). Analiza po razredima govori da ispitanici svih razreda najčešće jedu brzu hranu ponekad, pri čemu u prvom i trećem razredu ima najviše ispitanika koji brzu hranu jedu svakodnevno (8,33% i 11,76%).

Učestalost konzumacije mesnih prerađevina pokazuju da 82,50% ispitanika jede mesne prerađevine ponekad, 10,00% svakodnevno, dok 7,50% ispitanika ne konzumira mesne prerađevine nikada. Dječaci češće konzumiraju mesne prerađevine svakodnevno u odnosu na djevojčice (16,28% naspram 2,70%) ili ih ne jedu nikada (9,30% naspram 5,41%). Većina ispitanika svih razreda jede mesne prerađevine ponekad, pri čemu se najveći broj ispitanika koji ih konzumira svakodnevno javlja u trećem razredu (29,41%).

Navike hranjenja u školi pokazuju da 57,50% ispitanika nosi hranu od kuće, 32,50% kupuje hranu u školi, a 10,00% uopšte ne jede u školi. Dječaci nešto češće nose hranu od kuće (58,13%) u poređenju s djevojčicama (56,76%), dok je procenat ispitanika koji kupuju hranu gotovo jednak među dječacima (32,56%) i djevojčicama (32, 43%). Analiza po razredima pokazuje da ispitanici nižih razreda (prvog, drugog i trećeg) češće nose hranu od kuće. U četvrtom i petom razredu većina ispitanika kupuje hranu (69,23%, odnosno 55,56%).

Svi ispitanici izjavili su da se bave nekom vrstom fizičke aktivnosti, pri čemu se 65,00% bavi fizičkom aktivnošću svakodnevno, 31,25% dva do tri puta sedmično, dok samo 3,75% fizičku aktivnost upražnjava jednom sedmično. Djevojčice češće učestvuju u svakodnevnoj fizičkoj aktivnosti (75,68%) u poređenju s dječacima (55,81%). Analiza po razredima pokazuje određene razlike: svi ispitanici drugog razreda su svakodnevno fizički aktivni, za razliku od ispitanika trećeg razreda, kod kojih se svakodnevna fizička aktivnost javlja kod 29,41%.

Na posljednje pitanje anketnog upitnika ispitanici su odgovorili kako za vrijeme velikog odmora njih 43,75% uglavnom sjedi, 38,75% šeta, 13,75% trči, a 3,75% stoji. Analiza prema polu pokazala da je da dječaci u odnosu na djevojčice češće trče i šeću, dok djevojčice češće sjede i stoje. Najveći broj isitanika koji trči javlja se u prvom razredu (25,00%), dok se najveći broj ispitanika koji sjedi sreće u četvrtom razredu (61,54%).

DISKUSIJA

Ješić (2017) daje podatke sličnih istraživanja za ispitanike nižih razreda osnovne škole (prvog, drugog, trećeg i četvrtog razreda) sa područja Obrenovca (Srbija), pri čemu je prosječna visina dječaka iznosila 137,42 cm, masa 34,57 kg, a ITM 18,05 kg/m^2, dok je kod djevojčica prosječna visina iznosila 136,58 cm, masa 32,91 kg, a ITM 17,39 kg/m^2. Sve konstatovane vrijednosti su niže nego kod naših ispitanika, s obzirom na to da su u našem radu obuhvaćena i djeca petog razreda. Isti autor navodi da nije postojala statistički značajna razlika u odnosu na pol ni u jednoj ispitivanoj varijabli, što je konstatovano i u našem istraživanju.

Kada su u pitanju kategorije uhranjenosti, Banjević (2019) iznosi rezultate sličnih istraživanja kod djece osnovnoškolskog uzrasta (drugog i trećeg razreda) sa područja Nikšića (Crna Gora) gdje, po pitanju uhranjenosti u oba poduzorka ispitanika dominiraju oni sa normalnom uhranjenosti (84,38% dječaka i 82,14% djevojčica), a slijedili su prekomjerno uhranjeni (9,37% dječaka i 10,71% djevojčica), dok se zastupljenost gojaznih i pothranjenih

kretala oko 3%, kod oba pola. Ješić (2017) konstatuje da je na području Obrenovca (Srbija) prevalenca gojaznosti kod dječaka značajno veća nego djevojčica (10,9% prema 3,5%). Upoređujući vrijednosti naših istraživanja sa vrijednostima gore pomenutih studija, ustanovljen je znatno viši udio onih sa prekomjernom tjelesnom težinom i gojaznošću, koja je relativno ravnomjerno prisutna kod oba pola.

Na osnovu podataka Svjetske zdravstvene organizacije (WHO, 2007), za navedeni uzrast (od šest do 11 godina) se konstatuje raspon prosječnih vrijednosti tjelesne visine od 115,10 cm do 145,00 za djevojčice i od 116,00 cm do 143,10 cm za dječake. Za navedeni uzrast (od šest do 10 godina), tjelesna masa kod djevojčica se kreće od 20,20 kg do 31,90 kg, a kod dječaka od 20,50 kg do 31,20 kg. Referentni podaci o težini u odnosu na uzrast nakon 10-te godine nisu dostupni pošto tada mnoga djeca doživljavaju pubertetski skok rasta i može se činiti da imaju prekomjernu težinu (prema težini za uzarst), a zapravo su samo visoki (WHO, 2007). Poredeći ove vrijednosti sa našim rezultatima, uočavamo da su prosječne vrijednosti tjelesne visine kod ispitanika znatno veće (od 126,40 cm kod ispitanika uzrasta između šest i sedam godina do 154,80 cm za ispitanike uzrasta između 10 i 11 godina). Sličan trend prisutan je i kada je u pitanju tjelesna težina, čije su prosječne vrijednosti znatno veće kod naših ispitanika (od 26,02 kg kod ispitanika uzrasta između šest i sedam godina do 43,90 kg za ispitanike uzrasta između 10 i 11 godina). Stav Svjetske zdravstvene organizacije je da u smislu adekvatnog praćenja akceleracije rasta i razvoja, treba primjenjivati komparacije sa definisanim nacionalnim standardima za određeni uzrast, kako bi se anuliralo dejstvo etnografskih razlika (Banjević, 2019). S tim u vezi, poređeni su naši rezultati sa rezultatima Vučkovića i sar. (2019) koji su slično istraživanje provodili sa djecom iz drugog i trećeg. razreda osnovne škole (uzrast između 7 i 8 godina) sa područja Banja Luke. Tom prilikom, ustanovljene su nešto niže proječne vrijednosti ITM (17,33 kg/m^2 za dječake i 17,04 kg/m^2 za djevojčice) u odnosu na naša istraživanja (17,90 kg/m^2 za sve ispitanike drugog razreda).

Rezultati dobijeni u našem istraživanju su poređeni sa rezultatima sličnih istraživanja i dostupnih standarda za pojedine parametre. U SAD (Troiano i Flegal, 1999), kao i u evropskim zemljama poput Finske (Nuutinen i sar., 1991), Velike Britanije (Chinn i sar., 1998) i Holandije (Blokstra i Kromhout, 1991; Seidell i sar., 1995) prevalencija povećane tjelesne mase i gojaznosti kod djece i adolescenata se dramatično povećala. U našim istraživanjima konstatovana je zastupljenost djece sa prekomjernom tjelesnom težinom i gojaznošću od 41,18%. Trend djece s prekomjernom tjelesnom masom i gojaznošću zabilježen je i u velikom broju evropskih zemalja u tranziciji i zemalja centralne i istočne Evrope gdje su udjeli različiti, od Crne Gore (21,2%), Slovenije (48,1%), Turske (36,8%), Latvije (42,1%), Češke (38,4%), Slovačke (33,7%), Mađarske (33,3%), Poljske (32,9%), Litvanije i Ruske Federacije s 32,3%, pa do Bjelorusije sa 26,2% i Grčke sa 24,6% (VCG MZRISS, 2008; Bodzsar i Zskai, 2014; Tsiliou i sar., 2016). Ljubojević i sar. (2020) slična istraživanja vršili su kod djece uzrasta od 6 do 8 godina na području Banja Luke i tom prilikom su konstatovali da su prekomjerno uhranjeni zastupljeni sa 9,36%, a gojazni sa 5,37% što su svakako niže vrijednosti u odnosu na naše rezultate.

Djeca koja preskaču doručak sklonija su prekomjernoj tjelesnoj težini i gojaznosti u poređenju s djecom koja redovno doručkuju (Ortega i sar., 1998; Mushtaq i sar., 2011). Takođe, djeca koja ne doručkuju imaju znatno niži unos vitamina i minerala, i drugih hranjivih materija, u odnosu na one koji redovno konzumiraju doručak, te se smatra da bez obzira na konzumaciju ručka i večere, ne mogu nadoknaditi izgubljene hranjive materije (Nicklas i sar., 2004). Shaw (1998) je istakao da djeca koja preskaču doručak ometaju nastavu, imaju izraženije poteškoće kod čitanja, obavljanja različitih školskih zadataka i nepažljivija su u odnosu na djecu koja redovno konzumiraju doručak. Udio djece i mladih u Srbiji koji svakodnevno doručkuju iznosi 93,8%, (MZ IZJZS, 2014), dok podaci iz Hrvatske za područje

varaždinske županije (Golek Mikulić i Tkalec, 2015), ukazuju da 50% djece doručkuje ujutro prije polaska u školu, dok 39% djece sa područja Bjelovara doručkuje svaki dan tokom radne sedmice (Bertić, 2013). Rezultati našeg istraživanja ukazuju da 43,75% ispitanika doručkuje svaki dan ujutro prije polaska u školu.

U istraživanju Russel-a i sar. (2014) više od 90% roditelja izjavilo je da im je vrlo ili umjereno važno da pri izboru hrane odaberu onu za koju znaju da se djetetu sviđa. Usredsređenost na dijete i njegove želje pri izboru hrane može biti kontraproduktivno za razvoj preferencija prema zdravoj hrani, a pogotovo onoj koja zahtjeva višestruko probavanje da bi se djetetu svidjela (npr. povrće). Sve lošije prehrambene navike posljedica su nedostatka vremena i dinamike života, pri čemu se hrana često konzumira van kuće (Bertić, 2013). Postoje brojni pozitivni uticaji prehrane bogate voćem i povrćem na ljudsko zdravlje (He i sar., 2006), mada veliki broj djece ne zadovoljava preporučene dnevne unose voća i povrća od 400 g (Guenther i sar., 2006).

Kada je riječ o konzumaciji povrća kod djece i mladih u Srbiji, 56,6% konzumira povrće svakodnevno, dok njih 43,4% ne konzumira povrće u dovoljnoj količini ili ga pak ne konzumira uopšte (MZ IZJZS, 2014). Kod ispitanika iz našeg istraživanja 62,50% jede povrće ponekad, 36,25% konzumira povrće svaki dan, a 1,25% ne jede povrće nikada.

Ispitanici osnovnoškolskog uzrasta sa bjelovarskog područja najčešće konzumiraju voće više puta sedmično (52%) ispitanika, a rjeđe svaki dan (33%) i rijetko (15%), dok je situacija sa povrćem manje više ista (Bertić, 2013). Rezultati dobijeni u našem istraživanju pokazuju da 58,75% ispitanika jede voće ponekad, 40,00% svaki dan, a samo 1,25% ne jede voće nikada.

Kada su u pitanju slatkiši, ispitanici sa područja Bjelovara konzumiraju slatkiše redovno, odnosno čak 31% jede ih svaki dan (Bertić, 2013). Rezultati naših istraživanja govore da 71,25% ispitanika jede slatkiše ponekad, a 26,25% svakodnevno.

Gazirane sokove više puta sedmično konzumira 43% ispitanika sa područja Bjelovara, svaki dan 18%, a vrlo rijetko 36%. Situacija je bolja kada se radi o konzumaciji brze hrane: 60% ispitanika konzumira brzu hranu vrlo rijetko, 23% više puta sedmično, 10% svaki dan, a 7% nikada. Rezultati koje se odnose na konzumaciju grickalica govore da je veliki procenat ispitanika koji ovu vrstu hrane jedu svaki dan (23%), ali i više puta sedmično (46%). Većina ispitanika (37%) konzumira suhomesnate proizvode više puta sedmično, 4% ih ne jede, 23% ih vrlo rijetko jede, dok ih svaki dan jede 13%. Također, 59% ispitanika se hrani u školi, 8% nosi hranu od kuće, 30% kupi sendvič, a 3% ne jede ništa (Bertić, 2013). Kod ispitanika iz Varaždinske županije 40% njih konzumira brzu hranu jednom sedmično, 30% jednom mjesečno, 36% konuzumira grickalice svaki drugi dan, 50% ispitanika od pića najčešće konzumira vodu, dok mlijeko svakodnevno konzumira 40% ispitanika (Golek Mikulić i Tkalec, 2015). U okviru naših istraživanja, 61,25% podjednako pije i vodu i sokove, dok 73,75% ispitanika pije gazirane sokove ponekad. Kada je u pitanju konzumacija brze hrane, 85,00% naših ispitanika jede brzu hranu ponekad, kao i mesne prerađevine (82,50%).

Istraživanja provedena u Kantonu Sarajevo (BiH) su pokazala da je najveći broj ispitanika u školi koji jedu sendvič iz škole, tj. njih 34,63%, udio ispitanika koji konzumiraju hranu iz pekare je 23,36%, dok 23,64% nosi sendvič od kuće (Dinarević i sar., 2011). Rezultati naših istraživanja govore da 57,50% ispitanika nosi hranu od kuće, 32,50% kupuje hranu u školi, a 10,00% ispitanika uopšte ne jede u školi.

Prilikom uvođenja interventnih mjera koje mogu doprinijeti zdravlju na nivou populacije, procjena nivoa tjelesne aktivnosti danas se smatra prvom fazom (Heimer, 2013). Istraživanje provedeno u Srbiji pokazuje da se najmanje djece i mladih iz Beograda bavi sportom jednom, dva puta nedjeljno ili češće (73,8%), potom slijede djeca i mladi iz Vojvodine (76,6%), južne i istočne Srbije (86,2%), dok je ovaj udio najveći u Šumadiji i Zapadnoj Srbiji (90,9%) (MZ IZJZS, 2014). Kod ispitanika iz Hrvatske, iz Varaždinske županije, 64% ispitanika slobodno

vrijeme provodi u nekoj od sportskih aktivnosti u trajanju od najmanje sat vremena, svakodnevno (Golek Mikulić i Tkalec, 2015). Svi naši ispitanici bave se nekom vrstom fizičke aktivnosti, pri čemu se 65,00% bavi fizičkom aktivnošću svakodnevno, a 31,25% dva do tri puta sedmično.

ZAKLJUČAK

Djeca oba pola su u najvećem procentu normalno uhranjena. Zabrinjava velika zastupljenost gojazne djece, pri čemu su djevojčice gojaznije od dečaka. Podaci o životnom stilu ispitanika ukazuju da prehrambene navike nisu na zadovoljavajućem nivou. Svaki dan doručkuje 43,75% ispitanika, ali je zabrinjavajuće što 22,50% djece nikada ne doručkuje. Najveći broj ispitanika voće i povrće konzumira redovno do povremeno, ali isto tako i gazirane sokove, slatkiše, grickalice, brzu hranu i mesne prerađevine. S druge strane, upražnjavanje jedne, a često i više različitih fizičkih aktivnosti, redovnom dinamikom u toku sedmice, evidentirano je kod svih ispitanika. Smatramo da permanentnim edukativnim aktivnostima o važnosti zdrave ishrane treba uticati kako na djecu tako i na roditelje i da bi takve aktivnosti trebale da imaju značajnije mjesto u školama, ali i kroz različite oblike medijskih kampanja. S obzirom da je ovo istraživanje obuhvatilo svega 80 ispitanika, dalja istraživanja svakako treba provesti na većem uzorku kako bi rezultati bili pouzdaniji.

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NUTRITIONAL STATUS AND LIFESTYLES OF LOWER GRADE PRIMARY CHILDREN IN THE ŠAMAC

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Abstract The study of the nutritional status of lower grade primary school children (from first to fifth grade) in the municipality of Šamac, along with the presence of specific lifestyle patterns (eating habits and physical activity), was conducted in 2021. The sample included 80 children of whom 43 were boys and 37 were girls, aged 6 to 11 years. Basic anthropometric parameters—body height and weight—were measured, and the Body Mass Index (BMI) was calculated. Data on eating habits and physical activity levels were collected through an anonymous questionnaire. The average BMI for boys was 18.29 kg/m^2, and for girls, 18.40 kg/m^2. The average BMI values by school grade were: 17.43 kg/m^2 in first grade, 17.90 kg/m^2 in second grade, 18.26 kg/m^2 in third grade, 19.07 kg/m^2 in fourth grade, and 18.99 kg/m^2 in fifth grade. Although the majority of participants were within the normal weight category, a considerable number of children were found to be overweight or obese, which is a cause for concern. The questionnaire analysis indicated that eating habits often differed from recommended dietary guidelines, while the level of physical activity was generally at a satisfactory level.

Keywords: BMI, children, eating habits, physical activity

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SIGNIFICANCE OF EARLY MARKERS OF PREGNANCY FOR PRETERM DELIVERY

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Abstract The goal of the paper is to examine the clinical utility of the first-trimester maternal serum levels of pregnancy-associated plasma protein-A and free beta-human chorionic gonadotropin with regards to their ability to predict preterm delivery. As part of the screening for trisomy 21, 700 pregnant women were tested for values of pregnancy-associated plasma protein-A (PAPP-A) and free beta- human chorionic gonadotropin (freeβ-hCG), combined with the nuchal translucency (NT). The analysis showed that the age of the pregnant women correlated with whether or not they had preterm delivery, whereby a statistically highly significant difference was found ($\chi 2 = 19.320$; $df = 2$; $p < 0.01$), as consequence of the fact that the younger women did not have preterm delivery much more frequently (100.0%) in comparison with the middle-aged and older women from the study (90.5% and 77.9% respectively). Furthermore, the analysis in which we took account of the number of earlier abortions presented a statistically substantial difference ($F = 14.384$; $df = 5$; $p < 0.05$), showing that women with no preterm delivery often had no earlier abortions (80.1%), as opposed to those who did (70.0%). Therefore, hormones could not be used individually as a clinical test for the preterm delivery prediction; nevertheless, this is an important finding because it may enable the development of an algorithm to estimate an individualized risk of preterm delivery based on multiple factors, including the first-trimester biochemical markers. The clinical value of using these hormonal levels to predict and manage preterm delivery requires further investigation.

Key words: preterm delivery; pregnancy-associated plasma protein-A; free beta-human chorionic gonadotropin; first-trimester biochemical markers

INTRODUCTION

Preterm delivery is defined as a delivery which occurs before 37 gestation weeks have been completed. This occurence complicates almost 10% of all deliveries and it is to be blamed for about 70% of all neonatal deaths worldwide (Kugelmal et al., 2013; Mor et al., 2017). Preterm delivery is consider as leading to be the cause of neonatal morbidity and mortality around the world, including the USA (Jaremek et al., 2021), South Brazil (Barrios et al., 2021) and subsaharan Africa (Abu-Raya et al., 2020), even though these regions are situated at a significant distance from each other. Its immense impact on human health has not been

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reduced even though obstretics and neonatal care are constantly improving (Barrios et al., 2021).

A high percentage of preterm delivery today may be associated with a lack of more efficient therapies which would treat the causes of preterm delivery and involve mechanisms that prevent it. About a third of preterm delivery cases is related to an intrauterine infection, which is a preventable cause, having in mind that testing and treating infections of the lower genital tract reduces the incidence of preterm deliveries (Dodd et al., 2013). However, many cases are still called idiopathic and this is why rational prophylaxis may not be used. Another limitation to preventing preterm delivery is poor predictive value of assessing clinical risk when identifying women who need permanent monitoring and prophylactic interventions.

Preterm delivery is a syndrome which may be brought about by a number of causes, all of them reflecting the fact that either the mechanisms in charge for keeping the uterus still are not functioning or that the normal parturition cascade is overwhelmed (Nunthapiwat et al., 2019). A number of hormones and cytokines have been found to change during pregnancy and have therefore been considered to be potential indicators of this syndrome, even though many of these indicators have not been proven to have a role in the delivery mechanism.

The rationale behind these tests varies, as some are designed to detect the elevation of hormones whose level increases as the parturition is nearing for precautionary purposes – for instance corticotropin-releasing hormone (CRH) and estriol; whereas others, such as hCG and hPL, are interpreted as markers of placental dysfunction and/or fetal influence. In addition, proinflammatory cytokines have been designated as potential markers, taking into account the fact that they signal immune and inflammatory reactions following an intrauterine infection.

Elevated β-hCG levels in the serum predict a high risk of perinatal death (Argente et al., 2017;Preston et al., 2024), small body weight at birth, children who are small for their gestation period, premature (preterm) membrane rupture, and preterm birth (Green et al., 2020; Chiu et al., 2022; Paquette et al., 2023), but not the preterm delivery itself (before 34th gestation week) (Wu et al., 2022). Pregnant women with a high risk of preterm delivery may be tested for hCG values both in the cervical and vaginal discharge. An individual β-hCG value higher than 50mIU between 24th and 28th gestation week in the cervical-vaginal secretions predicts a double risk for a delivery prior to 34th week (Celik et al., 2022).

MATERIALS AND METHODS

This was a prospective observational study of subjects attending the first-trimester combined screening program for Down syndrome in a university policlinic over a 1-year period. It included 700 pregnant women between ten and thirteen weeks of pregnancy. As part of the screening for trisomy 21, all patients were tested for values of pregnancy-associated plasma protein-A (PAPP-A) and free beta- human chorionic gonadotropin (free-βHCG), combined with the nuchal translucency (NT).

The study excluded examinees whose fetuses showed chromosomal or structural changes, as well as patients who suffered from chronic pathologies, i.e. chronic hypertension and insulin-related diabetes.

The analysis included processing a database consisting of: medical history, demographic data, ultrasound findings and biochemical results.

We analyzed a database of coded variables in order to have better readability and understanding of the results.

Tablle 1. Review of variables in database

Title	Value
Place	University policlinic
Age groups	<25 25-35 35+
Previous deliveries	pd 0 pd 1 pd 2 pd>2 pd-previous deliveries
Previous abortions	No Yes
Complications in pregnancy	No Yes
Delivery terms	Preterm In term Prolonged

For the purpose of our study two aforementioned biochemical parameters, free hCGβ and PAPP-A, were analysed, and the data on preterm delivery along with other data were recorded in a specially designed database.

The biochemical analysis of PAPP-A was performed using the immunofluorescence kit (Wallac DELFIA Xpress). The immunofluorescence kit (DELFIA Xpress Wellac) was used for free hCGβ. Doses were analyzed in the dual degree. Results are expressed as multiple median (MoM) of specific gestational periods (EG). Measurements of Crown-Rump Length (CRL) and nuchal translucency (NT) were performed using an endovaginal probe. The risk for Down syndrome was assessed using a LifeCycle software (version 2.2.4 PerkinElmer Life Sciences, Wallac Oy). Patients positive for screening were grouped in the category of the real risk of Down syndrome, within extent over 1:380, in accordance with indicative benchmarks, advising them to do a chromosome analysis, both by villocentesis (CVS) and amniocentesis (A).

Pearson's correlation coefficient was used for the association between variables (PAPP-A-free HCGβ) and each of the opposite outcomes. The statistical analysis was done by using Microsoft Excel ND Sigmastat version 3.1. Sensitivity and false positivity for all gynaecological complications were tested to identify any contradictory outcome. Statistically, the p-value under 0.05 was taken into account.

RESULTS

During the study period, 700 women were eligible, according to the inclusion criteria. The analysis of the number of preterm deliveries in the pregnant women from our study showed that 620 women did not have them (88.6%), whereas 80 did.

Table 2. Descriptive parameters for numerical values or the entire group of the pregnant women (examinees).

Values	N	Minimum	Maximum	Average	SD
PAPP-A values	700	,160	15,000	2,737	2,086
Free βhCG values	700	6,000	941,000	70,726	62,227
Gestation week of the biochemical analysis	700	10,000	13,430	11,902	,783
Age	700	18,000	45,000	31,160	3,739
Delivery week	700	9,000	42,000	39,071	2,912
PAPP-A MoM	700	,110	5,350	1,170	,721
freeβhCG MoM	700	,130	79,600	1,393	3,144

The analysis showed that the distribution of numerical values obtained in our study is nearly normal (Kolgomorov Smirnov z was less than 1.96, i.e. $p > 0.05$ everywhere), which allowed for an application of the parameter methods in our further analysis.

Table 3 presents descriptive parameter values for the results of our study in relation to the distribution of preterm delivery (PRET) for the whole group of the pregnant women.

Table 3. Descriptive parameters for numerical values in relation to the distribution according to the PRET parameter (1 – preterm delivery occured, 0 – preterm delivery did not occur).

Values		N	Average	SD	SE
PAPP-A values	**0**	620	2,719	1,968	,079
	1	80	2,877	2,853	,319
FreeβhCG values	**0**	620	71,097	59,623	2,394
	1	80	67,851	79,985	8,942
Gestation week of the biochemical analysis	**0**	620	11,890	,774	,031
	1	80	11,989	,849	,094
Age	**0**	620	30,970	3,672	,147
	1	80	32,680	3,929	,439
Delivery week	**0**	620	39,520	2,679	,108
	1	80	35,600	2,237	,250
PAPP-A MoM	**0**	620	1,181	,696	,027
	1	80	1,086	,893	,099
FreeβhCG MoM	**0**	620	1,408	3,303	,132
	1	80	1,279	1,395	,155

Descriptive parameters for numerical values in our study in relation to the distribution according to the PRET parameter (1 – preterm delivery occured, 0 – preterm delivery did not occur).

The analysis showed that in the group of the pregnant women with preterm delivery there were no statistically significant differences in the average values for all the analyzed parameters presented in Table 4, except for the values related to the average age of the women ($t = 3.886$, $p < 0.01$), which is a consequence of somewhat higher average values for the older women. Other numerical values which were compared did not differ to a statistically significant degree ($p > 0.05$).

The analysis of comparing the age of the pregnant women and the distribution by preterm delivery (Figure no. 1) resulted in a statistically very significant difference ($\chi 2 = 19.320$, $df = 2$, $p < 0.01$) – a consequence of the fact that the younger women had no PRET (100.0%) in

contrast to the middle-aged and older women from our study (90.5% and 77.9% respectively). Thus, preterm delivery was completely absent among the youngest women, whereas as the age of the pregnant women increased there were more PRET findings.

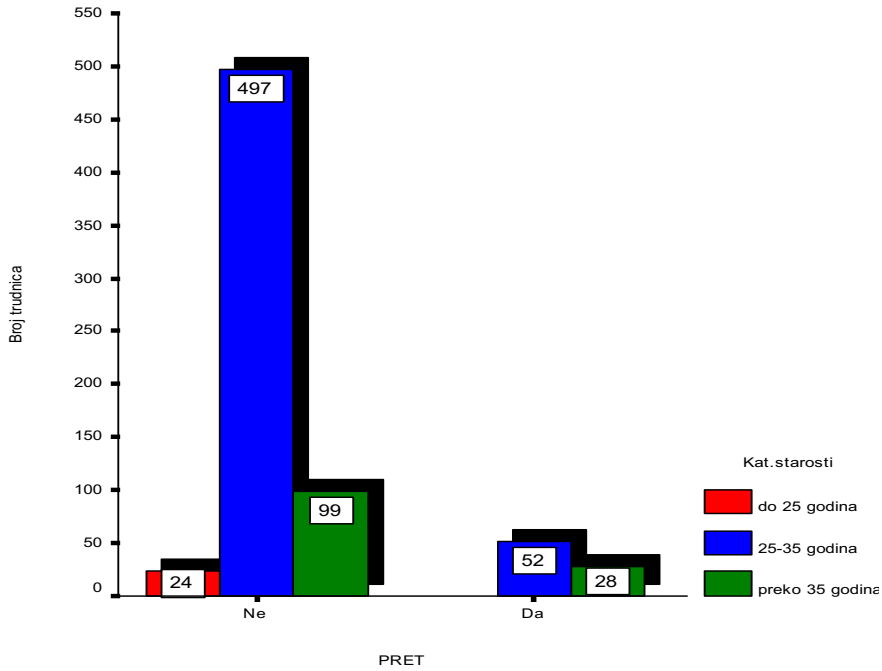

Figure 1. The age of the pregnant women and the distribution by preterm delivery

Table 4. The frequencies of the examined pregnant women obtained by cross-examining the findings of preterm delivery and the number of previous abortions in this study.

Values		Abortions						Total
		0	1	2	3	4	5	
Preterm delivery	No	494	107	14	3	1	1	620
	Yes	56	16	5	3	0	0	80
Total		550	123	19	6	1	1	700

The analysis of comparing the category of the presence of preterm delivery (PRET) with the category regarding the distribution by the number of previous abortions resulted in a statistically significant difference ($F = 14.384$, $df = 5$, $p < 0.05$), which is a consequence of the fact that women who did not have a preterm delivery (PRET) had no previous abortions (80.1%) as opposed to those who did (PRET) (70.0%).

Table 5. The frequencies of the examined pregnant women obtained by cross-examining the PAPP-A MoM and PRET categories in our study.

Values		Preterm delivery		Total
		No	Yes	
PAPP-A MoM	**up to 2,5**	594	75	669
	over 2,5	26	5	31
Total		620	80	700

The comparison of the PAPP-A MoM results for the pregnant women and the division by PRET did not show any statistically significant difference ($\chi2 = 0.798$, df = 1, p> 0.05), due to the fact that in all PAPP-A MoM categories (including the values of up to and over 2.5), the incidence of PRET proved to be nearly the same.

Table 6. The frequencies of the examined pregnant women obtained by cross-examining the freeβhCG MoM and preterm delivery findings in our study.

Values		Preterm delivery		Total
		No	Yes	
freeβhCG MoM	**up to 2,5**	583	75	658
	over 2,5	37	5	42
Total		620	80	700

The cross-examination of the categories related to freeβhCG MoM in the pregnant women and the division by PRET showed no statistically significant difference ($\chi2 = 0.011$, df = 1, p> 0.05), due to the fact that in all freeβhCG MoM categories (including the values of up to and over 2.5), the incidence of PRET proved to be nearly the same.

DISCUSSION

The results obtained in the cross-analysis of the women age and the incidence of preterm delivery showed a statistically highly significant difference ($\chi2 = 19.320$, df = 2, p < 00:01), which is a consequence of the fact that none of the pregnant women younger than 25 had a premature delivery, in contrast to the women aged between 25 and 35 and those older than 35 (90.5% and 77.9% respectively). However, the presence of preterm delivery was not the highest in the oldest category of the pregnant women tested in this study. Also, the analysis of the results suggested a statistical significance when it comes to the number of previous abortions. Namely, this analysis suggests that the number of previous abortions negatively affects the occurrence of preterm delivery (80.1% of women who did not have a preterm delivery did not have any previous abortions either).

It is more difficult, however, to explain the more frequent occurrence of preterm delivery in the pregnant women with elevated free β-hCG. It is assumed that placental insufficiency with hypoxia and early vascular disorders, which are reflected in the increased hCG / free β-hCG values in the second trimester, may be a trigger for preterm delivery with premature amnion rupture. Namely, there is evidence that in the process of the delivery initiation and fetal membrane rupture, the cervix and decidua cells are involved, as well as the cells of the chorioamniotic membranes (Harrison et al., 2016). The secretion of the enzyme protease, which acts on the degradation of extracellular matrix and cervical chorioamniotic membranes, could be due to certain pathological occurrences that activate the cervix, decidua and amniochorion cells. At the same time, the cytokines activation enhances the protease ability to break down the extracellular matrix, all of which may cause the rupture of fetal membranes and preterm delivery (Costa, 2016; Yan et al., 2023). However, this study does not support

earlier findings from the literature stating that pregnant women with preterm delivery have increased freeβ-hCG level values. Namely, the average freeβ-hCG MoM value in the pregnant women with a preterm delivery in this study was 1.279, while in the control group, i.e. the group of physiologically healthy women with a term delivery, that level was 1.408. Regardless of this difference between them, the analysis showed that there was no statistical significance in the finding. Also, the values of another prognostic parameter – PAPP-A, i.e. its average multiple medians, were lower in this study group than in the control group on average (1.086 compared to 1.181), but still there was no statistical significance.

Many of the papers herein cited suggest a significant correlation between the high hCG and free β-hCG levels and pregnancy-related complications, such as IUGR, hypertension, and preterm delivery (Palacio et al., 1993; Wenstorm et al. , 1994; Morssink et al., 1997; Stojilković Mikic et-Johnson., 1999; Giorgione et al.,2022). However, there are papers in which the said connection is not established (Pinette et al., 1997; Raty et al., 1999). It is likely that such a discrepancy is due to a different selection of the groups that were examined.

The use of predictive indicators of preterm delivery has two complementary goals. The first is the early identification of women who have a higher risk of having preterm delivery, which is a prerequisite for planning prevention strategies, if not to delay the delivery, then at least to improve the perinatal outcome. The ideal indicator should not only be accurate, but also present early enough to perform effective prophylaxis. Such an ideal test is difficult to achieve because the syndrome of preterm delivery is heterogeneous in nature and probably due to the fact that the pathological process leading to preterm delivery often develops late in pregnancy, making it more difficult to predict its emergence. The second goal is predicting preterm delivery after the start of spontaneous premature birth labour, which can help in the clinical decision to improve the preventive and therapeutic measures in order to avoid adverse consequences.

Before accepting one or several hormonal indicators as predictors of the preterm delivery risk, we have to be aware of the power and limitations of these tests in order to isolate the best piece of information they can offer. One should bear in mind that in addition to the fact the test must be accurate and be able to provide more significant results than false ones, the probability of the emergence or development of problems (or lack thereof) after a positive or negative test must be significantly different from the expected distribution of problems in the population; otherwise, the test would be useless. Assessing which test is useful and which is not may vary depending on whether the test is to be used on selected individuals or to test an entire population.

Hormonal tests to assess the risk of premature delivery and predict the outcome of gestational diseases may provide relevant information to the doctor, if interpreted carefully.

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ZNAČAJ RANIH MARKERA TRUDNOĆE ZA PREVREMENI PORODAJ

Apstrakt Cilj ovog rada jeste ispitivanje kliničke vrijednosti nivoa za trudnoću vezanog plazma proteina-A i slobodnog beta humanog horionskog gonadotropina u serumu majke u prvom tromjesečju, kao indikatora koji mogu da predvide prevremeni porođaj. U pitanju je prospektivna opservacijska studija subjekata koji su prošli skrining program otkrivanja Daunovog sindroma u prvom tromjesečju trudnoće, koja je obavljena u trajanju od jedne godine na univerzitetskoj Poliklinici Umberto I u Rimu. Kao dio skrininga na trizomiju 21, kod 700 trudnica testirane su vrijednosti za trudnoću vezanog plazma proteina-A (PAPP-A) i slobodnog beta humanog horionskog gonadotropina (slobodni β-hCG), i mjerene vrijednosti nuhalne translucence (NT). Iz studije su iskljucene sve trudnice kod kojih je rizik na Daunov sindrom bio veći od 1:380. Analiza kategorija starosne dobi trudnica u odnosu na podjelu po kategoriji prevremeni porođaj pokazala je da postoji statistički visoko značajna razlika ($\chi 2 = 19{,}320$; $df = 2$; $p < 0{,}01$), koja nastaje kao posljedica toga što mlađe žene češće nisu imale prijevremeni porođaj (100,0%), za razliku od sredovječnih i starijih (90,5% odnosno 77,9%). Analiza po broju ranijih abortusa pokazala je da postoji statistički značajna razlika ($F = 14{,}384$; $df = 5$; $p < 0{,}05$), a ona nastaje kao posljedica toga što žene koje nisu imale prijevremene porodjaje nisu imale ranije abortuse (80,1%), za razliku od onih koje imaju prijevremene porođaje (70,0%). Stoga se može zaključiti da se hormoni ne mogu individualno koristiti kao klinički test za predviđanje prevremenog porođaja; ipak, nalazi su od značaja budući da postoji mogućnost kreiranja algoritma koji bi procijenjivao individualizovani rizik za prevremeni porođaj na osnovu više faktora, uključujući i biohemijske markere iz prvog tromjesečja.

Ključne riječi: prevremeni porođaj; za trudnoću vezan plazma protein-A; slobodni beta humani horionski gonadotropin; biohemijski markeri prvog tromjesečja.

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SOME ANTHROPOMETRIC AND BIOCHEMICAL PARAMETERS AS POTENTIAL PREDICTORS FOR TYPE 2 DIABETES IN PATIENTS IN MONTENEGRO

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Abstract Anthropometric parameters, which include body measurements such as body mass, height, waist circumference, skinfolds, body mass index (BMI), and other measures, play a crucial role in biomedical research, including research on type 2 diabetes. The application of anthropometric measurements in biomedical studies helps to better understand the pathophysiological mechanisms of type 2 diabetes and other diseases. For example, studies have shown that high BMI and abdominal obesity can increase the production of pro-inflammatory cytokines and free fatty acids, contributing to insulin resistance. Additionally, specific measurements such as waist circumference can indicate specific types of fat (visceral or subcutaneous) that have different effects on metabolism. Anthropometric parameters, especially when combined with certain biochemical markers, can also have predictive value in specific populations, such as individuals with a family history of diabetes, older adults, or ethnic groups at higher risk for type 2 diabetes. The use of these parameters can enable early identification of individuals at increased risk and the implementation of preventive measures before the disease becomes clinically manifest. Therefore, in epidemiological studies, anthropometric parameters are essential for analyzing the distribution and prevalence of type 2 diabetes in different populations. With these measurements, researchers can compare disease frequency in groups with different anthropometric characteristics and analyze potential risk factors, which can assist in shaping public health strategies for diabetes prevention.

Key words: anthropometric parameters, biochemical parameters, predictors of type 2 diabetes.

INTRODUCTION

Type 2 diabetes (T2D) is currently present in over half a billion people, with the prevalence among adults in Montenegro being 10.1% (WHO, 2016; ADA, 2016). The primary pathophysiological mechanism in many metabolic disorders, such as metabolic syndrome (Elulu et al., 2016), type 2 diabetes (T2D) (Vargas-Uricoechea et al., 2018), and cardiovascular diseases (CVD) (Adukauskienė er all., 2016), is inflammation. Given that obesity is an established risk factor for the onset of all these disorders, it is suggested that inflammation may be the link between hypertrophic adipose tissue and a wide range of metabolic disturbances (Papaetis, 2015; Klisic et al., 2014). Furthermore, subcutaneous fat tissue also has a distinct effect on insulin resistance in this disease (Maitra et al., 2005; Chandra, 2016). A secondary consequence of insulin resistance (or factors such as adiposity) is dyslipidemia. Increased pro-inflammatory adipokines and cytokines from enlarged adipose tissue may be the primary factors in this interrelationship (Ikeda et al., 2013; Klisic et al., 2020). Recent studies suggest that dyslipidemia can predict HbA1c levels and may be an important factor in the pathogenesis of T2D (ADA, 2016; Ikeda et al., 2013; Klisic et al., 2020). Glycated hemoglobin (HbA1c) is a well-established marker for long-term glycemic control and an independent risk factor for CVD in both diabetic and non-diabetic populations. Additionally, high-sensitivity C-reactive protein (hsCRP) is a widely recognized inflammatory marker associated with an increased risk of CVD, which are common complications in individuals

with T2D (Yousuf et al., 2013). In addition to adipose tissue, other anthropometric parameters have already been identified as risk factors for the development of this disease (Henninger et al., 2014; Chandra et al., 2016), primarily waist circumference and BMI. The aim of this study was to examine and consolidate the results and conclusions regarding the relationship between well-established biochemical inflammation markers such as hsCRP, HbA1c, and lipid parameters with anthropometric parameters in individuals with T2D, through studies conducted in Montenegro over the past few years (Scepanovic et al., 2019; Klisic et al., 2017). Additionally, considering the contradictory results of different studies in various population groups (Hou et al., 2013; Dagan et al., 2013), it is not yet fully clarified which anthropometric parameter could be the best predictor of an unfavorable cardiometabolic profile.

METHODS AND PARTICIPANTS

By reviewing the published results, three independent studies conducted at the Primary Health Care Center in Podgorica, Montenegro, were analyzed. The studies included patients with T2D who were recruited by endocrinologists. The inclusion criterion for participation in the study was a diagnosis of type 2 diabetes. Diabetes cases were defined according to the criteria of the American Diabetes Association (ADA, 2016). All participants provided written informed consent. The study protocol was approved by the Institutional Ethics Committee of the Primary Health Care Center in Podgorica, and the study was conducted in accordance with the principles of the Helsinki Declaration. The biochemical analyses included measurements of fasting glucose, HbA1c, lipids, and hsCRP, and samples were processed according to standard procedures (Scepanovic et al., 2019; Klisic et al., 2017). The basic anthropometric measurements included: waist circumference (WC) (cm), height (cm), weight (kg), and the thickness of subcutaneous fat tissue at the biceps, triceps, iliac, and subscapular sites (Scepanovic et al., 2019). The first study (Šćepanović et al., 2019) examined the association between anthropometric and metabolic parameters with hsCRP and the risk of cardiovascular diseases. A total of 184 participants with T2D participated in this study. Pearson's correlation was performed to investigate the potential relationship between hsCRP and anthropometric and metabolic parameters. Multiple linear regression analysis was further performed to examine the prediction of hsCRP. In the second study (Klisic et al., 2017), the aim was to assess the impact of anthropometric and lipid parameters on long-term glycemic control. A total of 220 individuals with T2D participated. Statistical analyses were performed using PASW® Statistics version 18 (Chicago, Illinois, USA). For the analysis of long-term glycemic control, HbA1c was analyzed using ordinal regression. HbA1c (dependent variable) was ranked and divided into tertiles. Lipid status parameters (TC, HDL-c, LDL-c, and TG) were used as independent variables. In the third study (Scepanovic et al., 2019), the goal was to examine the predictive potential of 4 points of subcutaneous fat (biceps, triceps, abdomen, and subscapular) in relation to T2D patients (108 individuals and 112 control group) diagnosed with diabetes before the age of 60. Potential predictors were analyzed through the obtained odds ratio, regression coefficients, and Wald's statistic with corresponding p-values. Statistical analysis was performed using the IBM SPSS software package 21 for Windows 10.

RESULTS

The results of the study (Scepanovic et al., 2019) show that hsCRP is positively correlated with anthropometric parameters (i.e., BMI and WC) and glucose levels, both in men and women. Additionally, hsCRP was positively correlated with age, subscapular fat thickness,

and the TG/HDL-c ratio only in women (Table 1). Variables that showed a significant correlation (such as WC and glucose in both genders, and subscapular skin thickness and the TG/HDL-c ratio only in women) were further analyzed using multiple linear regression to predict hsCRP (Table 2). Backward selection allowed for the identification of the best model consisting of 2 parameters [i.e., WC (Beta=0.205, p=0.045) and glucose (Beta=0.305, p=0.003)] in men, and WC alone in women (Beta=0.405, p=0.003).

Table 1. Pearsons correlation coefficients (r) of log hsCRP with studied parameters

Variable	Females r	p	Males r	p
Age (years)	-0.233	0.030	0.038	0.709
BMI (kg/m^2)	0.549	<0.001	0.193	0.058
WC (cm)	0.496	<0.01	0.200	0.050
SF biceps (mm)	0.185	0.096	0.065	0.130
SF triceps (mm)	0.192	0.084	-0.023	0.822
SFsuprailiac (mm)	0.215	0.053	0.015	0.888
SFsubscapul (mm)	0.252	0.022	0.095	0.360
Fasting glucose (mmol/L)	0.286	0.070	0.271	0.007
HbA1c (%)	0.177	0.101	0.139	0.176
TC (mmol/L)	0.104	0.337	-0.154	0.132
HDL-c (mmol/L)	-0.121	0.266	-0.197	0.053
LDL-c (mmol/L)	0.051	0.638	-0.126	0.217
TG (mmol/L)	0.174	0.108	0.025	0.806
Non-HDL-c (mmol/L)	0.146	0.179	-0.123	0.230
TG/HDL-c ratio	0.267	0.012	0.041	0.687
Duration of diabetes (years)	0.022	0.838	-0.092	0.370

BMI- Body mass index;WC-waist circumference; SF-skinfold thickness;HbA1c-glycated hemoglobin;TC-total cholesterol; HDL-c-high density lipoprotein cholesterol; LDL-c-low density lipoprotein cholesterol; TG-triglycerides; hsCRP-high sensitivity C-reactive protein. (Scepanovic et all., 2019)

Women with T2D have a higher risk of cardiovascular diseases (CVD), measured by hsCRP levels, compared to men. **Additionally, waist circumference (WC)** is independently associated with hsCRP, both in men and women, suggesting that this simple parameter could be a reliable and cost-effective tool for assessing the risk of cardiovascular diseases (CVD) in the population with T2D.

Table 2. Multiple linear regression analysis for the association of several parameters with log hs CRP as dependent variable

Coefficients				
Model for males	Unstandardized Coefficients	Standardized Coefficients	t	p
	B Std.Error	Beta		
Constant	-8.187	2.925	-2.799	0.006
Glucose (mmol/L)	0.251	0.081 0.305	3.096	0.003
WC (cm)	0.046	0.023 0.205	2.034	0.045
WC-waist circumference				
Model for females	Unstandardized Coefficients	Standardized Coefficients	t	p
	B Std.Error	Beta		
Constant	-14.020	4.582	-3.060	0.003
WC (cm)	0.107	0.034 0.405	3.134	0.003
WC-waist circumference				

(Scepanovic et all., 2019)

A low level of high-density lipoprotein cholesterol (HDL-c) in the study (Klisic et al., 2017) was found to be an independent predictor of higher HbA1c values (OR = 0.44, 95% CI [0.20–0.67], P = 0.039), and an increase in HDL-c by 1 mmol/L reduced the probability of a higher HbA1c by 56%. As expected, significantly higher levels of total cholesterol (TC), LDL-c, and triglycerides (TG) (5.75 [4.94–6.38]; 3.48 ± 1.09; 2.02 [1.54–3.17]; P = 0.029, P = 0.018, and P = 0.001, respectively) were found in the group with the highest tertile of HbA1c, compared to the groups with the lowest and middle tertile of HbA1c. Additionally, HDL-c was significantly negatively correlated with HbA1c ($\rho = -0.209$; $P < 0.001$) (Table 3).

Table 3: Associations between glycated hemoglobin and clinical parameters using Spearman's correlation analysis

Variable	p	P
Age (years)	0.008	0.898
BMI (kg/m^2)	0.092	0.130
WC (cm)	0.105	0.084
Body weight (kg)	0.056	0.353
Body height (cm)	-0.036	0.550
WHtR	0.108	0.073
VAI	0.272	<0.001
LAP	0.26	<0.001
TC (mmol/L)	0.186	0.002
HDL-c (mmol/L)	-0.209	<0.001
LDL-c (mmol/L)	0.136	0.024
TG (mmol/L)	0.271	<0.001
Creatinine (μmol/L)	-0.056	0.356
eGFR(mL/min/1.73m)	0.088	0.146
Duration of diabetes (years)	0.041	0.499

BMI- Body mass index;WC-waist circumference; WHtR- waist-to-height-ratio; TC-total cholesterol; HDL-c-high density lipoprotein cholesterol; LDL-c-low density lipoprotein

cholesterol; TG-triglycerides; LAP lipid accumulation product; VAI visceral adiposity index; eGFR Estimatedglomerular filtration rate (Klisic et all., 2017)

Ordinal logistic regression analysis was conducted to examine the predictive role of lipid status parameters in relation to long-term glycemic control, represented as HbA1c (Table 4).

Table 4. Estimated odds ratios after ordinal regression analysis for glycemic control risk

Model 1				
	unadjusted		adjusted	
	OR (95% CI)	P	OR (95% CI)	p
TC (mmol/L)	1.46 (1.18–1.81)	0.001	1.30 (1.02–1.66)	0.032
TG (mmol/L)	1.44 (1.18–1.76)	<0.001	1.34 (1.07–1.67)	0.010
Model 2				
	unadjusted			adjusted
	OR (95% CI)	P	OR (95% CI)	p
HDL-c (mmol/L)	0.47 (0.23–0.98)	0.043	0.44 (0.20–0.67)	0.039
LDL-c (mmol/L)	1.42 (1.11–1.83)	0.006	1.42	(1.10–1.83) 0.006

For internal validation of the models and to determine if our data approximate the true population data, the bootstrap method with 10000 permutations was used. Model 1= included TC,TG, age and BMI. Model 2=included HDL-c; LDL-c, age and BMI. HDL-c-high density lipoprotein cholesterol; LDL-c-low density lipoprotein cholesterol; TG-triglycerides; OR=Odds ratio; CI=Confidence interval; BMI- Body mass index; (Klisic et all., 2017)

The analysis showed that as TC (OR = 1.46, 95% CI [1.18–1.81], P = 0.001), TG (OR = 1.44, 95% CI [1.18–1.76], P < 0.001), and LDL-c (OR = 1.42, 95% CI [1.11–1.83], P = 0.006) increase by 1 mmol/L each, the probability of higher HbA1c increases by 46%, 44%, and 42%, respectively. High TC and TG were independent predictors of higher HbA1c when age and BMI were included as covariates in Model 1 ([OR = 1.30, 95% CI (1.02–1.66), P = 0.032], [OR = 1.34, 95% CI (1.07–1.67), P = 0.010], respectively).

After testing Model 2 for predicting LDL-c, the estimated adjusted OR for higher HbA1c remained significant (OR = 1.42, 95% CI [1.10–1.83], P = 0.006). On the other hand, an increase in HDL-c (OR = 0.47, 95% CI [0.23–0.98], P = 0.043) by 1 mmol/L reduced the probability of higher HbA1c by 53%. An increase in HDL-c by 1 mmol/L decreased the probability of higher HbA1c by as much as 56% (OR = 0.44, 95% CI [0.20–0.67], P = 0.039). Spearman's correlation analysis tested the association between lipid parameters and long-term glycemic control and showed that triglycerides (TG) positively correlate highly with HbA1c ($\rho = 0.271$; $\rho = 0.260$; $\rho = 0.272$; $P < 0.001$ for all). Additionally, a significant positive correlation was found between total cholesterol (TC) and LDL-c with HbA1c ($\rho = 0.186$; $P = 0.002$ and $\rho = 0.136$; $P < 0.001$).

Table 5. presents the predictive analysis of subcutaneous fat parameters conducted by Scepanovic et al. (2019). Logistic regression revealed that all analyzed parameters are potential predictors for T2D. Larger measurements of subcutaneous fat predicted increased probabilities for the disease at all four sites, showing an increase in probability with each millimeter in the regression, OR=1.08 (biceps), 1.055 (triceps), 1.058 (abdominal), and 1.034 (subscapular), in the overall sample, adjusted for age, gender, and smoking percentage. Similar results were obtained regarding body mass index (BMI), which predicted a higher probability for T2D with an OR value of 1.085 for an increase of 1 kg/m², as well as with an OR value related to waist circumference (OR=1.041 for an increase of 1 cm).

Table 5. Logistic regression analysis of T2D predictors

N=220	OR	95% CI for OR		
		Lower	Upper	p
Biceps brachii SAT(mm)	1.080_a	1.025	1.138	0.004**
Male	1.032_b	0.950	1.120	0.456
Female	1.139_b	1.057	1.227	0.001**
Triceps brachii SAT(mm)	1.055_a	1.016	1.096	0.006*
Male	1.004_b	0.944	1.069	0.892
Female	1.091_b	1.037	1.149	0.001**
Abdominal SAT (mm)	1.058_a	1.020	1.098	0.002*
Male	1.029_b	0.979	1.082	0.254
Female	1.099_b	1.038	1.164	0.001**
Subscapular SAT (mm)	1.034_a	1.001	1.068	0.040*
Male	0.987_b	0.939	1.036	0.591
Female	1.088_b	1.035	1.145	0.001**
Body mass index (kg/m^2)	1.085_a	1.027	1.148	0.004*
Male	1.017_b	0.926	1.116	0.725
Female	1.139_b	1.057	1.228	0.001**
Waist circumferenece	1.041_a	1.017	1.064	0.001*
Male	1.011_b	0.977	1.045	0.528
Female	1.075_b	1.038	1.113	<0.001**

Values as presented as Wald statistic, odds ratio and confidence interval.T2D =type 2 diabetes mellitus; N=number, statistically significant (*); SAT=subcutaneous adipose tissue; OR odds ratio; CI=confidence interval; *p<0.05, **p<0.01, ***p<0.001, a=adjusted for age, sex and percentage of smokers; b=adjusted for age (Scepanovic et all, 2019)

The odds ratio values show that the OR values were non-significant in men, indicating a lower predictive potential ($p > 0.05$). In women, the OR values were significant and higher than in the overall sample: OR=1.139 (biceps), 1.091 (triceps), 1.099 (abdominal), and 1.088 (subscapular) ($p < 0.001$). The results were similar regarding the predictive potential of body mass index (BMI) and waist circumference, with significant ($p < 0.001$) OR values of 1.139 and 1.075, indicating a higher probability for T2D associated with an increase of one unit in women, while no significant predictive potential was found in men ($p > 0.05$). BMI and waist circumference also showed a significant predictive role for T2D in women ($p < 0.05$).

DISCUSSION

In the first study presented (Scepanovic et al., 2019), hsCRP was significantly associated with anthropometric indices in patients with T2D, but central obesity, measured by waist circumference (WC), was a better predictor compared to general measures (e.g., BMI) and four skinfold thickness measurements (e.g., biceps, triceps, subscapular, and suprailiac) in people with T2D. The current study extends these observations, suggesting that this simple and cost-effective anthropometric parameter could be a reliable tool for assessing cardiovascular disease (CVD) risk, not only in the healthy population but also in the population with diabetes. Furthermore, the majority of the diabetes population in our study had excess

body weight (88.6%), which is in line with previous studies indicating obesity as a key determinant for the onset and progression of T2D (Papaetis et al., 2015). In the current study, around 42% of individuals with T2D had CRP levels ≥2 mg/L, and about a third (29%) of participants had hsCRP levels ≥3 mg/L, which categorized them into medium and high-risk groups for CVD. Similarly, Halcox and colleagues (2014) reported that approximately 50% of non-diabetic patients had CRP levels ≥2 mg/L, and around 30% had CRP levels ≥3 mg/L. Salazar and colleagues (2014) observed that individuals with hsCRP >3 mg/L had twice the risk of CVD. With the exception of WC, women in our study had higher anthropometric indices than men. Also, a significantly higher number of women with T2D were in the high hsCRP subgroup compared to men. Although men are considered to have a higher risk of CVD than women (Karam et al., 2015), there are studies showing that this gender difference diminishes in patients with T2D (McEwan The results of the second study (Klisic et al., 2017) indicate the predictive role of traditional lipid parameters on long-term glycemic control (through HbA1c) in patients with diabetes. A reduction in HbA1c by just 1% reduces diabetes-related mortality by 21%. A significant increase in all tested lipid parameters was observed among groups according to HbA1c tertiles, except for HDL-c, which decreased as the HbA1c tertiles increased. Ordinal regression analysis showed the predictive role of lipid status parameters on long-term glycemic control represented as HbA1c. Specifically, the analysis showed that as TC, TG, and LDL-c increase by 1 mmol/L, the probability of higher HbA1c increases by 46%, 44%, and 42%, respectively. In line with our results, Gupta et al. (2008) showed that higher TG levels predict the onset of T2D independently of BMI. Surprisingly, some studies support the hypothesis that high LDL-c levels, although considered an established predictor of CVD (Chogtu et al., 2015), may be associated with a lower risk of T2D (Chogtu et al., 2015; Besseling et al., 2015). However, it was noted that high LDL-c is a predictor of higher HbA1c levels. This contrasts with previously reported studies suggesting that low LDL-c levels may be associated with an increased risk of T2D, both in participants using hypolipidemic drugs and in those who do not (Besseling et al., 2015; Andersson et al., 2015). Additionally, our study showed that an increase in HDL-c by 1 mmol/L reduces the probability of higher HbA1c by 53%, and after adjusting for factors that could affect the results, it reduces it by as much as 56%. When it comes to adipose tissue, studies have shown that larger adipocytes, i.e., increased thickness of abdominal subcutaneous fat, represents a significant risk for the development of T2D (Sarr et al., 2017). Subscapular subcutaneous fat is often more pronounced, while biceps and triceps subcutaneous fat are often degraded in diabetics with a disease duration longer than 10 years, making these points potential predictors of T2D (Chandra et al., 2016). It has been determined that there are differences between men and women in the metabolic function of adipose tissue (Karastergiou et al., 2012). The study in Montenegro shows a highly diversified predictive potential of measuring skin folds of subcutaneous fat for T2D. The concept of the connection between subcutaneous fat and diabetes has already been confirmed in various studies (Sarr et al., 2017; Jensen et al., 2006; Despres et al., 2006; Oda et al., 2012), which is consistent with the results of this research, especially in terms of the stronger predictive potential in women. All four sites of the analyzed subcutaneous fat in the study showed significant predictive potential for T2D, with waist circumference and abdominal subcutaneous fat being strong predictors of the disease, in line with previous studies showing their particularly important role in the development of T2D (Henninger et al., 2014; Devulapally et al., 2017). Although the thickness of subcutaneous fat was higher only at the abdominal and subscapular levels, after adjusting for age, gender, and smoking percentage, biceps and triceps subcutaneous fat were also identified as potential predictors. The study demonstrated a strong predictive potential of subcutaneous fat at the biceps brachii site, which is consistent with earlier generalized research (Özdirenç et al., 2003).

The significance of the current results is highlighted by the Wald statistic for the predictive potential of the biceps subcutaneous fat thickness, which was greater than the predictive potential of BMI.

In conclusion, women with T2D have a higher risk of cardiovascular diseases (CVD), measured by hsCRP, compared to men. Among the examined anthropometric indices, waist circumference (WC) as a simple parameter can be a reliable and cost-effective tool for assessing cardiovascular disease risk in the T2D population. Taking into account all contradictory reports when considering causality between dyslipidemia and T2D, there is an urgent need for careful design of clinical trials involving lipid-modifying drugs to improve insulin sensitivity and reduce the risk of T2D, its complications, and cardiovascular diseases. Our results show a new finding that an unfavorable lipid profile may predict HbA1c levels in patients with T2D. Subcutaneous fat of the upper body also has significant predictive potential regarding T2D, with abdominal, biceps, triceps, and subscapular sites demonstrating strong predictive value. The centralization of fat tissue in the abdominal region is the most important risk factor for T2D, while the thickness of skinfolds at the biceps and triceps remains a statistically significant predictor. Measurements of subcutaneous fat in the upper body are stronger predictors of T2D in female participants.

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NEKI ANTROPOMETRIJSKI I BIOHEMIJSKI PARAMETRI KAO POTENCIJALNI PREDIKTORI ZA DIJABETES TIP2 KOD PACIJENATA U CRNOJ GORI

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Apstrakt Antropometrijski parametri, koji uključuju mjerenja tijela kao što su tjelesna masa, visina, obim struka, kožni nabori, indeks tjelesne mase (BMI) i druge mjere, igraju ključnu ulogu u biomedicinskim istraživanjima, uključujući i istraživanja dijabetesa tipa 2. Primjena antropometrijskih mjerenja u biomedicinskim istraživanjima pomaže u boljem razumijevanju patofizioloških mehanizama dijabetesa tipa 2, a i drugih oboljenja. Na primjer, istraživanja su pokazala da visoki BMI i abdominalna gojaznost mogu povećati proizvodnju pro-upalnih citokina i slobodnih masnih kiselina, što doprinosi insulinskoj rezistenciji. Takođe, određena mjerenja poput obima struka mogu ukazivati na specifične tipove masti (visceralnu ili subkutanu) koji imaju različite učinke na metabolizam. Antropometrijski, a naročito u kombinaciji sa odredjenim biohemijskim parametrima, mogu takođe imati prediktivnu vrijednost u specifičnim populacijama, kod osoba s porodičnom istorijom dijabetesa, starijih osoba ili etničkih grupa s većim rizikom za dijabetes tipa 2. Korištenje tih parametara može omogućiti ranu identifikaciju osoba s povećanim rizikom i implementaciju preventivnih mjera prije nego što bolest postane klinički manifestna. Stoga, u epidemiološkim istraživanjima, antropometrijski parametri su ključni za analizu distribucije i prevalencije dijabetesa tipa 2 u različitim populacijama. Pomoću tih mjerenja istraživači mogu uporediti učestalost bolesti u grupama s različitim antropometrijskim karakteristikama i analizirati potencijalne faktore rizika, što može pomoći u oblikovanju javnozdravstvenih strategija za prevenciju dijabetesa.

Ključne reči: antropometrijski parametri, biohemijski parametri, prediktori dijabetesa tip2