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ORIGINAL ARTICLE

GENETIC POLYMORPHISMS IN CYP2 GENE FAMILY IN BULGARIAN INDIVIDUALS AND THEIR CLINICAL IMPLICATIONS

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Abstract. *The cytochrome P450 superfamily consists of hemeproteins involved in the detoxication of different xenobiotics, including drugs. The CYP2 gene family is responsible for the metabolism of 80% of the drugs in clinical use. There are considerable interindividual and interethnic variabilities in the rate of drug metabolism as a result of genetic polymorphisms. The goal of our study was to determine the frequency of 10 genetic polymorphisms in CYP2 family genes to give light on the pharmacogenetic defects of the main CYPs, involved in drug metabolism, in Bulgarian individuals. We detected high allele frequency for CYP2D6*10 (0.27), CYP2D6*4 (0.22), and CYP2B6*9 (0.24), followed by CYP2C19*2 (0.14), CYP2C9*3 (0.11) and CYP2C9*2 (0.09). The genotype frequencies were also determined for all investigated variants. In total 47.2% of the analyzed individuals carried CYP2D6 genetic polymorphisms – 5.6% carried a single variant and 41.6% were found to have two or more such variants. Homozygotes for CYP2D6 variants were established among 14% of Bulgarian individuals. Determination of the prevailing pharmacogenetic polymorphisms of the CYPs, most responsible for drug metabolism, will lead to a lower risk of drug toxicity, increased drug efficacy, and drug dose optimization.*

Key words: *cytochrome P450, genetic polymorphisms, pharmacogenetics, drug safety*

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INTRODUCTION

The human cytochrome P450 superfamily is composed of heme-containing enzymes, responsible for the biotransformation of different xenobiotics, including drugs. Although CYP enzymes are expressed in different organs – kidneys, placenta, gonads, adrenal glands, etc. [1], they are most concentrated in the liver and intestines. CYPs are the main enzymes involved in drug detoxication. They perform the reactions of oxidation during phase I of drug metabolism, converting lipophilic compounds to water-soluble ones that can be easily excreted from the body. There are at least 57 functional genes and 58 pseudogenes, associated with CYPs, organized in 18 families and 44 sub-families, based on the amino acid sequence homology of the enzymes [2, 3]. Of all human CYPs, CYP1, 2, and 3

are involved in the metabolism of 80% of the drugs in clinical use [2-4]. CYP2D6 is highly polymorphic, which determines a considerable interindividual difference in drug response. The gene is located on the long arm of chromosome 22 (22q13.2) and is the most common mutant isoform [3]. The CYP2D6 enzyme is involved in the metabolism of nearly 25% of clinically prescribed drugs. Substrates for the enzyme include tricyclic antidepressants (clomipramine, imipramine, desipramine, nortriptyline), selective serotonin reuptake inhibitors (fluoxetine, paroxetine), certain neuroleptics (chlorpromazine, thioridazine, olanzapine, haloperidol), opioid analgesics (codeine, tramadol, methadone), antiarrhythmic drugs (propafenone, lidocaine, procainamide, mexiletine), anticancer drugs (cyclophosphamide), etc. The CYP2B6 gene is part of the CYP2 cluster (together with CYP2A) located on chromosome 19 (19q13.2) and is one of the most polymorphic genes in humans [11]. The CYP2B6 enzyme constitutes up to 10% of the functional monooxygenases in the liver and is involved in 10-12% of the clinically used drugs, including the antiretroviral drugs (efavirenz, nevirapine), antimalarial (artemisinin), anticancer (cyclophosphamide, ifosfamide), antidepressants (fluoxetine, sertraline), antiseizure drugs (phenytoin, mephenytoin), opioids (methadone) and others [12]. CYP2C19 is involved in the bioactivation of the antiplatelet drug clopidogrel. Clopidogrel is a purinergic receptor inhibitor, preventing platelet aggregation and reducing the risk of thrombosis. As a prodrug, clopidogrel must be activated in the liver by the CYP2C19 enzyme isoform. The carriers of two loss-of-function copies are considered as CYP2C19 poor metabolizers, and exhibit reduced antiplatelet effect of clopidogrel.

Evidence is constantly accumulating for their clinical significance in terms of drug toxicity, efficacy, and dose determination. There is considerable variability in CYPs'activity among individuals and populations. The main types of genetic variations in CYP genes that were determined are loss-of-function and gain-of-function variants [3, 7]. Loss-of-function variants predispose to a high risk of adverse drug reactions, due to reduced elimination and enhanced plasma concentrations [8]. The gain-of-function variants increase the rate of drug elimination, resulting in subtherapeutic plasma concentration and inefficient drug response. Loss- and gain-of-function variants are a result of multiallelic polymorphisms. These polymorphisms depend highly on ethnicity and lead to various pharmacogenetic phenotypes, classified as poor, intermediate, extensive, and ultra-rapid metabolizers [3, 9].

AIM OF THE STUDY

The goal of our study was to determine the frequency of genetic polymorphisms in CYP2 family genes to give light on the pharmacogenetic defects of main CYPs, involved in drug metabolism, in Bulgarian individuals. Determination of the allele and genotype frequencies in the population will lead to significant improvements in pharmacotherapy.

MATERIALS AND METHODS

We collected data from 200 unselected Bulgarian patients and attended the laboratory for genomic diagnostics. An informed consent form was signed by all patients before testing. The study adhered to the tenets of the Declaration of Helsinki for research involving human subjects. DNA was isolated from peripheral venous blood, using CHEMAGEN® Magnetic Separation Station (PerkinElmer) following the manufacturer's instructions. The concentration of the genomic DNA was determined with the Qubit dsDNA BR Assay Kit on the Qubit 2.0 fluorimeter. DNA samples of index patients were processed for targeted sequencing. TruSight One panel by Illumina, which includes 4127 genes associated with hereditary diseases, was used for library preparation. Accurate concentrations of the dilutions were calculated followed by denaturation and dilution of libraries according to MiSeq System Denature and Dilute Libraries Guide. Samples were loaded for sequencing by use of a cartridge as described in MiSeq System User Guide (part # 150276) and sequencing was performed on a MiSeq System using the MiSeq Reagent Kit v3. Data aligned to the Human reference sequence – Genome Reference Consortium Human Build 37 (GRCh37/hg19) led to a list of variants that in terms of filtering was annotated by the VariantStudio Software.

We extracted the data for genetic polymorphisms in twenty-two genes belonging to CYP 1, 2, and 3 families. The examined CYP allele variants are presented in Table 1.

RESULTS

Allele and genotype frequencies of the studied polymorphisms

We determined allele and genotype frequencies for all studied genetic polymorphisms among Bulgarian individuals – Table 2. The minor allele frequencies (MAF) were comparable with those in the European population.

The highest allele frequency of drug response-associated polymorphisms was identified in the CYP2D6 gene – CYP2D6*10 (0.27) and CYP2D6*4 (0.22), encoding enzymes with reduced activity. We estab-

lished 38.8% heterozygotes and 2.25% homozygotes for CYP2D6*4 and 30.3% heterozygotes and 11.8% homozygotes for CYP2D6*10 among Bulgarian individuals – Figure 1. For CYP2D6*3 we found very low allele frequency (0.008) and only 1.7% heterozygotes among investigated Bulgarians.

Table 1. Characteristics of the studied allele variant

Allele	Annotation	Variant type	Functional consequence
CYP2D6 - metabolism of dextromethorphan, sparteine, metoprolol, nortriptyline and many other antidepressants and codeine			
CYP2D6*3	rs35742686	Frameshift	Inactive
CYP2D6*4	rs3892097	Splicing defect	Homozygotes are poor metabolizers
CYP2D6*10	rs1065852	Missense (Pro34Ser)	Homozygotes are poor metabolizers
CYP2C9 – warfarin, piroxicam, lesinurad, phenytoin, flurbiprofen response			
CYP2C9*2	rs1799853	Missense (Cys144Arg)	Reduced metabolism
CYP2C9*3	rs1057910	Missense (Ile359Leu)	Reduced metabolism
CYP2C8 – acute gastrointestinal bleeding during the use of NSAIDs			
CYP2C8*3	rs10509681	Missense (Lys329Arg)	Increased toxicity
CYP2C19 – mephenytoin, proguanil, clopidogrel, amitriptyline, citalopram, clomipramine response			
CYP2C19*2	rs4244285	Splice acceptor activation (p.Pro227=)	Poor metabolizers
CYP2C19*4	rs28399504	Missense (Met1Val)	Poor metabolizers
CYP2B6 – nevirapine, efavirenz, methadone response			
CYP2B6*9	rs3745274	Missense (Gln172His)	Increased toxicity
CYP2A6 – warfarin, nicotine response			
CYP2A6*2	rs1801272	Missense (Leu160His)	Reduced metabolism

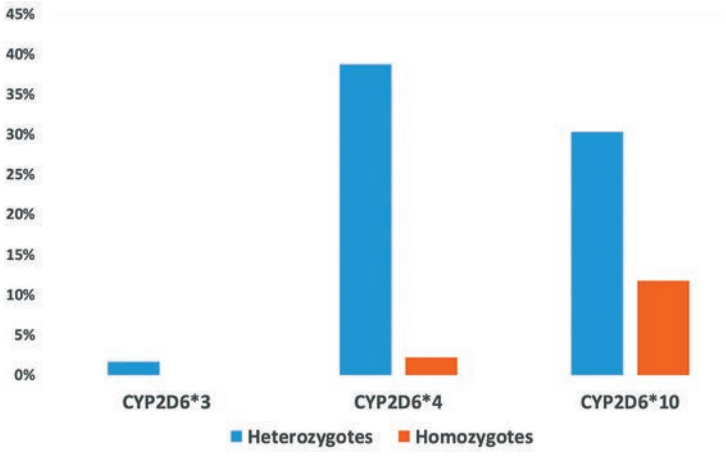

Fig. 1. Incidence of CYP2D6 functional polymorphisms' genotype in Bulgarian population

Table 2. Genotype and allele frequency for the studied variants

Allele	% of heterozygotes	% of homozygotes	MAF (BG)	MAF (EU)
CYP2D6*3	1.68%	0	0.008	0.02
CYP2D6*4	38.76%	2.25%	0.22	0.22
CYP2D6*10	30.34%	11.80%	0.27	0.24
CYP2C9*2	16.29%	1.12%	0.093	0.13
CYP2C9*3	18.54%	1.68%	0.11	0.068
CYP2C8*3	15.73%	1.12%	0.09	0.11
CYP2C19*2	25.28%	1.68%	0.14	0.15
CYP2C19*4	0.56%	0.00%	0.003	0.003
CYP2B6*9	35.40%	6.74%	0.24	0.24
CYP2A6*2	6.18%	0.56%	0.04	0.025

The two CYP2C9 polymorphisms - CYP2C9*2 and CYP2C9*3, have an allele frequency of 0.09 and 0.11, respectively. We detected 16.3% heterozygotes and 1.12% homozygotes for CYP2C9*2 and 18.5% heterozygotes and 1.7% homozygotes for CYP2C9*3 – Figure 2. Similar allele (0.09) and genotype frequencies (15.7% heterozygotes and 1.12% homozygotes) were established for CYP2C8*3 – Figure 3.

The highly prevalent were the genetic polymorphisms CYP2B6*9 and CYP2C19*2 with allele frequencies 0.24 and 0.14, respectively. The heterozygotes were 35.4% for CYP2B6*9 and 25.3% for CYP2C19*2, and homozygotes – 6.7% and 1.7%, respectively. Very low was the allele frequency for CYP2C19*4 (0.003) with 0.6% heterozygotes – Figure 4.

CYP2A6*2 has an allele frequency of 0.04 with 6.2% heterozygotes and 0.6% homozygotes among Bulgarian individuals – Figure 3.

Combined carriership of different drug response variants of CYP2D6

The simultaneous carriership of two CYP2D6 variants (Inactive + Decreased) was determined. In total 47.2% of the analyzed individuals carried CYP2D6 genetic polymorphisms. We detected a considerably higher frequency of combined carriership (41.6% – 41% for two variants and 0.6% for three variants) compared to a single one (1.7% for each CYP2D6 variant) – Table 3, Figure 5.

DISCUSSION

CYP2 is the largest family of all human CYPs, with CYP2D6, CYP2C9, CYP2C19, and CYP2B6 contributing to drug metabolism the most. Our research showed that 47.2% of the analyzed individuals carried CYP2D6 genetic polymorphisms. There is only one Bulgarian study on the frequency of these poly-

Table 3. Single and combined carriership of CYP2D6 variants

CYP2D6 variant (s)	Number of individuals (%)
Only 1 CYP2D6 variant	10 (5.6%)
rs35742686 Inactive rs3892097 Inactive rs5030655 Inactive rs1065852 Decreased	3 (1.7%) 2 (1.1%) 2 (1.1%) 3 (1.7%)
2 CYP2D6 variants	73 (41%)
rs3892097+ rs1065852 Inactive+Decreased rs5030655+ s1065852 Inactive+Decreased	72 (40.4%) 1 (0.6%)
3 CYP2D6 variants	1 (0.6%)
rs35742686+rs3892097+ rs1065852	1 (0.6%)

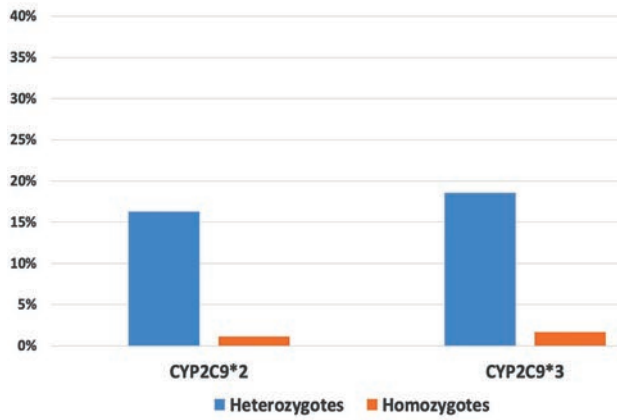

Fig. 2. Incidence of CYP2C9 polymorphisms' genotypes in Bulgarian population

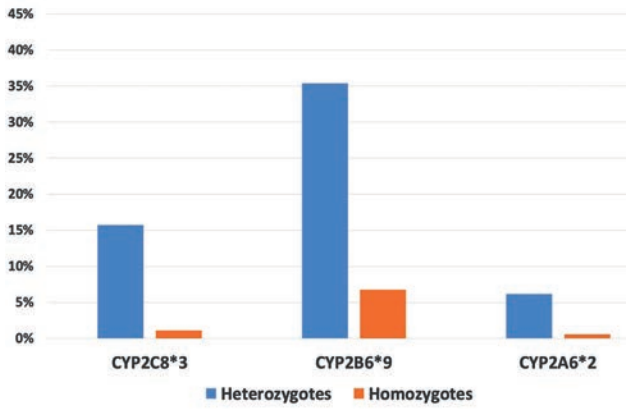

Fig. 3. CYP2C8, CYP2B6, and CYP2A6 polymorphisms' genotypes in Bulgarian population

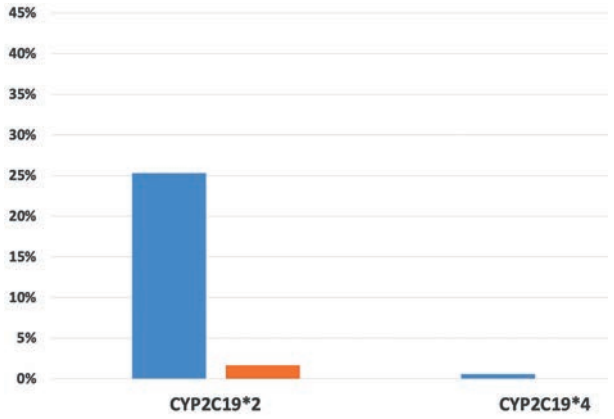

Fig. 4. CYP2C19 polymorphisms' genotypes in Bulgarian population

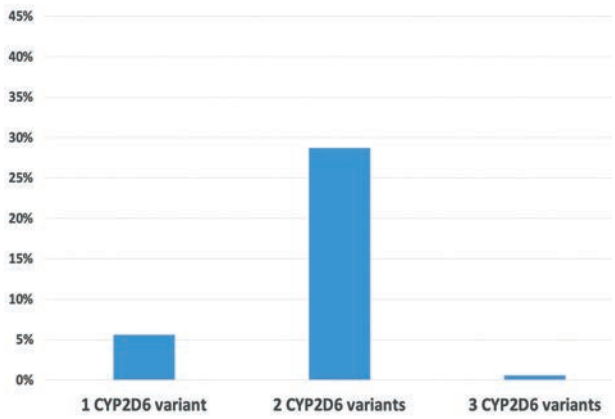

Fig. 5. Incidence of single, double, and triple carriership of CYP2D6 variants

morphisms, but among patients with psychiatric disorders [10] and they found 48.6% frequency of CYP2D6 polymorphisms among these patients. Our results showed that the most frequent drug response variants are the polymorphisms in CYP2D6 – CYP2D6*4 and CYP2D6*10. According to the existing data, CYP2D6*10 is the most common non-functional allele variant in the world population and is identified with the highest frequency in the Asian population (allele frequency more than 50%), followed by Chinese (50.7%) and Japanese (23-43%) cohorts [11, 12]. This allele variant in all its variations is rarer identified in the European population (24%), as well as in our Bulgarian cohort (27%) and it predisposes to supratherapeutic plasma levels and increased risk of drug toxicity. The CYP2D6*10 enzyme is unstable with reduced metabolic activity (60% of normal) and decreased affinity for its substrates. CYP2D6*4 is the second most common CYP2D6 allele variant established among Bulgarian individuals (22%), which coincides with the Caucasian population. In the Caucasian population, the CYP2D6*4 is the most common non-functional allele variant (20% of the population), established in 75%-90% of all poor metabolizers [12, 13]. In the Spanish and Turkish populations, this allele variant is established at a lower rate of 11-12%. CYP2D6*4 is rarely identified in Chinese and Japanese cohorts – less than 1% [13]. In the Asian population, the CYP2D6*4 is established in only 1-2% of the poor metabolizers. Homozygote carriers of both CYP2D6 variants were detected in 11.8% and 2.2%, respectively – that means 14% of Bulgarian individuals are poor metabolizers due to homozygosity of these genetic polymorphisms. The double carriership of non-functional CYP2D6 allele variants was established with high frequency in the Bulgarian population (41%), as it is worth investigating if these variants exist at cis- or trans-position in the same individual. Their compound heterozygosity additionally will increase the frequency of poor metabolizers in the Bulgarian population.

We found a 24% allele frequency of CYP2B6*9, as heterozygotes were 35% of the individuals, and 6.74% – were homozygotes. CYP2B6*9 encodes an enzyme with reduced activity. It is established with different frequencies amongst the ethnic groups. A relatively high frequency of the allele is observed in the German, British, and Swiss populations, 28%, 28%, and 26%, respectively. It is established in more than 40% of the populations of South India and Indonesia (Timorian), but less than 5% of the Asian population. The CYP2B6 enzyme is involved in the metabolism of many clinically used drugs, including the non-nucleoside reverse transcriptase inhibitors – efavirenz and nevirapine. Efavirenz and nevirapine are applied as first-line treatment for human immunodeficiency virus (HIV) –infected patients [14]. Considerable interindividual variability in the plasma concentrations of both drugs was determined. Efavirenz and nevirapine are metabolized by the CYP2B6 enzyme in the liver. The carriership of loss-of-function CYP2B6 gene variants is associated with a higher risk of Steven-Johnson syndrome and toxic epidermal necrolysis. Longer and massive exposure to these drugs, observed in poor metabolizers, could cause significant immune reactions, resulting in severe adverse effects [15]. CYP2B6 is the major enzyme responsible for the bioactivation of cyclophosphamide, converting it to 4-hydroxy-cyclophosphamide. Cyclophosphamide is an alkylating anticancer prodrug, which requires enzymatic activation in the liver. Carriers of CYP2B6*9 are considered to have worse pharmacotherapeutic outcomes from treatment with cyclophosphamide [16]. Determination of the CYP2B6 genotype of these

patients could improve their pharmacotherapeutic effects and anticancer therapy outcomes. Another metabolic pathway with clinical significance, associated with the CYP2B6 enzyme, is the biotransformation of methadone. CYP2B6 is the major enzyme involved in methadone metabolism and its activity determines the rate of drug's clearance and plasma concentration [17-20]. According to previous research data, there is a strong correlation between rs3745274 SNP of the gene CYP2B6 and opioid addiction. Some authors considered a higher rate of opioid addiction relapse in rs3745274 carriers. The CYP2B6*9 allele variant is also associated with fatalities caused by methadone application [6].

Another cytochrome enzyme associated with drug response is CYP2C19. Approximately 2% of Caucasians, 14% of Chinese, and 57% of Oceanians are CYP2C19-poor metabolizers. Our data (14% allele frequency) coincided with the allele frequency in the European population [21]. The therapeutic effect is reduced in intermediate metabolizers, as well. These individuals are carriers of one loss-of-function copy of the CYP2C19 gene, and one normal or gain-of-function allele variant. The frequency of intermediate metabolizers is relatively high in East Asia (45%), Central and South Asia (40%), Oceanians (36%), and 20-26% in American and European populations. The allele variants CYP2C19*2 and CYP2C19*4 encode non-functional enzymes. The results of our research revealed a lower frequency (25%) of CYP2C19*2 heterozygotes among Bulgarian individuals [22]. It is considered that 6-12% of the observed variability in the antiplatelet effect of clopidogrel is a result of the CYP2C19 polymorphism [23]. The allele variant CYP2C19*4 is established in less than 1% of the general population [21].

CYP2C9 is involved in the metabolism of acidic drugs including S-warfarin, phenytoin, and nonsteroidal anti-inflammatory drugs. The CYP2C9 gene is highly polymorphic with over 60 allele variants being identified. CYP2C9*2 is the most common mutant allele, encoding an enzyme with decreased activity and up to 40% reduction in S-warfarin biotransformation. CYP2C9*3 is caused by a missense mutation, encoding an enzyme with conformational changes and significantly reduced affinity for its substrates. This allele variant results in a considerable reduction in S-warfarin metabolism by up to 95% [24]. The results of our study revealed a frequency of these alleles (9% and 11%, respectively) in the Bulgarian population comparable to this one in Europe (13% and 7%) and higher than in Asian populations (< 5%). The CYP2C9*2 and *3 allele variants are frequent among the Caucasian population – 1% homozygotes and 22% heterozygotes, and 0.4% homozygotes and 15% heterozygotes for CYP2C9*2 and CYP2C9*3, respectively [24]. Warfarin is an anticoagulant that inhibits the vitamin K epoxide reductase complex and the carboxylation of factors II, VII, IX, and X as well as proteins C and S. The drug has a narrow therapeutic window requiring strict monitoring and precise dosing. Patients with reduced CYP2C9 enzyme activity are exposed to a higher risk of supratherapeutic anticoagulation and bleeding.

CONCLUSIONS

CYPs are the main enzymes responsible for drug biotransformation. Determining the most frequent polymorphisms of certain enzyme isoforms may help develop personalized medicine, and improve the individual approach towards the patients. Studying the most common pharmacogenetic defects of CYPs in the population will lead to a lower risk of drug toxicity, increased pharmacotherapeutic efficacy, and dose optimization, especially for drugs with narrow therapeutic windows.

Informed Consent Statement: *Informed consent was obtained from all subjects involved in the study.*

Disclosure Summary: *The authors have nothing to disclose.*

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ORIGINAL ARTICLE

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PERMANENT PACEMAKER IMPLANTATION: EARLY POST-IMPLANTATION DATA

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Abstract. Introduction: *Data on the development of left ventricular dysfunction after permanent pacemaker implantation are available. Myocardial collagen deposition is a well-known mechanism that occurs in left ventricular remodelling. This gave us reason to dynamically monitor the levels of the main molecules involved in collagen synthesis, PIPC (carboxy-terminal propeptide of type I procollagen) and PIIINP (amino-terminal propeptide of type III procollagen).* ***Materials and Methods:*** *PIPC and PIIINP levels were studied using enzyme-linked immunoassays in plasma from 45 patients (25 men, 20 women, 72.1 ± 9 years) and 46 controls (24 men, 22 women, 71.9 ± 8.7 years) without known cardiovascular diseases (except arterial hypertension, conduction disorder, indication for the procedure) at baseline (immediately before PPM implantation for patients), at 12 and 24 weeks.* ***Results:*** *There was no difference in baseline levels of PICP and PIIINP between patients and controls ($p > 0.05$, Table abstract). At week 12, PICP levels increased significantly in patients compared to baseline in controls ($p < 0.05$, Table abstract). At week 24, values continued to increase and were again significantly higher than baseline in the controls ($p < 0.001$, Table abstract). At the 12-week follow-up visit, PIIINP values in patients were significantly higher than those at baseline in controls ($p < 0.001$, Table abstract). At week 24, the values of the patients were still higher than those of the controls, but the difference was not significant ($p > 0.05$, Table abstract).* ***Conclusion:*** *This study showed early activation of collagen synthesis < 6 months after PPM (permanent pacemaker) implantation. Due to the selection of patients without concomitant cardiovascular pathology, we have reason to assume that it is a result of the procedure itself and a serious prerequisite for increased collagen deposition in the myocardium.*

Key words: *PIPC, PIIINP, permanent pacemaker*

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INTRODUCTION

Since the introduction of PPM implantation as part of the therapeutic approach in patients with bradyarrhythmias, data on its undisputed benefits have rapidly accumulated [1, 2, 3]. Over the years, dual chamber pacing has become a standard approach for the treatment of permanent or paroxysmal third- or high-degree atrioventricular block (AVB) [4, 5]. Life expectancy of heart disease patients has also increased significantly with improved primary and secondary prevention [6, 7]. This led to an in-

crease in the number of PPM implantations and allowed us to analyze its long-term effects. In addition to the indisputable benefits of dual chamber pacing, there are convincing data on the negative influence of apical right ventricular stimulation on heart pump parameters [8, 9, 10]. Reduction in left ventricular (LV) stroke volume, inefficient LV emptying, and appearance of functional mitral regurgitation were observed. Their manifestation is associated with asynchronous apical right ventricular (RV) stimulation [11]. At the end of the tenth year, development of heart failure (HF) was observed in approximately 20% of patients with an implanted dual chamber pacemaker [12, 13]. According to some authors, changes in LV function occurred as early as the end of the first year, when a decrease in ejection fraction (EF) of more than 10% was recorded [14].

These results predetermine the need to look for opportunities to overcome the established limitations of apical RV pacing, one of which is the development of alternative pacing methods, that approximate as closely as possible the physiological pathway of myocardial depolarisation in areas close to the heart conduction system (His bundle (HB), left bandle area (LBA), etc.), as well as multipoint pacing [15, 16, 17, 18, 19, 20, 21]. The pathophysiological mechanisms involved in myocardial remodelling during pacemaker stimulation and how to overcome their influence have been increasingly discussed [22]. Some authors emphasise that the process is individual and probably multifactorial [23]. A common principle in the development of myocardial contractile disorders is the remodelling of the extracellular matrix at the expense of increased interstitial replacement fibrosis [24]. There is activation of myofibroblasts and increased collagen production in the interstitium, which is an expression of contractile dysfunction [25, 26]. The intimate mechanisms of this process, as well as the timing of their development, remain unspecified [27, 28, 29, 30].

The data presented above gave us reason to conduct the present study, the objective of which was to investigate the fibrotic response in patients after PPM implantation with apical RV stimulation by dynamically examining the main molecules of collagen synthesis: PIPC and PIIINP.

MATERIALS AND METHODS

Study design

The study was conducted in the Cardiology Department at the Virgin Mary University Hospital, Burgas, Bulgaria for the period March 2019 – August 2021. Inclusion of patients and controls began after approval of the Research Ethics Committee at the Medical University of Varna, No 82, March 28th, 2019 and the Virgin Mary University Hospital, Burgas, No. 502, March 21st, 2019, in compliance with the requirements of the Declaration of Helsinki (The World Medical Association, Declaration of Helsinki, 2008). Participants over 18 years of age were included after prior explanation and signing of an informed consent to participate.

Two groups were formed, patients and controls. Selection of study participants was based on clearly formulated inclusion and exclusion criteria (see below).

The study was designed to equalise the demographic and clinical characteristics of both groups in order to minimise the possibility of selection bias and compare them objectively [31]. This contributed to the reliability of the conclusions, as well as the established cause-and-effect relationships. The control group was created similar to the patient group in terms of gender, age, and comorbidities.

For the purpose of the study, peripheral venous blood was drawn from a cubital vein, and the levels of PICP and PIIINP were examined in each participant. Transthoracic echocardiography was performed on the day after PPM implantation to avoid the effect of atrioventricular asynchrony on LV pump parameters.

Collagen synthesis parameters were determined thrice in patients: immediately before PPM implantation (baseline value or visit 1 – V1), at 12 weeks (visit 2 – V2) and 24 weeks (visit 3 – V3) after implantation. The same parameters were also examined thrice in controls: at baseline (visit 1 – V1), at 12 weeks (visit 2 – V2) and 24 weeks (visit 3 – V3) after selection for the study. Blood was centrifuged, and the resulting plasma was frozen and stored according to the requirements of the assays used. The control group underwent ECG and echocardiography examination after their inclusion in the study. At follow-up visits, blood was drawn to examine fibrosis indices, and participants were also questioned about new complaints and diseases.

Indication for implantation in the patients included in the study was presence of complete AVB. After signing informed consent, they were implanted with a dual chamber pacemaker (PPM in DDDR mode) according to the requirements described in the EHRA (European Heart Rhythm Association) expert consensus for this type of procedure [5]. This ensured sustained apical RV pacing above 80% in all participants, which was verified by telemetry at each follow-up visit. For the purposes of the study all participants underwent transthoracic echocardiography on the day after implantation to assess LV pump parameters and rule out structural heart disease.

Study population

For the purpose of the study, 144 patients were screened from whom 45 patients (25 men, 20 women, 72.18 ± 1.35 years) without known cardiovascular disease (except arterial hypertension and conduction disorder, indication for the procedure) were selected. 99 were excluded from the study due to exclusion criteria (see below).

The control group was formed after screening 102 patients, and 46 (24 men, 22 women, 71.96 ± 1.29 years) were selected according to the set inclusion and exclusion criteria and included in the study after signing informed consent. The controls had no history and ECG evidence of current rhythm-conduction pathology. 39 of them had arterial hypertension as a comorbidity, which was optimally controlled with medications.

For the purposes of the study, it was of utmost importance to minimise the impact of medications and comorbidities on fibrotic response in selected patients and controls. For this reason, both patients and controls were treated with pharmaceuticals for which there is currently no evidence of a direct effect on the renin-angiotensin aldosterone system (RAAS). After selection, participants were treated with one or a combination of the following medications: a dihydropyridine calcium antagonist (amlodipine), thiazide diuretic (hydrochlorothiazide), and, if necessary, a centrally acting medication (methyldopa), in doses needed to achieve blood pressure control.

Inclusion criteria for the patient group

1. Presence of complete AVB as an indication for implantation of a dual chamber pacemaker.
2. Eligible comorbidity: moderate arterial hypertension that was medically well-controlled.
3. Absence of exclusion criteria.

Inclusion criteria for the control group

1. No history or ECG evidence of rhythm-conduction pathology.
2. Eligible comorbidity: moderate arterial hypertension that was medically well-controlled.
3. Absence of exclusion criteria.

Exclusion criteria:

1. Presence of cardiovascular disease: coronary artery disease (acute coronary syndrome; history of myocardial infarction, regardless of age; coronary revascularisation PCI/CABG; stable angina pectoris); heart failure with depressed pump function; uncontrolled hypertension; inflammatory heart disease: myocarditis, pericarditis, infective endocarditis; congenital heart disease; clinically significant acquired valvular heart disease; cardiomyopathies; thromboembolic events.
2. Presence of other diseases: renal or liver failure; diseases of the central nervous system; inflammatory and/or infectious diseases in the last three months; neoplastic or autoimmune diseases; nutritional pulmonary disease; diseases of the endocrine system; surgical intervention in the last three months;
3. Presence of pregnancy, systemic intake of NSAIDs (non-steroidal anti-inflammatory drugs) and antithrombotic drugs and mineralocorticoid antagonists.

Collection and storage of blood samples

Blood samples were obtained after puncture of the cubital vein (left or right) with a vacutainer system. Venous blood samples were centrifuged for 15 min at 3500 rpm. The separated serum was frozen at -20 C and after 3 to 4 weeks transferred for storage at -80 C. Included patients had 3 blood samples taken as follows: at baseline before pacemaker implantation, at 12 and at 24 weeks after implantation. Patients in the control group had identical amounts of blood samples taken, processed, and tested according to the same protocol.

Laboratory procedures

Factors tested: PICP and PIIINP were quantified by the ELISA method with MyBioSource kits from Human PICP Sandwich – ELISA and Human PIIINP Sandwich – ELISA (MyBioSource, Inc. San Diego, USA) as follows:

1. Human carboxy-terminal propeptide of type I procollagen, PICP ELISA Kit with a sensitivity of 2.26 ng/ml
2. Human aminoterminal propeptide of type III procollagen, PIIINP ELISA Kit with a sensitivity of 1.0 ng/ml.

Statistical analysis

All analyses were performed with STATISTICA 13.3.0, StatSoft Inc, USA.

Continuous variables were expressed as mean ± Standard Error of the Mean (SEM) and categorical variables were expressed as percentage of the total group. Two-tailed Student's t-test for independent samples was used to compare quantitative variables measured in controls and patients. Values $p < 0.05$ were adopted for statistically significant.

RESULTS

There were no statistical differences between patients and controls in terms of number, mean age, sex, and BMI ($p > 0.05$) as seen in Table 1.

Table 1. Demographic characteristics of patient and control groups

	Patients	Controls	P value
Number of participants	45	46	> 0.05
Mean age	72.18 ± 1.35	71.96 ± 1.29	> 0.05
Men/Women	25/20	24/22	> 0.05
BMI (kg/m^2)	27.45 ± 0.64	26.51 ± 0.49	> 0.05

According to the study design, the patient and control groups had no significant differences in comorbidities ($p > 0.05$) and antihypertensive therapy ($p > 0.05$) as seen in Table 2.

Table 2. Clinical characteristics of patient and control groups

	Patients (%)	Controls (%)	P value
Comorbidities			
Hypertensive disease	39 (86.66 %)	37 (80.43%)	> 0.05
Antihypertensive therapy			
Dopegit	23 (51.11%)	24 (52.17%)	> 0.05
Amlodipine	35 (77.78%)	33 (71.74%)	> 0.05
Hydrochlorothiazide	35 (77.78%)	35 (76.09%)	> 0.05

Transthoracic echocardiography did not reveal significant differences between LV end-diastolic and end-systolic volume, as well as in ejection fraction in the patient and control groups. Additionally, the measured values were within the normal range accepted by the European Association of Cardiovascular Imaging [32].

Table 3. Transthoracic echocardiography data

Echocardiographic indicator	Patients	Controls	P value
LVEDV	51.98 ± 1.97	52.17 ± 1.65	> 0.05
LVESV	24.50 ± 0.77	24.52 ± 0.76	> 0.05
EF%	57.36 ± 0.66	55.98 ± 0.33	> 0.05

2.2.1 PICP deviations

It is clear from Figure 1 that baseline values in patients were not different from those of controls (85.13 ± 4.68 vs 79.34 ± 3.49 ng/ml, $p > 0.05$). At week 12 (patients V2), PICP levels had increased significantly compared to controls (90.51 ± 4.28 vs 79.34 ± 3.49 ng/ml, $p = 0.0445$), and at week 24 (patients V3) the increasing trend was sustained (161.35 ± 14.05 vs 79.34 ± 3.49 ng/ml, $p < 0.001$).

Comparison of values in the patient group (Figure 2) showed that at week 12 (patients V2) PICP levels

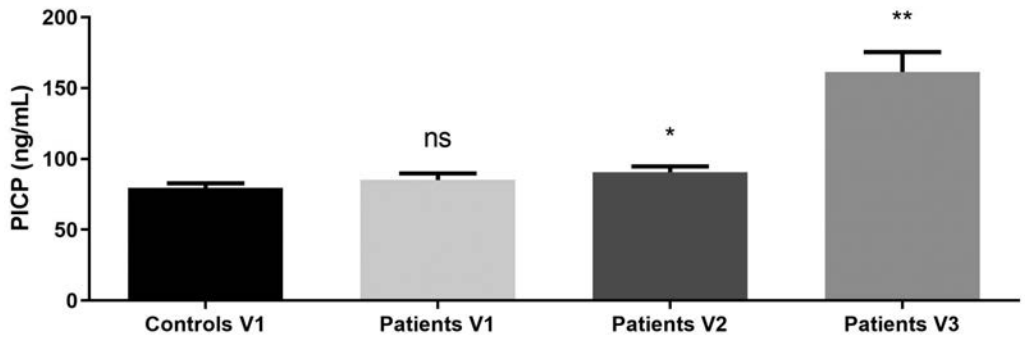

Fig. 1. Comparison of PICP values at baseline (patients V1), week 12 (patients V2) and week 24 (patients – V3) versus baseline in the control group (controls V1). (* $p < 0.05$; ** $p < 0.001$; ns- statistically insignificant difference)

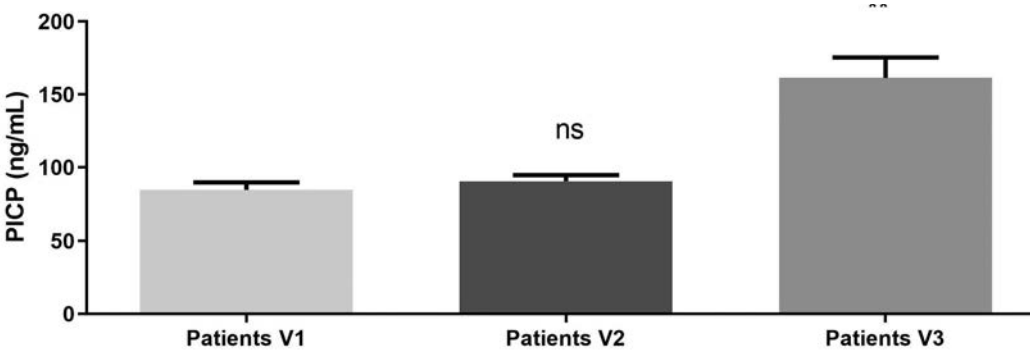

Fig. 2. Dynamics of PICP levels in the patient group: baseline (patients V1), at week 12 (patients V2) and at week 24 (patients V3). (* $p < 0.05$; ** $p < 0.001$; ns- statistically insignificant difference)

had increased from baseline (patients V1) (90.51 ± 4.28 vs 85.13 ± 4.68 ng/ml, p < 0.05), but this increase was not significant. At week 24 (patients V3), absolute values continued to increase and were now significantly higher compared to baseline levels (patients V1) (161.35 ± 14.05 vs 85.13 ± 4.68 ng/ml, p < 0.001).

There were no significant changes in plasma levels of the indicator during follow-up in the control group. There was also no significant difference between the values at the second and third visits compared to baseline (80.91 ± 4.14 vs 79.34 ± 3.49 ng/ml; 85.26 ± 4.75 vs 79.34 ± 3.49 ng/ml, p > 0.05). There were no significant differences in levels between the third and second visit follow-ups (85.26 ± 4.75 vs 80.91 ± 4.14 ng/ml, p > 0.05) (see Figure 3).

2.2.2 PIIINP deviations

Figure 4 shows that baseline values in patients did not differ from controls (4.11 ± 0.20 vs 3.94 ± 0.24 ng/ml, p > 0.05). At week 12, levels in patients (patients V2) had increased significantly compared to baseline levels of controls (6.95 ± 0.56 vs 3.94 ± 0.24 ng/ml, p < 0.001). At week 24, patient values (patients V3) had fallen to levels that were higher than controls, but the difference was not significant (4.56 ± 0.20 vs 3.94 ± 0.24 ng/ml, p > 0.05).

PIIINP levels in the patient group at week 12 (Figure 5) (patients V2) had increased significantly from baseline (patients V1) (6.95 ± 0.56 vs 4.11 ± 0.20 ng/ml, p < 0.001). At week 24 (patients V3), PIIINP levels had decreased, still higher than baseline, although the difference was not statistically significant (4.56 ± 0.20 vs 4.11 ± 0.20 ng/ml p > 0.05)

There were no significant changes in the plasma levels of the indicator during the follow-up period in the control group – no significant differences were found between the values of the second and third visits compared to baseline (4.60 ± 0.32 vs 3.94 ± 0.24 ng/

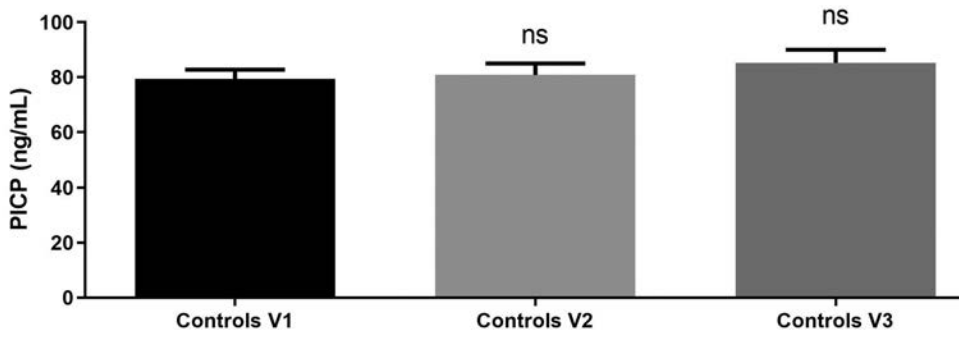

Fig. 3. Dynamics in PICP levels in the control group: baseline (controls V1), at week 12 (controls V2) and at week 24 (controls V3); ns- statistically insignificant difference

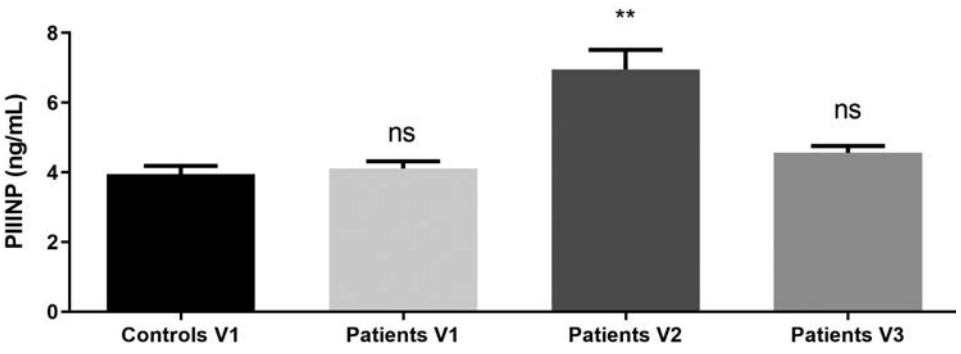

Fig. 4. Comparison of PIIINP values at baseline (patients V1), at week 12 (patients V2) and week 24 (patients V3) with baselines in the control group (controls V1). (*p < 0.05; **p < 0.001; ns – statistically insignificant difference)

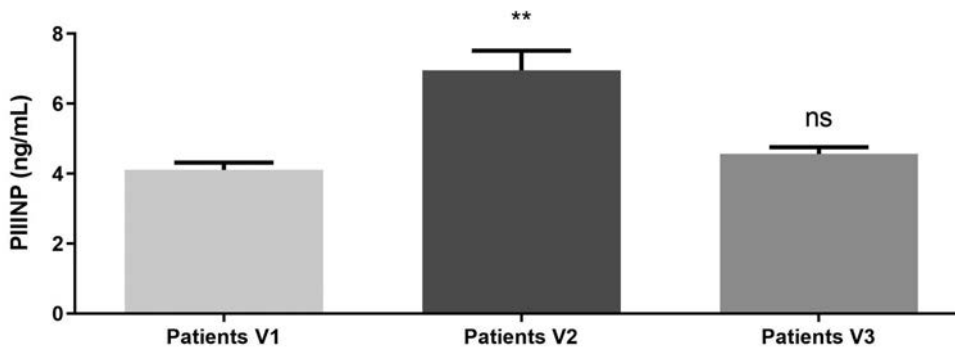

Fig. 5. Dynamics of PIIINP levels in the patient group: baseline (patients V1), at week 12 (patients V2) and at week 24 (patients V3). (*p < 0.05; **p < 0.001; ns – statistically insignificant difference)

ml; 4.06 ± 0.29 vs 3.94 ± 0.24 ng/ml, p > 0.05). There were no significant differences in follow-up levels at the third and second visit (4.06 ± 0.29 vs 4.60 ± 0.32 ng/ml, p > 0.05) (Figure 6).

DISCUSSION

In 1994, experimental models showed that constant RV apical pacing for 14 weeks resulted in regional changes in myocardial perfusion, increased catecholamine activity, and development of diastolic dysfunction [33]. There is now strong evidence of negative consequences on cardiac function as a result of pacing-induced asynchronous LV contraction [34]. ECM remodelling underlies the development of systolic and diastolic dysfunction over time in PPM patients [35, 36]. Data from studies have shown that after 12 weeks of constant RV stimulation, heterogeneous changes in ECM, increased MMP-2 and MMP-9 activity and increases in collagen type I mRNA levels were observed [37]. According to the implemented study design, our team aimed to look for early changes in collagen synthesis biomarkers after PPM implantation. The results showed that PICP levels increased at week 12 after implantation compared to controls, although with a cutoff value of p = 0.0445 (Figure 1). At week 24, there was a significant increase in PICP, both relative to baseline values in patients (p < 0.001) (Figure 2) and baseline values in controls (p < 0.001) (Figure 1). This gave us reason to assume that PPM implantation leads to an extremely early activation of collagen metabolism. The ECM in the myocardium has been shown to have a specific composition and contains mainly type I (85%) and type III collagen (11%) [38]. Current understanding is that the cardiac interstitium is a dynamic reticular structure with important metabolic activity [39]. Under the influence of a pathological stimulus, fibroblast activation occurs, leading to increased collagen synthesis [25,40]. Patients with advanced heart failure have an increased collagen content in the interstitium, leading to deeper pathophysiological consequences and disease progression [38,41]. In recent years, PICP and PIIINP levels have established themselves as reliable molecules for assessing collagen metabolism [42,43]. Data from previous studies suggest that both the amount of interstitial collagen and type I/III collagen ratio are important for development of cardiac dysfunction [44]. An increase in this ratio is associated with reduced LV wall elasticity and cavity dilatation. In the patient group, there was an increase in PIIINP levels at week 12, both relative to baseline in patients (p < 0.001) (Figure 5) and baseline in controls (p < 0.001) (Figure 4). However, at week 24 after implantation, there was a decrease to similar, but higher, levels than at baseline. Although there was a trend to increase toward the end of the follow-up period, the difference was not significant. Regarding the significant increase at week 12 after implantation, we can suggest that it might probably be a result of the surgical intervention. It is an undeniable fact that collagen type III is involved in the construction of the skin and subcutaneous tissue and plays an active role in the healing of the surgical cicatrix [45]. A study of samples taken from surgical wounds found a significant increase in PIIINP levels in the days following

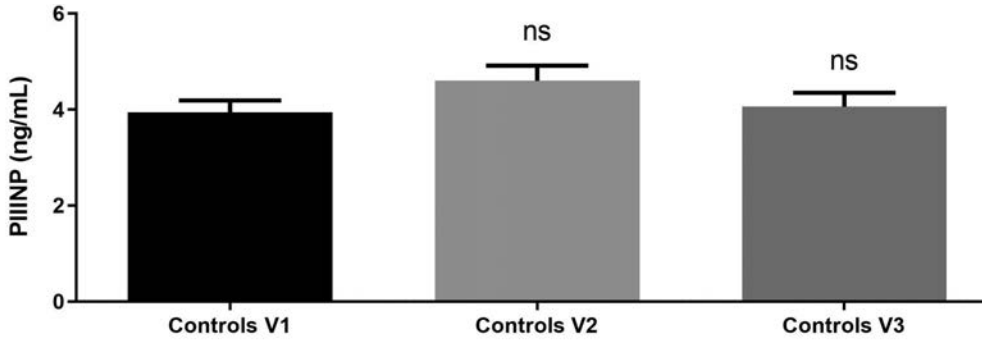

Fig. 6. PIIINP dynamics in the control group: baseline, at inclusion in the study (V1 controls V1), at week 12 (V2 controls) and at week 24 (V3 controls); ns- statistically insignificant difference

Table 4. Plasma PICP and PIIINP concentrations in patients after dual-chamber PPM implantation and controls

	PICP/V1 ng/ml	PICP/V2 ng/ml	PICP/V3 ng/ml	PIIINP/V1 ng/ml	PIIINP/V2 ng/ml	PIIINP/V3 ng/ml
Patients	85.13 ± 4.68	90.51 ± 4.28	161.35 ± 14.05	4.11 ± 0.20	6.95 ± 0.56	4.56 ± 0.20
Controls	79.34 ± 3.49	80.91 ± 4.14	85.26 ± 4.75	3.94 ± 0.24	4.60 ± 0.32	4.06 ± 0.29

V1 – Visit 1, baseline; V2 – Visit 2, 12 weeks after V1; V3 – Visit 3, 24 weeks after V1

intervention [46]. Serum levels of this marker have also been found to rise several times above those of nonoperated patients, and then gradually decline to baseline values. Despite the lack of significant differences between the two groups, there was a trend toward higher values in patients, as indicated by the cutoff value of p = 0.15. Whether the increasing trend will be maintained can be ascertained by monitoring the levels of this indicator over a longer period.

Collagen deposition in reactive myocardial fibrosis increases myocardial stiffness, leading to expression of systolic and diastolic dysfunction [47, 48, 49]. This is due to a greater amount of type I collagen, which has large-diameter fibers and is highly cross-linked compared to type III [43]. In patients with congestive heart failure, there is a positive correlation between PICP levels and LV size, deterioration of LV systolic function, BNP levels, and appearance of intraventricular asynchrony [50]. However, these results were determined only once in patients with clinical signs of congestive heart failure already demonstrated. Similar results have been found in patients with an implantable cardioverter-defibrillator (ICD) [51]. Data show that patients with registered tachycardia, requiring device therapy, had lower LV ejection fraction and higher PICP/PIIINP ratio. In this study, serum markers of collagen synthesis were also examined once before defibrillator implantation, and the change in values over time was unknown.

According to our study design, all patients had preserved LV systolic function at baseline, determined by echocardiography on the day after pacemaker implantation. As has been shown, changes in the myocardial interstitium can occur in various pathological conditions [52, 53]. Therefore, the included patients were free of serious comorbidities that might have affected fibrotic activity during follow-up. On the other hand, with natural processes that occur in the biological ageing of the body, there is an increase in collagen deposition in different tissues and organs [25]. This was the reason for tracing collagen synthesis markers in parallel and dynamically in both the patient and control groups. Based on the above, we can assume that the increase in PICP and PIIINP levels at week 24 was due to PPM-induced asynchronous ventricular contraction.

CONCLUSION

Our study showed early activation of collagen synthesis as early as 6 months after PPM implantation. Due to the selection of patients without concomitant cardiovascular disease, we have reason to assume that these changes were probably the result of asynchronous LV contraction. From the data we obtained in the dynamic follow-up of collagen synthesis markers, the development of a profibrotic state was evident, which we can assume to be an expression of ECM remodelling.

Disclosure Summary: *The authors have nothing to disclose.*

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ORIGINAL ARTICLE

PREVALENCE OF METABOLIC SYNDROME AND ITS COMPONENTS IN PATIENTS WITH CONTROLLED GRAVES' DISEASE

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Abstract. Aim: *Our aim was to assess the prevalence of the metabolic syndrome (MetS) and its components in patients with controlled Graves' disease (GD).* ***Methods:*** *This was a cross-sectional study involving 95 consecutive patients with GD referred to our tertiary care inpatient clinical center meeting the following inclusion criteria: controlled hyperthyroidism, treatment with antithyroid drugs, untreated Graves' orbitopathy (GO), if present. Patients' anthropometric parameters were evaluated and laboratory tests were performed with measurement of fasting blood glucose, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, thyroid hormone and antibody levels. The presence of the MetS and its components as defined by the International Diabetes Federation from 2009 were evaluated.* ***Results:*** *In our patient cohort 82.1% were females, 17.9% were males, mean age 50.2 ± 13 years, with median duration of GD 16.5 months. The MetS was observed in 32.6% of our patients, obesity – in 34.7%, hyperglycemia in 38.9%, arterial hypertension – in 36.8%, low HDL-cholesterol – in 23.2% and hypertriglyceridemia – in 13.7%. There was not statistical difference neither between the prevalence of the MetS, nor between the prevalence of its individual components in female and male GD patients. The MetS was significantly more frequent in older patients, as well as abdominal obesity, hyperglycemia and arterial hypertension. There was not statistical difference in the frequency of the MetS and its components between GD patients with and without GO, except for waist circumference, which was significantly higher in patients with GO.* ***Conclusions:*** *The presence of the MetS and its components among GD patients are to great extent similar to those reported in the general population, which underlines the need for their screening and proper treatment in this subpopulation.*

Key words: *Graves' disease, metabolic syndrome, obesity, arterial hypertension, hyperglycemia, dyslipidemia*

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INTRODUCTION

Graves' disease (GD) is a chronic organ-specific autoimmune disorder and is the commonest cause of hyperthyroidism accounting for 70-80% of all cases [1]. It affects 0.5-2% of women in developed countries without iodine deficiency, while men show 5-10-fold lower prevalence. Patients suffering from GD are usually between 30 and 60 years of age. Its etiology is not fully elucidated, but

the combination between genetic susceptibility and environmental factors is considered to play a crucial role in GD development [2]. The major factor in GD pathogenesis are TSH-receptor antibodies (TRAb) [3]. They possess different biological activities and some of them are capable of stimulating and activating thyroid hormone production, as well as thyroid cell division resulting in thyrotoxicosis and goiter. Usual complaints of patients with active GD are related to increased catabolic rate (weight loss, sweating, heat intolerance), overstimulated central nervous system (nervousness, anxiety, irritability, sleep deprivation, tremor, enhanced reflexes), increased sympathetic tone (increased and/or irregular heartbeat, hypertension) and enhanced protein catabolism (muscle weakness, osteoporosis) [4]. There are three therapeutic options for GD – antithyroid drugs (ATDs), radioiodine therapy and surgery [5].

Weight loss is one of the most frequent symptoms of active GD. Several studies reported weight regain during and after treatment for GD, which sometimes exceeded the weight loss at GD presentation [6-10]. Persistently increased appetite, changes in neurotransmitter levels and/or adipocytokine levels, diminished lean body mass with concomitantly increased fat mass and decrease in resting energy expenditure are some of the proposed mechanisms [8, 10]. Pre-existing obesity, prior weight loss due to thyrotoxicosis, iatrogenic hypothyroidism (even transient) and long follow-up period independently predicted weight gain in patients with GD treated with one of the three therapeutic options [7]. Some, but not all, studies showed that patients who underwent thyroidectomy or radioiodine therapy tended to gain more weight compared to patients treated with ATDs [8]. Thyroid hormones are also involved in the regulation of blood pressure [11]. The effects of hyperthyroidism include increased cardiac output and contractility, tachycardia, widened pulse pressure, decreased systemic vascular resistance, which leads to increased systolic and decreased diastolic blood pressure. Thyroid hormones also affect glucose homeostasis by increasing hepatic glucose output, decreasing glycogen stores in the liver and skeletal muscle, altering glucose intestinal absorption and metabolism, decreasing active insulin output from the pancreas, and increasing renal insulin clearance [12]. In thyrotoxicosis all these actions are exaggerated leading to hyperglycemia and predisposing diabetic patients to develop diabetic ketoacidosis. The thyrotoxic state affects the lipid profile as well, causing acquired hypocholesterolemia and probably hypotriglyceridemia [13]. Indeed, even within the normal range of TSH values, a linear decrease in total cholesterol, LDL-cholesterol and triglycerides, and a linear increase in HDL-cholesterol has been observed with decreasing TSH.

Metabolic syndrome (MetS) is a combination of interrelated metabolic disturbances that increases the risk of type 2 diabetes mellitus and cardiovascular morbidity and constitutes a significant health and socioeconomic problem worldwide [14]. According to the definition of the International Diabetes Federation, the MetS is diagnosed in case of presence of three or more of the following criteria: increased body weight, more specifically central obesity, elevated fasting plasma glucose or previously diagnosed diabetes mellitus, lowered HDL-levels, increased triglycerides and raised blood pressure [15]. The prevalence of the MetS is between 25%-50% and varies widely depending on sex, age, ethnicity, socioeconomic and cultural factors [16-18]. As a result of its progressively increasing incidence, it comes as no surprise that it is more and more often seen in patients with other endocrinopathies, such as thyroid diseases, including GD. The combination of the two disorders additionally affects patients' quality of life and well-being and leads to increased burden for the Health Care System.

Our aim was to assess the prevalence of the MetS and its components in patients with controlled GD.

MATERIALS AND METHODS

This was a cross-sectional study involving 95 consecutive patients with GD referred to our tertiary care inpatient clinical center (University Hospital of Endocrinology, Sofia). The patients met the following selection criteria: inclusion criteria – controlled hyperthyroidism, treatment with ATDs, untreated GO, if present; exclusion criteria – uncontrolled hyperthyroidism or iatrogenic hypothyroidism, previous radioiodine therapy or thyroid surgery, Graves' orbitopathy (GO) treated with glucocorticoids. The confidence level and margin of error for the sample size of 95 patients with GD were 99% and 4%, respectively.

The diagnosis of GD was previously established based on the typical clinical manifestations and confirmed hormonally and immunologically, as well as by thyroid ultrasound. Data on GD duration, previous and current treatment, concomitant diseases, including GO, arterial hypertension, diabetes mellitus, prediabetes, dyslipidemia and their therapy, and smoking habits was acquired by patients' medical history. Then, patients' anthropometric parameters were evaluated – height in meters and weight in kilograms, on the basis of which the body mass index (BMI) was calculated according to the formula: BMI [kg/m^2] = body weight [kg]/height [m^2]. Waist cir-

cumference was measured in centimeters with non-stretchable tape at the level of the midpoint between the bottom of the ribcage and the highest points of the iliac crest. Blood pressure was measured twice in a sitting position, at rest, with a manual sphygmomanometer with a 5-minute interval between individual measurements.

Subsequently, laboratory tests were performed after overnight fasting with measurement of fasting blood glucose by hexokinase enzyme method, total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides by enzymatic colorimetric method, thyroid hormone levels (TSH by immunoradiometric method and fT4 by electrochemiluminescence immunoassay) and antibody levels (TRAb by radioreceptor method, thyroid-peroxidase antibodies and antithyroglobulin antibodies by electrochemiluminescence immunoassay).

After all tests were performed, the presence of the MetS and its components as defined by the International Diabetes Federation from 2009 were evaluated [15] (Table 1).

Table 1. Diagnostic criteria of metabolic syndrome

Diagnostic criteria (3 or more)		
Obesity	Waist circumference	♂ ≥ 94 см ♀ ≥ 80 см
Hyperglycemia	Fasting blood glucose	≥ 5.6 mmol/l or known diabetes mellitus
Dyslipidemia	HDL-cholesterol	♂ < 1.0 mmol/l ♀ < 1.3 mmol/l or antilipidemic therapy
	Triglycerides	≥ 1.7 mmol/l or antilipidemic therapy
Arterial hypertension	Blood pressure	Systolic blood pressure ≥ 130 mm Hg or diastolic blood pressure ≥ 85 mm Hg or antihypertensive therapy

Ethical approval

The study protocol was approved by the Institutional Ethical Committee and was in accordance with the 1964 Declaration of Helsinki and its later amendments.

Statistical analysis

The results were analyzed using SPSS version 19 (IBM SPSS, v.19.0. Armonk, NY: IBM Corp.). First, descriptive analysis and the Shapiro-Wilk test for normality were performed. Continuous variables were presented as means and standard deviations or as medians with minimum and maximum values according to the data distribution. Categorical variables were presented as count and/or proportion. When comparing two continuous variables, the Student's t-test or the non-parametric Mann-Whitney test were used depending on the distribution of the data. Categorical variables were tested by χ^2 test. Bivariate correlation test with Spearman's coefficient was used to assess the correlations between some continuous variables. A p-value of less than 0.05 was considered an indicator of statistical significance.

RESULTS

Baseline parameters

In our patient cohort 82.1% were females, 17.9% were males, mean age 50.2 ± 13 years, with median duration of GD 16.5 months (Table 2). In Table 2 are also presented the baseline metabolic parameters.

Table 2. Baseline characteristics

Demographics	N = 95
Sex, n (%) Females Males	 78 (82.1) 17 (17.9)
Age, yearsa	50.2 (± 13)
Smoking habits, n (%) ● Current ● Ex-smokers ● Non-smokers	 42 (44.2) 12 (12.6) 41 (43.2)
Clinical characteristics of GD	
Duration of GD, months[b]	16.5 (2-134)
GO, n (%)	48 (50.5)
TSH, mIU/l[b]	0.89 (0.5-4.2)
fT4, pmol/l[b]	14.6 (9-21)
TSH-receptor antibodies, IU/l[b]	2.9 (1-40)
Anti-TPO antibodies positivity, %	56.6
Anti-Tg antibodies positivity, %	38.3
Metabolic parameters	
BMI, kg/m^2[a]	25.3 (± 5)
Waist circumference, cma	87.9 (± 14.6)
Total cholesterol, mmol/L[b]	5.3 (3.6-10.8)
LDL-cholesterol, mmol/L[b]	3.3 (2-8.5)
HDL-cholesterol, mmol/L[b]	1.5 (0.9-2.9)
Triglycerides, mmol/L[b]	0.9 (0.4-3.1)
Blood glucose, mmol/L[a]	5.5 (± 0.52)
Patients on antihypertensive therapy, n (%)	32 (33.7)
Patients on antidiabetic therapy, n (%)	10 (10.5)
Patients on antilipidemic therapy, n (%)	6 (6.3)

[a]Data are presented as means, data in the parenthesis are standard deviations. [b]Data are presented as medians, data in parenthesis are minimum and maximum value.

GD Graves' disease, GO Graves' orbitopathy, BMI Body mass index

Prevalence of the MetS and its components

The frequency of the MetS and its components is presented in Fig. 1. The MetS was observed in 32.6% of our patients, obesity – in 34.7%, hyperglycemia in 38.9%, arterial hypertension – in 36.8%, low HDL-cholesterol – in 23.2% and hypertriglyceridemia – in 13.7%.

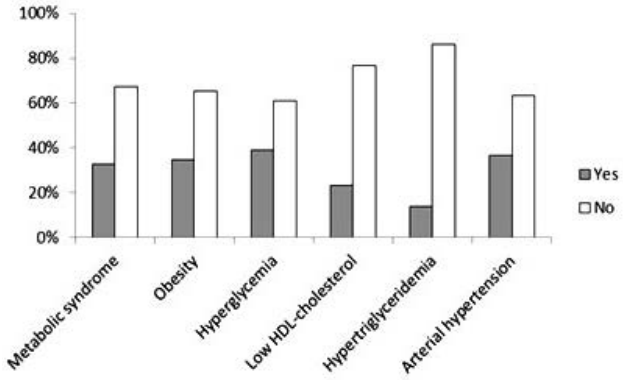

Fig. 1. Prevalence of metabolic syndrome and its components among patients with Graves' disease

Prevalence of the MetS and its components depending on sex and age

There was no statistical difference neither between the prevalence of the MetS, nor between the prevalence of its individual components in female and male GD patients (Fig. 2).

When comparing the prevalence of the MetS in patients < 50 years and > 50 years, we found that it was significantly more frequent in older patients (49% vs 11.9%, $p < 0.01$) (Fig. 3). Abdominal obesity, hyperglycemia and arterial hypertension were also significantly more often observed in older patients (73.6% vs 54.8%, $p = 0.04$; 54.7% vs 19%, $p < 0.01$ and 58.5% vs 9.5%, $p < 0.01$, respectively), whereas the rates of dyslipidemia were similar in the two age groups.

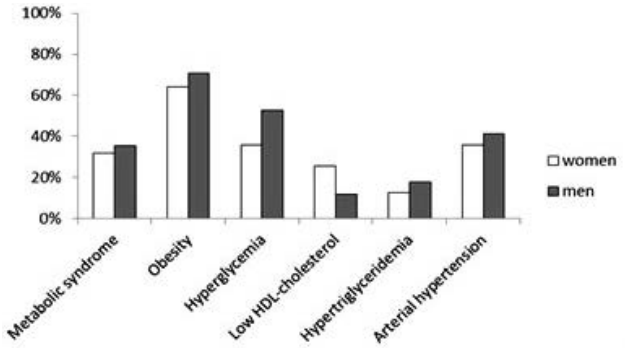

Fig. 2. Prevalence of metabolic syndrome and its components among patients with Graves' disease depending on sex

Metabolic disturbances in patients with GD and GO

Baseline characteristics of patients with GD with and without GO did not differ significantly, except for TAT positivity, which was higher in patients with GO (55% vs 22%, $p < 0.01$) (Table 3). In terms of the metabolic parameters, waist circumference was significantly higher in patients with GO (91.4 ± 14.9 vs 84.3 ± 13.5, $p = 0.04$), as well as BMI (27 ± 5.6 vs 24.7 ± 5, $p = 0.04$). However, when comparing the frequency of the MetS and its components, we did not find a significant difference between GD patients with and without GO (Fig. 4).

Associations between some clinical and metabolic parameters

Weight, BMI and waist circumference correlated positively with age and duration of GD (Table 4). Blood glucose correlated positively with age. Total cholesterol correlated positively with age and TSH and negatively with fT4. Triglycerides correlated positively with age. Total cholesterol, LDL-cholesterol and triglycerides correlated positively with waist circumference, while HDL-cholesterol – negatively.

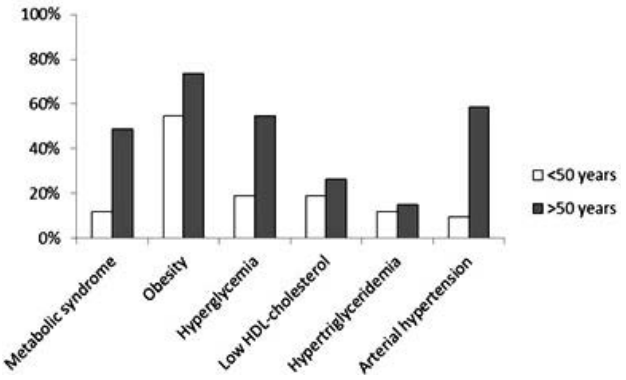

Fig. 3. Prevalence of metabolic syndrome and its components among patients with Graves' disease depending on age

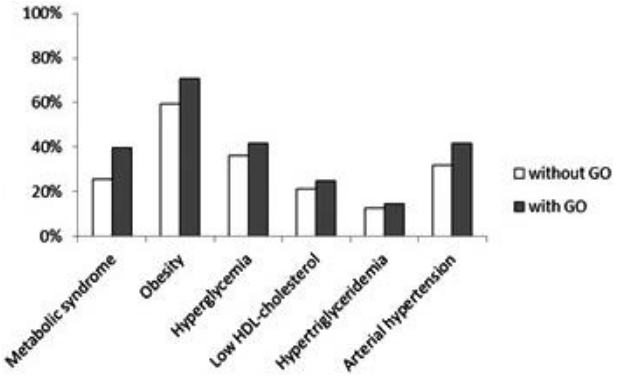

Fig. 4. Prevalence of metabolic syndrome and its components among patients with Graves' disease with and without Graves' orbitopathy

Table 3. Comparison between the baseline characteristics of patients with Graves' disease with and without Graves' orbitopathy

Demographics	GD without GO, n = 47	GD with GO, n = 48	p
Sex, n (%)[c] Females Males	 40 (85.1) 7 (14.9)	 38 (79.2) 10 (20.8)	0.45
Age, yearsa	49 (± 14.4)	53 (± 12.7)	0.16
Smoking habits, n (%)[c] ● Current ● Ex-smokers ● Non-smokers	 19 (44.2) 6 (12.6) 22 (43.2)	 23 (44.2) 6 (12.6) 19 (43.2)	0.75
Clinical characteristics of GD			
Duration of GD, months[b]	13.5 (2-108)	21 (3-134)	0.17
Duration of GO, months[b]	48 (50.5)		
TSH, mIU/l[b]	1.2 (0.7-4.2)	0.7 (0.5-4)	0.43
fT4, pmol/l[b]	14.3 (12-21)	14.8 (9-21)	0.73
TSH-receptor antibodies, IU/l[b]	2.1 (1-40)	3.3 (1-40)	0.33
Anti-TPO antibodies positivity, % [c]	58.5	45.2	0.73
Anti-Tg antibodies positivity, % [c]	55	22	< 0.01
Metabolic parameters			
BMI, kg/m^2[a]	24.7 (± 5)	27 (± 5.6)	0.04
Waist circumference, cm[a]	84.3 (± 13.5)	91.4 (± 14.9)	0.02
Total cholesterol, mmol/L[b]	5.1 (3.6-8.8)	5.4 (3.6-10.8)	0.45
LDL-cholesterol, mmol/L[b]	3.3 (2-6.1)	3.5 (2-8.5)	0.30
HDL-cholesterol, mmol/L[b]	1.5 (0.9-2.3)	1.4 (0.9-2.9)	0.69
Triglycerides, mmol/L[b]	0.9 (0.4-2.5)	1.0 (0.4-3.1)	0.25
Blood glucose, mmol/L[a]	5.5 (± 0.5)	5.5 (± 0.6)	0.72

[a]Data are presented as means, data in the parenthesis are standard deviations, p-value is calculated using Student T-test. [b]Data are presented as medians, data in parenthesis are minimum and maximum value, p-value is calculated using Mann-Whitney U test. [c]P-value is calculated using the Chi-Square test.

GD Graves' disease, GO Graves' orbitopathy, BMI Body mass index

Table 4. Relationship between some clinical, biochemical and metabolic parameters

	Weight	BMI	Waist	Age	GD duration	Glucose	TC	LDL-C	HDL-C	TG	TSH	fT4
Weight		ρ = 0.89 p < 0.01	ρ = 0.84 p < 0.01	ρ = 0.21 p = 0.04	ρ = 0.29 p = 0.01	ρ = 0.22 p = 0.03	ρ = 0.09 p = 0.41	ρ = 0.15 p = 0.16	ρ = -0.25 p = 0.02	ρ = 0.19 p = 0.07	ρ = 0.07 p = 0.49	ρ = 0.15 p < 0.16
BMI	ρ = 0.89 p < 0.01		ρ = 0.86 p < 0.01	ρ = 0.24 p = 0.02	ρ = 0.28 p = 0.01	ρ = 0.24 p = 0.02	ρ = 0.16 p = 0.13	ρ = 0.18 p = 0.08	ρ = 0.18 p = 0.07	ρ = 0.26 p = 0.01	ρ = 0.07 p = 0.48	ρ = 0.03 p = 0.77
Waist	ρ = 0.84 p < 0.01	ρ = 0.86 p < 0.01		ρ = 0.21 p = 0.04	ρ = 0.34 p < 0.01	ρ = 0.30 p < 0.01	ρ = 0.25 p = 0.02	ρ = 0.29 p = 0.01	ρ = -0.25 p = 0.02	ρ = 0.28 p = 0.01	ρ = 0.08 p = 0.45	ρ = 0.01 p = 0.91
Age	ρ = 0.21 p = 0.04	ρ = 0.24 p = 0.02	ρ = 0.21 p = 0.04		ρ = 0.05 p = 0.61	ρ = 0.39 p < 0.01	ρ = 0.32 p < 0.01	ρ = 0.33 p < 0.01	ρ = -0.01 p = 0.92	ρ = 0.31 p < 0.01	ρ = 0.02 p = 0.83	ρ = 0.08 p = 0.45
GD duration	ρ = 0.29 p = 0.01	ρ = 0.28 p = 0.01	ρ = 0.34 p < 0.01	ρ = 0.05 p = 0.61		ρ = 0.05 p = 0.64	ρ = 0.04 p = 0.73	ρ = 0.04 p = 0.74	ρ = -0.14 p = 0.20	ρ = 0.04 p = 0.73	ρ = 0.31 p < 0.01	ρ = 0.03 p = 0.81
Glucose	ρ = 0.22 p = 0.03	ρ = 0.24 p = 0.02	ρ = 0.30 p < 0.01	ρ = 0.39 p < 0.01	ρ = 0.05 p = 0.64		ρ = 0.15 p = 0.15	ρ = 0.14 p = 0.17	ρ = -0.01 p = 0.89	ρ = 0.30 p < 0.01	ρ = 0.06 p = 0.57	ρ = -0.11 p = 0.27
TC	ρ = 0.09 p = 0.41	ρ = 0.16 p = 0.13	ρ = 0.25 p = 0.02	ρ = 0.32 p < 0.01	ρ = 0.04 p = 0.73	ρ = 0.15 p = 0.15		ρ = 0.93 p < 0.01	ρ = 0.24 p = 0.01	ρ = 0.40 p < 0.01	ρ = 0.23 p = 0.03	ρ = -0.23 p = 0.03
LDL-C	ρ = 0.15 p = 0.16	ρ = 0.18 p = 0.08	ρ = 0.29 p = 0.01	ρ = 0.33 p < 0.01	ρ = 0.04 p = 0.74	ρ = 0.14 p = 0.17	ρ = 0.93 p < 0.01		ρ = 0.01 p = 0.95	ρ = 0.42 p < 0.01	ρ = 0.14 p = 0.17	ρ = -0.21 p = 0.04
HDL-C	ρ = -0.25 p = 0.02	ρ = -0.18 p = 0.07	ρ = -0.25 p = 0.02	ρ = -0.01 p = 0.92	ρ = -0.14 p = 0.20	ρ = -0.01 p = 0.89	ρ = 0.24 p = 0.01	ρ = 0.01 p = 0.95		ρ = -0.33 p < 0.01	ρ = 0.15 p = 0.16	ρ = -0.05 p = 0.60
TG	ρ = 0.19 p = 0.07	ρ = 0.26 p = 0.01	ρ = 0.28 p = 0.01	ρ = 0.31 p < 0.01	ρ = 0.04 p = 0.73	ρ = 0.30 p < 0.01	ρ = 0.40 p < 0.01	ρ = 0.42 p < 0.01	ρ = -0.33 p < 0.01		ρ = -0.11 p = 0.30	ρ = 0.08 p = 0.45
TSH	ρ = 0.07 p = 0.49	ρ = 0.07 p = 0.48	ρ = 0.08 p = 0.45	ρ = 0.02 p = 0.83	ρ = 0.31 p < 0.01	ρ = 0.06 p = 0.57	ρ = 0.23 p = 0.03	ρ = 0.14 p = 0.17	ρ = 0.15 p = 0.16	ρ = -0.11 p = 0.30		ρ = -0.25 p = 0.02
fT4	ρ = 0.15 p < 0.16	ρ = 0.03 p = 0.77	ρ = 0.01 p = 0.91	ρ = 0.08 p = 0.45	ρ = 0.03 p = 0.81	ρ = -0.11 p = 0.27	ρ = -0.23 p = 0.03	ρ = -0.21 p = 0.04	ρ = -0.05 p = 0.60	ρ = 0.08 p = 0.45	ρ = -0.25 p = 0.02	

DISCUSSION

In the present study we found that approximately one third of our patients with controlled GD had the MetS. Sengupta et al. conducted a study in India having similar to our design including GD patients with minimum duration of GD of 12 months [19]. The authors found that the prevalence of the MetS was 36%, which is comparable with our results, as well as with the reported prevalence of the MetS in the Indian population (30%) [20]. The frequency of the MetS in the general population actually varies a lot and is influenced by several factors, such as: age, sex, ethnical, cultural and socio-economic background [16-18]. The latest data on the prevalence of the MetS in the Bulgarian population are from 2012, when the prevalence of the MetS was 35.7% – similar to that of our GD patients. However, there is a well-known trend for a gradual increase in the frequency of the MetS over the years [16]. For example, over a five-year period, from 2007 to 2012, the prevalence of the MetS in Bulgaria increased by approximately 5% according to the results reported by Borissova et al. [21, 22]. We could assume that the prevalence of the MetS in Bulgaria increased further during the last decade. Unfortunately, due to the lack of current data on this topic, we are not able to directly compare our results on GD patients with the general population.

The prevalence of visceral obesity assessed by measurement of waist circumference in our GD patient cohort was approximately 35%, which is lower than the reported by Borissova et al. in their study on the features of the MetS in the unselected Bulgarian population (62.5%) [23]. Although the median duration of GD in our patients cohort was 16.5 months, in some of them the disease was actually recently diagnosed. The latter may be the cause for undercompensated weight after the initial catabolic weight loss due to hyperthyroidism. The frequency of hyperglycemia in Bulgarian population in 2012 reported by Borissova et al. is lower than what we observed in our study (25.2% vs 38.9%) [23]. However, our results are comparable with the findings of more recent studies evaluating the prevalence of diabetes and prediabetes in the general population [24, 25]. So, the discrepancies between the results of Borissova et al. and ours might be due the fact that the prevalence of hyperglycemia had changed over the last decade being much more common now. In our study the proportion of patients taking antidiabetic therapy was relatively low compared to the prevalence of hyperglycemia, which might be due to the lack of active search for diabetes and prediabetes among the GD population by endocrinologists. The prevalence of arterial hypertension in our study was 36.8% which is similar to that in the general population in Bulgaria (38.9%) [26]. Almost all patients having arterial hypertension were already on antihypertensive therapy at the time of the inclusion in the study. The reported prevalence of dyslipidemia in Bulgarian population is 33.7% for hypertriglyceridemia and 32.9% for low HDL-cholesterol, and these percentages are much higher than what we observed in our study (13.7% and 23.2%, respectively) [27]. Interestingly, only 6.3% of our patients were on antilipidemic therapy. Unlike arterial hypertension, dyslipidemia is asymptomatic and it appears to be underdiagnosed or undertreated in GD patients. This finding underlines the importance of the active screening for dyslipidemia in this group of patients. We found that the level of triglycerides and that of HDL-cholesterol correlated positively and negatively, respectively, with waist circumference – findings confirmed in previous studies [28, 29]. Therefore, it is not surprising that our patient cohort, characterized by lower presence of visceral obesity compared to the general population, had a lower frequency of dyslipidemia, as well.

When evaluating the presence of the MetS and its components in different age and sex groups, we found that the MetS was much more common in older individuals, which is in accord with previous studies [16, 18, 23]. Borissova et al. reported higher prevalence of the MetS in men than in women (40.9% vs 31.1%), whereas in our study the rates of the MetS were similar in both sexes. It could be that the relatively small number of males included in our study lead to inability to establish existing patterns. Our results showed that the prevalence of abdominal obesity between the two genders was the same, but it was more frequently seen in older individuals, which is consistent with the observed positive correlation between weight, BMI and waist circumference, and age. Some population-based studies report similar results, including one Bulgarian study [23, 30-32]. We found that blood glucose level correlated positively with age and hyperglycemia was more commonly seen in older individuals. These results are comparable with those of Borissova et al., who studied the prevalence of diabetes and prediabetes in the Bulgarian population [33]. The prevalence of arterial hypertension increases with age as seen in our study and that of Borissova et al., which focused on the general population in Bulgaria (26). Additionally, in their study arterial hypertension was more frequent in men. According to our results, there was a positive correlation between triglyceride levels and age. However, we were not able to demonstrate a significant age- and sex-related difference between the preva-

lence neither of hypertriglyceridemia, nor of low HDL-cholesterol level. On the opposite, Borrisova et al. observed higher prevalence of hypertriglyceridemia in men (46.9 vs 22.2, $p < 0.01$), whereas the prevalence of low HDL-cholesterol was more common in women (35.8 vs 29.7, $p < 0.01$) [27]. Additionally, higher rates of hypertriglyceridemia were found in older individuals, while regarding HDL-cholesterol there was no difference between the different age groups.

Our results demonstrated positive correlation between total cholesterol level and TSH, even the latter being within the normal range. This finding is in accord with the results of previous studies, which also reported a linear increase of LDL-cholesterol and triglycerides and a linear decrease in HDL-cholesterol with increasing TSH within the normal range [34]. In addition, in our study a negative correlation between total cholesterol and fT4, again within the normal limits, was observed. These findings accentuate the great influence of thyroid function on lipid profile and the importance of adequate treatment doses.

When analyzing the metabolic parameters in patients with and without GO, we found that patients with GO had significantly higher BMI and waist circumference. It might be that the propensity for accumulation of adipose tissue in the visceral area is somehow related to the fat deposition in retroorbital spaces seen in GO. Zhang et al. found some common features between orbital fat tissue and white and brown adipose tissue [35]. Whether the obesity-related chronic low-grade inflammation could increase the risk for development of allergies and autoimmune disorders, is still debatable [36]. However, this is a relevant speculation, taking into account the changed levels of some visceral fat-derived adipocytokines and cytokines in obese individuals, which could alter the immune response [37]. It is possible that obesity promotes more severe immune disturbances in patients with GD, whose phenotypic manifestation could be GO.

LIMITATIONS

Our study has several limitations. First, the relatively low number of GD patients in the different age and sex groups decreases the statistical power of the conclusions. Further larger studies are needed to confirm our results. Second, in the present study we did not evaluate some additional MetS-related parameters, such as: fasting insulin level and insulin resistance, uric acid level and levels of adipocytokines. A major limitation of our study is the lack of sex-, age- and BMI-matched control groups, which does not allow a correct assessment of the influence of hyperthyroidism on the frequency and characteristics of the metabolic syndrome and reduces the informativeness and interpretability of the obtained results. Due to lack of current data on the MetS and its components in the general population in Bulgaria, we were opted to compare our results with those of earlier studies in Bulgaria or recent studies from other countries. Last, our study was a tertiary hospital-based study, which might have led to Berkson's bias, as patients referred to our center usually have more severe GD and/or GO.

CONCLUSIONS

The results of the present study indicate that the presence of the MetS and its components among GD patients are to a great extent similar to those reported in the general population, except for dyslipidemia, which appears to be the most sensitive component of the MetS to the action of thyroid hormones. There were age-related differences regarding the proportion of GD patients with the MetS, obesity, hyperglycemia and arterial hypertension. Both hyperglycemia and dyslipidemia are often underdiagnosed and undertreated in GD patients. The presence of GO is associated with significantly increased anthropometric indicators of obesity, but does not affect the frequency of the MetS and its individual components in the population of patients with GD. These findings underline the need for screening for the MetS and its components and their proper treatment in GD patients, especially the older ones.

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ORIGINAL ARTICLE

DETECTION OF PREDEFINED BACTERIAL SPECIES IN THE VAGINAL MICROBIOTA IN SARS-COV-2-POSITIVE PATIENTS

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Abstract. *Whether severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can be found in the vagina of infected women remains unclear; moreover, the impact of the virus on the normal vaginal microbiota is not known.* ***The aim*** *of our study was to identify the vaginal presence of SARS-CoV-2 and detection of predefined bacterial species changes in the vaginal flora of women that tested positive for SARS-CoV-2 infection.* ***Materials and Methods.*** *This prospective study included 40 women, aged 24-47 years, tested for SARS-CoV-2 via nasopharyngeal and vaginal culture (TaqPath ™COVID-19 CE-IVD RT-PCR), and vaginally tested for changes in the vaginal microbiota using the Femoflor® 16 REAL-TIME PCR Detection Kit.* ***Results.*** *No one of women in this study was tested positive for vaginal presence of SARS-CoV-2. Three (7.5%) women with sexually transmitted disease were excluded. Irregularities were observed in the vaginal microbiota of 8 (21.6%) out of 37 patients included in the study: 3 (8.1%) from the SARS-CoV-2-positive group and 5 (13.5%) from the SARS-CoV-2-negative group. The remaining 29 (78.4%) women had normal vaginal flora; lactobacilli were found to be dominant. Although results revealed a difference in the vaginal microbiota between the two groups, the differences were not statistically significant ($p \geq 0.05$).* ***Conclusions.*** *Even though it remains unclear whether SARS-CoV-2 invades the vagina of infected women, there is no significant evidence to suggest that it causes a more frequent disturbance in the vaginal microbiota of infected women compared to that in healthy women.*

Key words: *SARS-CoV-2, vaginal presence, vaginal flora, microbiota, microbial changes*

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INTRODUCTION

Vaginal flora in healthy women forms a dynamic ecosystem dominated by lactic acid bacteria (lactobacilli), and this ecosystem undergoes continuous changes in its structure and composition under the influence of many exogenous and endogenous factors [1-3]. Reduction or disappearance of vaginal lactobacilli unlocks a pathological microbial spiral, leading to a disruption in the existing equilibrium with the possibility of developing a local infection [1-3]. Besides local factors, such as exogenous

import of pathogenic microbes and viruses, allergic reactions, operative interventions, various other diseases, and external factors can cause a disturbance in the vaginal flora [4-11]. In 2019–2020, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was established as the causative agent for some life-threatening serious diseases [12]. In March 2020, the disease caused by SARS-CoV-2, termed coronavirus disease (COVID-19), was officially declared a pandemic by the World Health Organization [12]. COVID-19 exhibits different trends in terms of morbidity and symptoms in humans, based on their locations [13]. The symptoms also vary depending on individual genetics, ethnicity, age, and co-morbidities [14]. Recent studies reported that SARS-CoV-2 can be found in the vagina of infected women [15]. According to other studies, it is not found in the vagina of infected patients [16]. However, to the best of our knowledge, this is the first study that aimed to investigate the impact of the virus (direct or indirect) on vaginal microbial flora.

The purpose of our study was to identify the vaginal presence of SARS-CoV-2 and detection of predefined bacterial species changes in the vaginal flora of women that tested positive for SARS-CoV-2 infection.

MATERIALS AND METHODS

This prospective study was conducted in the Gynecology and COVID-19 Departments of Military Medical Academy, Sofia in October–December 2020. The study included a total of 40 women aged 24-47 who visited an emergency COVID-19 office of the Military Medical Academy with symptoms of mild and severe SARS-CoV-2 infection [17]. The medical history of each study participant was recorded; a general medical examination, gynecological examination, and microbiological tests were performed thereafter. A swab for the Multiplex real-time RT-PCR test intended for qualitative detection of nucleic acids from SARS-CoV-2 (TaqPath™ COVID-19 CE-IVD RT-PCR Kit, Applied Biosystems, Thermo Fisher Scientific, Life Technologies Corporation, 6055 Sunol Blvd, Pleasanton, CA 94566) was taken from the nasopharynx of every patient [18]. The women were divided into two groups depending on their nasopharyngeal PCR SARS-CoV-2 test results. The first group included 19 women who tested positive for SARS-CoV-2. The second group included 21 women who tested negative for SARS-CoV-2. During the gynecological check, a vaginal examination was performed to evaluate vaginal secretion for persistent infection according to clinical symptoms and following specifications: quantity, consistency, color, and odor. At the same time, a sample for the Femoflor® 16 REAL-TIME PCR test and a swab for the Multiplex real-time RT-PCR test intended for qualitative detection of nucleic acids from SARS-CoV-2 (TaqPath™) was taken from vagina of every study participant prior to any antibiotic treatment.

Inclusion criteria: Clinical symptoms suspected of SARS-CoV-2 infection, preserved ovarian steroidogenesis.

Exclusion criteria: Pregnant women; women taking corticosteroids, antibiotics, imidazoles, probiotics or vaginal medications in the last month; immunocompromised patients; and those with autoimmune diseases, endocrine diseases, or diabetes, contraception HIV infection and the researched and established sexual transmitted diseases (STD).

The patients were provided with information regarding the purpose of the study and the inclusion/exclusion criteria; all patients provided informed consent for participation in the study. Ethical approval (№ 27/12 Nov. 2020) was sought and granted by the Ethical Review Board of Military Medical Academy, Sofia.

A sample of vaginal secretion was collected with a dry sterile swab for microbiological testing with a Femoflor 16® REAL-TIME PCR Detection Kit (DNA-Technology Research & Production, LLC, Moscow, Russia) to detect vaginal microbiota changes, during vaginal examination from the back vaginal vault [19]. The sample was then transferred to plastic tubes containing 300 µl of physiological saline solution or in tubes containing the “DNA-Technology” PREP-RAPID DNA Extraction Kit (P-001/1EU) solution, according to the manufacturer’s instructions [19]. Overall time from the sample intake until analysis did not exceed 24 hours at storage temperatures between 2 °C and 8 °C. The Femoflor® Real-time PCR Kit is a qualitative in vitro nucleic acid test and uses one biological sample for quantitative assessment of the total bacterial mass, urogenital normoflora-lactobacilli, combinations of aerobic and anaerobic microorganisms typically found in the urogenital tract of women, mycoplasma, and fungi in the Candida genus, involved in the development of dysbiotic processes in urogenital microbiocenosis [19]. The test results reveal information about the total vaginal bacterial mass by measuring *Lactobacillus spp.; Enterobacterium spp.; Streptococcus spp.; Staphylococcus spp.; Gardnerella vaginalis/Prevotella bivia/Porphyromonas spp.; Eubacterium spp.; Sneathia spp./Leptotrichia spp./Fusobacterium spp.; Megasphaera spp./ Veillonella spp./Dialister spp.; Lachnobacterium spp./ Clostridium spp.; Mobiluncus spp./Corynebacterium spp.; Peptostreptococcus spp.; Atopobium vaginae;*

Mycoplasma hominis; Mycoplasma genitalium; Ureaplasma (urealyticum + parvum); Candida spp., and T. vaginalis; N. gonorrhea vaginal colonization [19].

Diagnostic sensitivity of Femoflor: 97%. Diagnostic specificity of Femoflor: 97% [19].

Statistical methods

The Chi-square test was used to evaluate independent variables. The result was considered statistically significant at $p < 0.05$.

RESULTS

None of the women in this study were tested positive for vaginal presence of SARS-CoV-2. Of the 40 women enrolled in our study, 3 (7.5%) were found to have a sexually transmitted disease (exclusion criteria) with T. vaginalis (1/2.5% from the SARS-CoV-2-positive group and 2/5% from the SARS-CoV-2-negative group); therefore, they were excluded from the study.

Consequently, we presented and discussed the results for 37 patients: 18 (48.6%) in the first SARS-CoV-2-positive group and 19 (51.4%) in the second SARS-CoV-2-negative group. None of the women tested positive for other sexual transmitted diseases (*Mycoplasma genitalium; N. gonorrhea*) after the Femoflor®-16 vaginal test.

The vaginal microbiota was found to be disturbed in 8 (21.6%) patients: 3 (8.1%) from the SARS-CoV-2-positive group and 5 (13.5%) from the SARS-CoV-2-negative group; the remaining 29 (78.4%) women showed normal vaginal flora dominated by Lactobacillus spp. Although a difference in vaginal microbiota disturbances was observed between the two groups, the results were not statistically significant ($p \geq 0.05$).

Table 1. Patient distribution based on nasopharyngeal and vaginal SARS-CoV-2 RT-PCR, and STD test results

All patients included n-40/100%	Vaginal SARS-CoV-2 positive n (%)	Vaginal SARS-CoV-2 negative n (%)	STD positive n (%)	STD negative n (%)
nasopharyngeal SARS-CoV-2 positive	0 (0)	19 (47.5)	1 (2.5)	18 (45)
nasopharyngeal SARS-CoV-2 negative	0 (0)	21 (52.5)	2 (5)	19 (47.5)
Total	0 (0)	40 (100)	3 (7.5)	37 (92.5)

Table 2. Patient distribution based on SARS-CoV-2 RT-PCR test results and vaginal microbiota status.

All patients included n-37/100%	Normal vaginal microbiota n (%)	Disturbed vaginal microbiota n (%)	Total n (%)
SARS-CoV-2 positive	15 (40.6)	3 (8.1)	18 (48.7)
SARS-CoV-2 negative	14 (37.8)	5 (13.5)	19 (51.3)
Total	29 (78.4)	8 (21.6)	37 (100)

*X2 (1, n = 37) = 0.5078, p = 0. 47608. *This statistic is used for all vaginal microbiota changes and all group I/group II patients

Table 3. Microbial findings of patients from the first and second groups with disturbed vaginal microbiota.

Patients with disturbed vaginal microbiota	Vaginal infections – patients n (%)	Detected microbial species – patients n (%)
n-3 (8.1%) SARS-CoV-2 positive	2 (5.4) Obligate anaerobes	1 (2.7) *Gardnerella vaginalis/ Prevotella bivia/Porphyromonas spp.*
		1 (2.7) *Mixed anaerobe infection: Gardnerella vaginalis/Prevotellabivia/Porphyromonas spp.; Peptostreptococcus spp.; Mycoplasma hominis; Lachnobacterium spp./ Clostridium spp.*
	1 (2.7) Candida spp.	1 (2.7) Candida albicans
n-5 (13.5%) SARS-CoV-2 negative	2 (5.4) Obligate anaerobes	1 (2.7) *Mixed anaerobe infection: Gardnerella vaginalis/Prevotellabivia/Porphyromonas spp.; Peptostreptococcus spp.; Mycoplasma hominis; Lachnobacterium spp./ Clostridium spp.*
		1 (2.7) *Atopobium vaginae*
	1 (2.7) Streptococcus species	1 (2.7) *Streptococcus species*
	2 (5.4) Mixed vaginal infections	2 (5.4) *C. albicans; Gardnerella spp.; Peptostreptococcus spp.; Ureaplasma spp.*
Total	8 (21.6)	8 (21.6)

Microbial species in patients with impaired vaginal microbiota presented at Table 3, were not significantly different between the two groups. There were the prevalence of obligate anaerobes, Candida spp, and mixed infections.

DISCUSSION

The world's most common pathogens that infect humans are viruses. The mechanism underlying viral infections includes promotion and suppression and other less-understood pathophysiological steps [20]. Many studies suggest mutual interactions between viruses and the human microbiota [7, 20-25]. Normal Lactobacillus spp. dominated the microbiota and could prevent and suppress some of the sexually transmitted and other viral infections through distinct mechanisms, such as competitive adhesion, interactions with the local immunity and plasminogen-plasmin system, and production of lactic acid, hydrogen peroxide, and antibacterial substances [2, 3, 20-23]. There is evidence that disturbance of the vaginal microbiota potentiated sexually transmitted virus infections such as HIV, HSV and HPV infections; however, it is still unclear whether these microbial disturbances are a result of local virus infections or they arise as a consequence of a different stimulus [23-25]. In this study we did not find vaginal presence of SARS-CoV-2 in none of the women even at those 3 (8.1%) with disturbed vaginal flora, nasopharyngeal SARS-CoV-2 positive patients. From this fact, we can make an assumption that normal Lactobacillus spp. dominated vaginal microbiota, have no impact on the vaginal invasion of SARS-CoV-2 in COVID-19 positive patients.

Microbial species detected in patients with impaired vaginal microbiota in both groups were almost the same. These were the anaerobes and Candida spp isolated most often in women with impaired vaginal flora. From these results, it can not be concluded that patients positive for SARS-CoV-2 have more often than negative, infection of a certain microbial species.

Some studies did, while others did not, report a vaginal presentation of SARS-CoV-2 in infected women [15, 16, 26]. Therefore, it is still unclear whether SARS-CoV-2 is found in the vagina of infected women; moreover, clarification is required regarding the type of impact of the virus on the normal vaginal microbiota, the circumstances and factors that facilitate the vaginal entry of this virus (direct or indirect), and whether COVID-19 can be transmitted sexually [27].

A limitation of our study is the small number of patients included. Similar randomized studies are needed to establish the vaginal presentation of SARS-CoV-2 in infected women and effect of SARS-CoV-2 infection on the vaginal microbiota.

In conclusion, even though it is still unclear whether SARS-CoV-2 invades the vagina of infected women, there is no significant evidence suggesting that it causes a more frequent disturbance in the vaginal microbiota of infected women when compared to that in healthy women.

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ORIGINAL ARTICLE

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SEROPREVALENCE OF IGG ANTIBODIES AGAINST SARS-COV-2 N PROTEIN AMONG VACCINATED AND UNVACCINATED SUBJECTS IN LAHORE, PAKISTAN

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Abstract. *The spread of SARS-CoV-2 represented a global health crisis. On May 5, 2023, the chief of the World Health Organization (WHO) officially announced the end of COVID-19 as a global health emergency. Serological assays can identify previously infected SARS-CoV-2 individuals, even if they did not go for testing while acutely ill. The current study sought to detect antibodies directed against the nucleocapsid of SARS-CoV-2 (IgG against the SARS-CoV-2 N protein) in both vaccinated and unvaccinated COVID-19 individuals. Of the 100 participants, 53 and 47 were vaccinated and unvaccinated, respectively. The vaccination status of the cohort based on gender data indicates that 41 (41%) of all participants were vaccinated males, whereas 12 (12%) were vaccinated females. We found that 42 (42%) were unvaccinated males and 5 (5%) were unvaccinated females. Of 53 vaccinated subjects, 42 and 11 participants were positive and negative for IgG against the SARS-CoV-2 N protein, respectively. Of 47 unvaccinated participants, 28 and 19 were positive and negative for IgG against the SARS-CoV-2 N protein, respectively. The average of S/P "Sample/Positive control" percentages, which correlate to levels of IgG against SARS-CoV-2 N protein, were significantly higher among the vaccinated patients (73.8%) as compared to non-vaccinated patients (57.1%), with p = 0.02. There was a downward trend in levels of IgG against the SARS-CoV-2 N protein with increasing age, except for the 60–69 age group.*

Key words: *SARS-CoV-2 N protein, COVID-19, SARS-CoV-2*

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INTRODUCTION

The emergence and spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to the coronavirus disease-2019 pandemic (COVID-19) [1]. On May 5, 2023, the chief of the World Health Organization (WHO) officially announced the end of COVID-19 as a global health emergency: https://news.un.org/en/story/2023/05/1136367. Antibody tests are useful diagnostic assays that are mainly applied to patients in their later disease courses and to people who have been infected with the virus in the past [2, 3]. Serological assays could help in identifying previously

infected COVID-19 individuals, in spite of the fact that they did not go for testing while acutely ill [4]. Serologic testing helps in determining immunity, stratifying vaccine receipt individuals, and documenting vaccine response, which can provide details about return-to-work status and travel decisions [2, 3]. They also have a pivotal role in the evaluation of the epidemiology at local, national, and global levels [5, 6].

Serological assays normally depend on the detection of antibodies against nucleoprotein (N) or spike protein (S) because these are highly immunogenic SARS-CoV-2 proteins [7]. The Spike protein is present on the SARS-CoV-2 surface, and it has the ability to attach to ACE-2 receptors. It is a good sign of a strong immune system to detect antibodies against spike protein because anti-spike protein antibodies were found to have neutralizing effects in vitro [8, 9]. A recent study, which detected both anti-N and anti-S antibodies, found an average seropositivity duration with anti-N assay specificity comparable to anti-S [10].

COVID-19 infection can elicit antibodies against nucleoprotein and spike protein [9, 11, 12], of which, based on animal virus-challenge research, the spike protein-specific antibodies were neutralized and lined with a protected immune response [13, 14]. All of the approved COVID-19 vaccines are able to produce strong antibody responses [15]. Although there is not enough data about the persistence of antibodies induced by the effects of vaccines, infection-induced neutralizing antibodies can be detected for an average of six months after the onset of symptoms [16].

Spike-specific and neutralizing antibodies showed a remarkable rise in people who took a single shot of vaccine after SARS-CoV-2 infection, which notably surpassed levels observed with only COVID-19 infection. In fact, COVID-19 patients who are recovered and vaccinated could be the best COVID-19 convalescent plasma donors [17]. Researchers also found that the production of antibodies is higher in severe diseases than in mild ones. Previous infections and vaccination are associated with a low rate of COVID-19 infections [18-20]. The majority of previous studies examined the antibody response to the spike protein of SARS-CoV-2 [21-24]. In the present study, we aim to measure and assess the antibody level produced against the SARS-CoV-2 nucleocapsid protein in both vaccinated and unvaccinated COVID-19 subjects.

MATERIALS AND METHODS

Participant blood samples

We recruited 100 volunteers at the Institute of Microbiology, University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan. Human serums from all 100 participants were collected in plastic serum separator gel blood collection tubes on April 9, 2021. Age, sex, and vaccination status were recorded for all participants. All subjects declared that they did not have any current physically identifiable COVID-19 symptoms at the time of sample collection. All participants in this study were Pakistanis and took two doses of the Sinopharm COVID-19 vaccine.

ELISA Procedure

The IgG antibody level produced against the nucleocapsid (N) protein of SARS-CoV-2 (IgG against SARS-CoV-2 N protein) in human serum was detected using indirect semi-quantitative ELISA (the ID-Screen® IgG against SARS-CoV-2 N protein indirect kit, Innovative diagnostic (ID), France). Optical densities (OD) were read and recorded at 450nm. The plates were read within 30–40 minutes as a way to avert loss of optical density.

The S/P ratio “Sample/Positive Control” for each sample was calculated and expressed as a percentage (S/P %):

$$\times\ 100 : \frac{\text{OD (Sample)-OD(Negative Control)}}{\text{OD (Postive Control)-OD (Negative Control)}} \times 100$$

As indicated by the kit, it is recommended to keep the threshold at S/P% > 60% to indicate positive for IgG against the SARS-CoV-2 N protein.

Statistical analysis

Statistical analysis was done using GraphPad Prism. Age was classified into the following five groups: 20-29 years old, 30-39 years old, 40-49 years old, 50-59 years old, and 60-69 years old. Seropositivity outcomes were analyzed in the different groups. The Student T test was used to assess the statistical significance.

RESULTS

Gender-wise vaccination status

Of 100 participants, we found that 83 (83%), and 17 (17%) were males and females, respectively. Out of the 100 participants, 53 were vaccinated and 47 participants were unvaccinated, respectively. All 53 vaccinated subjects confirmed that they received full COVID-19 vaccination shots. The subjects in each age group were as follows: 16 subjects in the 20-29 age group, 25 subjects in the 30-39 age group, 23 subjects in the 40-49 age group, 32 subjects in the 50-59 age group, and 4 subjects in the 60-69 age group. The vaccination status of the cohort based on

gender data indicates that 41 (41%) of all participants were vaccinated males, whereas 12 (12%) were vaccinated females (Figure 1). 42 (42%) were unvaccinated males, and 5 (5%) were unvaccinated females. Figure 2 shows the distribution of vaccination status and gender among all participants.

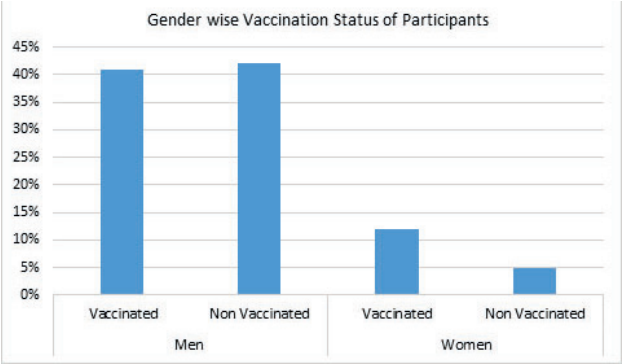

Fig. 1. Vaccination status of the cohort based on gender data

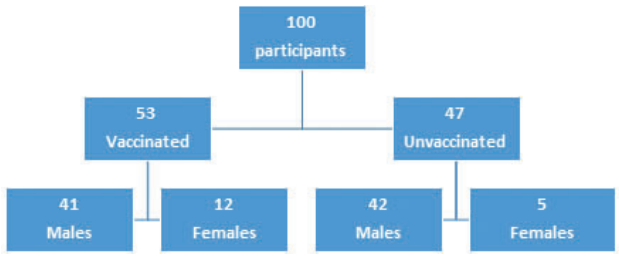

Fig. 2. Distribution of vaccination status in relation to gender among all participants

Antibody level, vaccination status, and age groups

Among all participants, 70 (70%) were positive for IgG antibodies against the SARS-CoV-2 N protein. Out of 53 vaccinated subjects, 42 and 11 participants were positive and negative for IgG antibodies against the SARS-CoV-2 N protein, respectively. Out of 47 unvaccinated participants, 28 and 19 were positive and negative for IgG antibodies against the SARS-CoV-2 N protein, respectively. Figure 3 shows the distribution of vaccination status in relation to IgG against the SARS-CoV-2 N protein among all participants. This study found that 11 out of 53 (20.7%) vaccinated participants were negative for IgG antibodies against the SARS-CoV-2 N protein, whereas 19 out of 47 (40.4%) unvaccinated participants were negative. This showed that 79.3% and 59.6% of vaccinated subjects and unvaccinated participants were positive, respectively. The average of S/P percentages, which correlate to IgG against SARS-CoV-2 N protein, were significantly higher among vaccinated patients (73.8%) as compared to non-vaccinated (57.1%), with p = 0.02 (Figure 4). There was a downward trend in IgG antibodies against the SARS-CoV-2 N protein with age, except for the 60-69 age group (Figure 5). No significant difference was detected among all the age groups with p > 0.05.

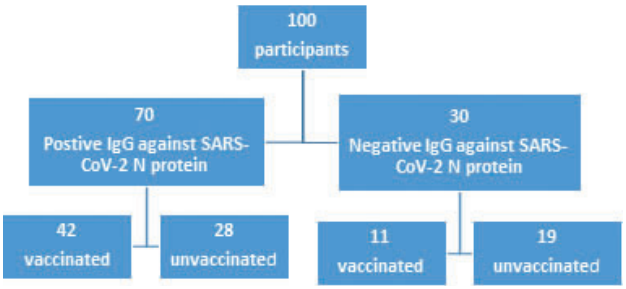

Fig. 3. Distribution of vaccination status in relation to IgG against SARS-CoV-2 N protein among all participants

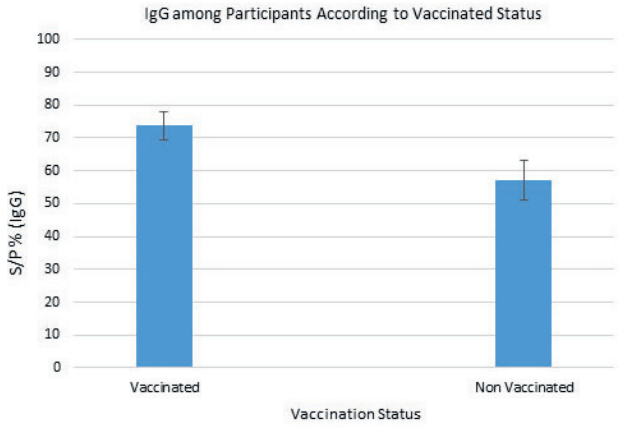

Fig. 4. The graph shows the IgG (S/P%) among vaccinated and non-vaccinated study participants. The IgG (S/P%) was significantly higher among the vaccinated patients as compared to the non-vaccinated, with p = 0.02

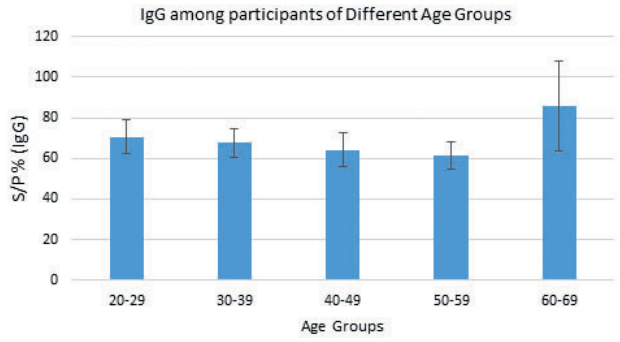

Fig. 5. The graph shows the IgG antibodies among the participants distributed among different age groups. There was no significant difference among all the age groups with p > 0.05

DISCUSSION

There is no conclusive information about antibody responses after COVID-19 infection [25-27], or among

COVID-19-vaccinated subjects [28-30]. IgG against SARS-CoV-2 N protein were detectable in the serum of infected COVID-19 people [31]. Our study reported that 28 out of 47 (59.6%) unvaccinated subjects were positive for IgG against the SARS-CoV-2 N protein, implying that they had been infected with COVID-19 before. These 28 unvaccinated individuals positive for IgG against the SARS-CoV-2 N protein did not give conclusive answers as to whether they had been infected with COVID-19 before or not. Previous study found that 76% (367/484) of recovered COVID-19 patients were positive for IgG against the SARS-CoV-2 N protein [32]. Our study found that 19 out of 47 (40.4%) unvaccinated subjects were negative for IgG against the SARS-CoV-2 N protein, giving two possibilities. The first is that they have not been infected with COVID-19 before. The second one is that they may have had COVID-19 a long time ago and the antibodies have waned. These 19 unvaccinated individuals, negative for IgG against the SARS-CoV-2 N protein, did not also give conclusive answers as to whether they had a previous infection with COVID-19 or not. A recent study reported that the rate of seroreversion at six months in mildly symptomatic or asymptomatic patients is higher than in symptomatic COVID-19 patients [33]. Waning antibody levels in previously infected COVID-19 patients have been previously reported [34], and this could make them prone to reinfection again.

We found that 11 vaccinated participants were negative for IgG against the SARS-CoV-2 N protein, which could be due to taking vaccines for more than 6 or 12 months prior to conducting the experiment. Subsequent antibody waning after vaccine administration is expected, increasing the possibility of reinfection if “booster” doses are not taken [35]. Seroprevalence generally indicates the number of patients who had values greater than the sensitivity threshold of the serological test used in the specific research and does not indicate the real number of patients who have totally lost their immunity or antibodies [34, 36]. A recent study reported a decline in antibody levels at twelve weeks and six months after vaccine administration, implying that the immunity waned with time [37], and this underscores the urgent need for monitoring possible booster vaccines.

Dong and colleagues reported that there is an inverse correlation between IgG titers to COVID-19 infection and age for those older than 18 years of age but a direct correlation with age in adults [38]. Moreover, 44-66-year-old adults are likely to have high IgG values, compared to younger adults (18-44-year-olds), within six months following the onset of symptoms, yet there is no difference seen at twelve months after the onset of symptoms [39]. We noticed that there was a downward trend in IgG against the SARS-CoV-2 N protein with age, except for the 60–69 age group. The reason for the high level of IgG against SARS-CoV-2 N protein in the 60-69 age group could be due to the fact that only 4 subjects participated in this study, and 3 out of them were positive (S/P % > 90%). Seroprevalence in 65-year-olds or older persons was usually lower than in 18- to 49-year-old persons [40]. Previous study found that the SARS-CoV-2 IgG values exhibited a positive correlation with age in adults and an inverse correlation with age in pediatric populations [41]. The strongest point in our data is that vaccinated people had higher levels of antibodies compared to unvaccinated people. Previous study reported that there were high levels of antibodies in 98% of the vaccinated cohort [42]. A recent study found that 46% of patients with hematological malignancy who received two mRNA vaccine doses did not produce SARS-CoV-2 IgG and were therefore vaccine non-responders [43].

The main strength of our study includes a clear indication of the immune responses and protection level against COVID-19 through measuring the IgG antibodies among vaccinated and non-vaccinated COVID-19 subjects. However, the present study also has limitations. First, we do not have the timeline of COVID-19 patients or vaccinated COVID-19 people. This means that we do not know how long they had COVID-19 infection or were vaccinated, or whether they had the vaccine before or after COVID-19. Previous studies showed that spike-targeting and neutralized antibodies showed a remarkable rise in people who administered a single vaccine shot after COVID-19 infection, which notably surpassed values seen in people who had only COVID-19 infection. Second, the sample was also limited to volunteers at the Institute of Microbiology at the University of Veterinary and Animal Sciences (UVAS) in Lahore and cannot give a clear picture of the Lahore population or the wider population of Pakistan. Third, there was underrepresentation of the female population, and conclusions on gender differences in both infection and vaccine responses require more studies. The study focused on measuring the IgG antibody level against nucleocapsid (IgG against SARS-CoV-2 N protein). In future studies, IgG antibodies targeting the SARS-CoV-2 spike, IgM antibodies, and viral nucleic acids should be assessed to study the kinetics of the SARS-CoV-2 prevalence.

CONCLUSION

The average of S/P “Sample/Positive control” percentages, which correlate to levels of IgG against

SARS-CoV-2 N protein, were significantly higher among the vaccinated patients (73.8%) as compared to the non-vaccinated (57.1%), with p = 0.02. There was a downward trend in levels of IgG against the SARS-CoV-2 N protein with age, except for the 60-69 age group.

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Author contributions: *AD and HB conceived and designed the study. AD, MFS, MN, RS coordinated, carried out the experiments, and analyzed the data. AD drafted the original manuscript. AD, MFS, RS, AJ, MN, TY, and HB did necessary editing of the manuscript. All authors read and approved the manuscript.*

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ORIGINAL ARTICLE

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CANALIS BASILARIS MEDIANUS AS AN ANATOMICAL VARIATION IN THE BASILAR PART OF THE OCCIPITAL BONE: A DESCRIPTIVE CONE BEAM COMPUTED TOMOGRAPHIC STUDY

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Abstract. Objectives: *Canalis basilaris medianus (CBM) is a unique anatomical variation located in the basal occipital region of the skull, rarely encountered in head and neck radiographic imaging. The aim of the present study was to evaluate the prevalence and types of CBM using Cone Beam Computed Tomography (CBCT) scans.* ***Materials and Methods:*** *CBCT (Full FOV) images of 200 patients aged between 10 to 70 years were selected for the current retrospective study following the inclusion and exclusion criteria. The image sections from the scan data were scrutinized for the presence of CBM, in addition to its classification based on the type of morphology. The presence and types of CBM were recorded based on the age and gender. The chi-square test was used to analyze the presence and types of CBM with regard to gender and age group.* ***Results:*** *The overall prevalence of CBM was estimated as 9.5%. CBM was present in 13% of males and 3% of females ($p = 0.021$). Considering the types of CBM, the superior recess type was predominantly observed followed by the inferior recess, superior and inferior type. However, there was no significant gender-based differences noted among the types of CBM ($p > 0.05$). Also, there was no statistically significant difference noted in the prevalence of CBM in different age groups ($p > 0.05$).* ***Conclusion:*** *It is necessary for maxillofacial radiologists to have a solid understanding of both normal and variant skull-base anatomy to facilitate recognition of variants such as CBM in order to recognize the associated anomalies. To our knowledge, this was the first study done which assesses the gender-based differences among the various types of CBM.*

Key words: *Canalis basilaris medianus, Skull base, Anatomic variation, Cone Beam Computed Tomography*

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INTRODUCTION

The anatomy of the skull has been studied extensively in the past centuries and many anatomical variations of the skull have been identified and well documented in the scientific literature [1, 2]. Minor variations within the parts of the cranial cavity, particularly in the basal occipital region, have been of great interest to anatomists and clinicians due to their neurological importance. Apart from the common foramina of the skull base, there are several other, rarely found foramina that have been identified as anatomical variations. This includes canalis basalis medianus (CBM), foramen meningo-orbitale, craniopharyngeal canals, palatovaginal canals, foramen of Vesalius and canaliculus innominatus [3, 4]. The majority of these variations result from the typical developmental process and their presence is primarily determined genetically [5].

CBM is unique anatomical variant that can be described as a well-defined, corticated canal structure in the basilar part of the occipital bone (basiocciput). CBM is an extremely rare variant that is encountered during head and neck imaging [6]. Computed Tomography (CT) is regarded as the best modality for imaging of osseous structures of the head and neck region [7]. However, the introduction of Cone Beam Computed Tomography (CBCT) in the field of maxillofacial imaging has led to a revolutionary change from two-dimensional (2D) to three-dimensional (3D) imaging due to its advantages over conventional radiography and medical CT. CBCT with typically large Field of View (FOV) can aid in the imaging of significant structures of the skull with diagnostic efficacy comparable to CT, along with the advantage of low radiation dosage, wide availability and cost effectiveness superior to CT [8].

Improved knowledge of dental practitioners in the identification of significant anatomic landmarks of the maxillofacial region has contributed to a rise in the incidental findings which are detected accidentally during routine radiographic examinations. Though CBM is a well-recognized anatomical variant, studies evaluating its morphology and prevalence are sparse. Hence, the present study is aimed to evaluate the radiographic characteristics of CBM and its prevalence using CBCT among general population.

MATERIALS AND METHODS

The present retrospective study was carried out in accordance with the principles of the Declaration of Helsinki. The images of large-volume CBCT scans were collected from the patients who reportedly underwent radiographic investigation in the Department of Oral and Maxillofacial Radiology, AB Shetty Memorial Institute of Dental Sciences, Nitte (Deemed to be University), Mangalore, in the period of January 2021 to December 2022. Full FOV CBCT volumes and clinical data of patients were utilized for the study based on the selection criteria. Our study included a total of 226 CBCT volumes that belonged to patients aged 10 to 70 years, taken with good diagnostic quality for a variety of maxillofacial indications. However, a total of 26 radiographs were excluded due to a lack of image clarity, poor visibility of the clivus as a consequence of the superimposition of artefacts and evidence of surgical interventions over the region of interest. Radiographs of subjects with a documented history of any syndrome, neurovascular diseases and trauma were also excluded from the study.

Image acquisition

All the CBCT volumes included in the study were procured using Promax 3D Mid model CBCT unit (Planmeca, Helsinki, Finland). The radiographs were taken under standard imaging protocols by a trained radiographer with a varying exposure parameter of 8 to 10 mA (tube current); 80 to 90 kVp (tube voltage) and an average exposure time of 27 seconds over a rotation of 360 degrees, depending on the built of the patient. The resultant voxel sizes of the radiographic images ranged from 200 µm to 400 µm. All the radiographs were obtained with a standardized head position (the Frankfort horizontal plane placed parallel to the floor), relaxed lips position and teeth in occlusion. The Scan data were studied in a full screen monitor using Planmeca Romexis software (Version 4.6.2) by two independent Oral and Maxillofacial Radiologists with a minimum of 10 years of clinical and radiological experience. Both radiologists examined no more than 10 longitudinal sets of the CBCT scans at a time to reduce bias caused by visual fatigue. In order to guarantee optimal viewing, the brightness, as well as the contrast of the radiographic images was modified with the aid of the software processing tool. Radiographic evaluation was done based on criteria such as gender, age, presence of CBM and its morphological types. There were no significant inter-examiner discrepancies in the radiographic interpretation among the evaluating radiologists.

Image analysis and interpretation

The obtained scan data were carefully inspected at different levels under appropriate lighting for the presence of CBM by scrolling across the radiographic images. Sagittal sections revealed the

presence of CBM in the form of a well-corticated osseous defect in the basi-occipital region of the clivus. Following the determination of the incidence of CBM, the CBCT sections were evaluated for the following six morphological types as described by Currarino [6]:

1. The complete variants of CBM, such as (a) inferior (Figure 1A), (b) superior (Figure 1B) and (c) bifurcating types have open ends on both sides.
2. The incomplete variants of CBM, such as (d) inferior basi-occiput recess (Figure 2A), (e) superior basi-occiput recess (Figure 2B) and (f) long channel in the basi-occipital region have an open end and a blind end on each side [6, 9].

Statistical Analysis

The presence and types of CBM were recorded based on the age and gender. The collected data were entered in Microsoft Excel-2010 and statistical analysis was carried out using the Statistical Package for Social Sciences software (IBM, Armonk, NY, USA), Version 26. The categorical data were represented as percentages. The chi-square test was used to analyze the presence and types of CBM with regard to gender and age group. A p-value of 0.05 was considered as statistically significant.

RESULTS

The analyses included a total of 226 CBCT scans and 200 were selected based on the inclusion and exclusion criteria. The mean age of the subjects was 34 ± 16.2 years with an age range of 10 to < 70 years. The scan volumes that were included in our study belonged to 131 males and 69 females. Table-1 displays the frequency of CBM based on gender and age groups. Out of 200 CBCT scans, 19 (9.5%) showed CBM, of which 17 (89.5%) belonged to males and 2 (10.5%) belonged to females. The CBCT scans were divided into five age groups, with group A including 10 to 20 years (18.5%), group B including 21 to 30 years (27%), group C including 31 to 40 years (20.5%), group D including 41 to 50 years (15%) and group E including 51 to 70 years (19%) of age. There was a statistically significant relationship between the occurrence of CBM with gender as we observed a male predominance (p =

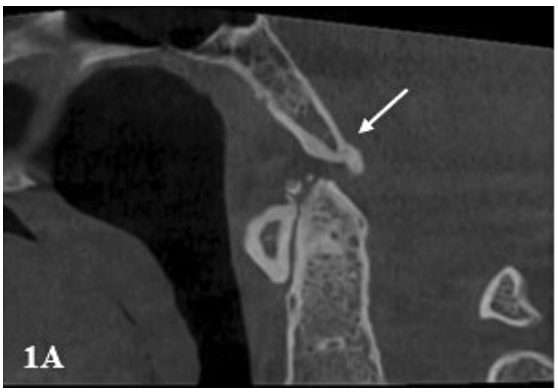

Fig. 1A. Sagittal (CBCT) section demonstrating the inferior type (complete variant) of CBM in the basi-occipital region

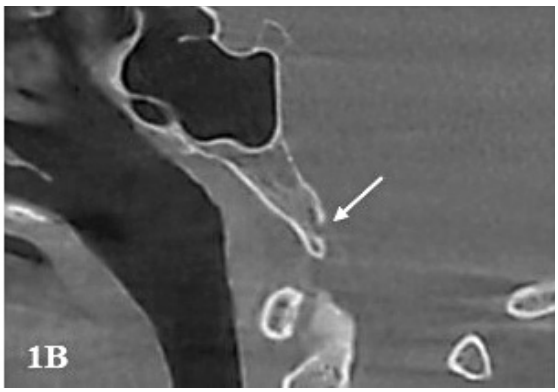

Fig. 1B. Sagittal (CBCT) section demonstrating the superior type (complete variant) of CBM in the basi-occipital region

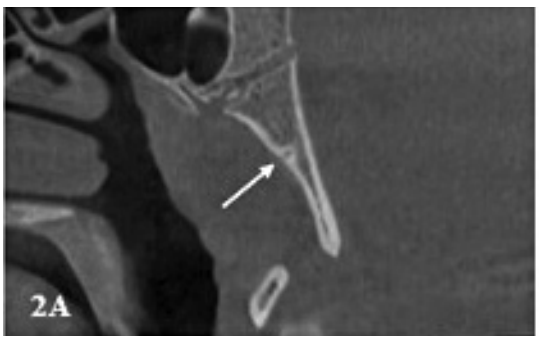

Fig. 2A. Sagittal (CBCT) section demonstrating the inferior recess type (incomplete variant) of CBM in the basi-occipital region

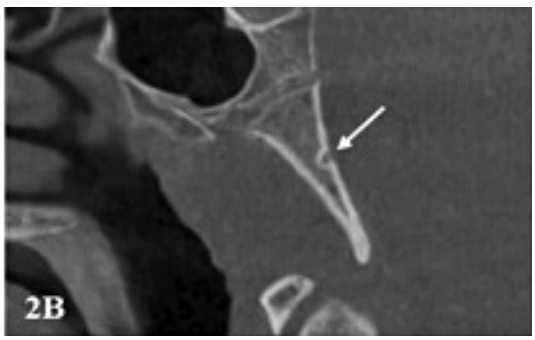

Fig. 2B. Sagittal (CBCT) section demonstrating the superior recess type (incomplete variant) of CBM in the basi-occipital region

0.021). On comparison of the presence of CBM in the different age groups, the present study showed the highest prevalence in the age group of 21 to 30 years (Group B) followed by 41 to 50 years (Group D), 10 to 20 years (Group A), 31 to 40 years (Group C) and 51 to 70 years (Group E), respectively (Figure-3). However, there was no significant relationship ($p > 0.05$) between the occurrence of CBM among the different age groups. On observing the various morphological types of CBM, the superior recess type (incomplete variant) was the most predominant among males followed by Inferior recess (incomplete variant). The complete variants of CBM such as superior and inferior type, were noted one in each among the males. Out of two females, both had superior recess type (incomplete variant) of CBM. Other types of CBM such as the bifurcating (complete variant) and channel type (incomplete variant) were not found among the scan volumes that were studied. However, there was no statistical significance noted among the different types of CBM ($p > 0.05$) (Table 2, Figure 4).

Table 1. Frequency of CBM based on Gender and Age groups

	Canalis basilaris medianus		Total	p-value
	Present	Absent		
Gender				
Male	17 (12.9%)	114 (87%)	131	0.021
Female	2 (2.9%)	67 (97.1%)	69	
AGE				
Group A 10-20 years	4 (10.8%)	33 (89.2%)	37	0.455
Group B 21-30 years	7 (12.9%)	47 (87%)	54	
Group C 31-40 years	3 (7.3%)	38 (92.7%)	41	
Group D 41-50 years	4 (13.3%)	26 (86.7%)	30	
Group E 51-70 years	1 (2.6%)	37 (97.4%)	38	

Table 2. Comparison of various types of CBM based on Gender

	Type of CBM				p-value
	Inferior	Superior	Inferior Recess	Superior Recess	
Male	1	1	7	8	0.570
Female	0	0	0	2	
Total	1 (5.3%)	1 (5.3%)	7 (36.8%)	10 (52.6%)	19

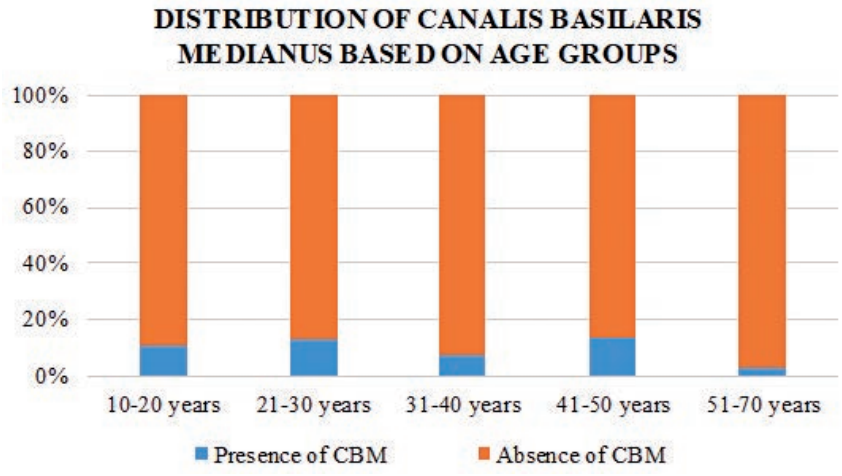

Fig. 3. Bar graph illustrating the distribution of various types of CBM based on age groups

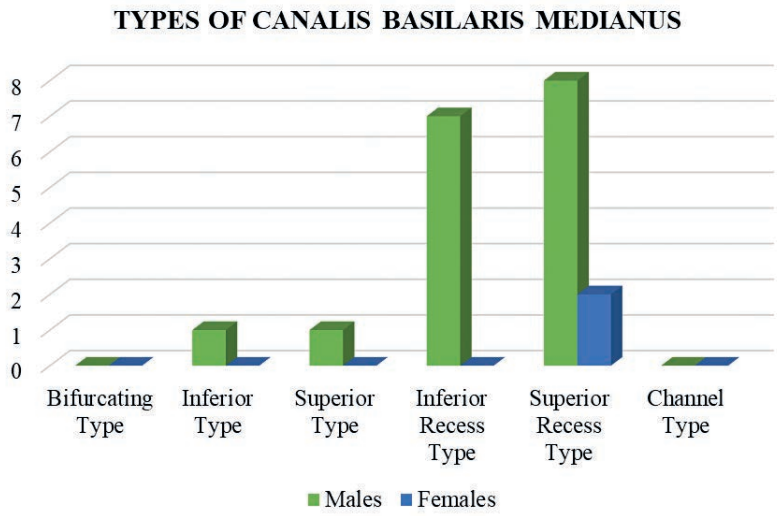

Fig. 4. Bar graph illustrating the distribution of various types of CBM among males and females

DISCUSSION

Over the last few decades, the relationship between anomalies of the clivus and their clinical impact has been gaining interest among various pathologies that are associated with the head and neck region. The clivus is a component of the cranial base that is formed by the body of the sphenoid bone and the basilar part of the occipital bone, connected by the spheno-occipital synchondrosis. It is situated along the anterior part of the occipital bone, sloping downwards from the dorsum sellae. Though clivus is a small part of the cranium, numerous anatomical variations are known to exist in the structure of the clivus, out of which fossa navicularis magna, craniopharyngeal canal and CBM are identified as the most prominent variants [10, 11].

In spite of the fact that a majority of individuals with CBM are asymptomatic, the clinical implication of this skeletal anomaly is controversial. However, considering its serious implications as a disseminating pathway for the spread of various pathologies from the pharyngeal to the intra-cranial region, clinicians should be aware regarding its identification using the routine maxillofacial imaging modalities such as CBCT which facilitates the multiplanar assessment of the craniofacial structures including the base of the skull region [12]. Sagittal CBCT sections are considered as the best plane for the demonstration of CBM along the base of skull [13]. Our study was undertaken primarily to assess the prevalence of CBM and their types using CBCT scans among the general population who reported to a tertiary dental hospital.

Origin of CBM

CBM which is considered as one of the incidental findings of the skull radiographs, was described as early as 1880 by Grubber in dry skulls. To date, the origin of CBM has been explained on the basis of two theories: 1. Theory of vascular origin which states that the formation of CBM is due to the persistent cranial vessels including the emissary veins. 2. Theory of notochordal origin which discloses the formation of CBM as a result of post-natal continuance of the canalis chordae (remnant of the notochord) [6].

Prevalence of CBM

The overall prevalence of CBM in our study population, aged between 10-70 years, was found to be 9.5%. Our finding is higher than the rates documented in previous literature reports, where Serindere et al. [14] reported a prevalence of 4% and Akkoca et al. [15] reported a prevalence of 4.3% both among the Turkish population. Abdolmaleki et al. reported a prevalence of 5.4% among the Iranian population [16]. Another study conducted in a South-Asian population had reported a prevalence of 5.33% [13]. The least prevalence of CBM reported by Bayrak et al. was 2.5% among the Turkish population [17]. Variations in the prevalence rates could be due to the difference in the age group and ethnicity of the population studied. In addition, radiographic factors like imaging modality and exposure parameters could also lead to variations in the identification of CBM.

In the present study, CBM was found to be more predominant in males (13%) than females (3%), with a statistically significant difference. Though there is no clear consensus reason regarding the gender-based prevalence of CBM, our findings were similar to that of Serindere et al. [14] who reported an increased prevalence among males. In addition, literature evidence report the prevalence to be higher in children than adults [6, 9]. However, we did not find any significant difference in the prevalence of CBM among different age groups. Moreover, CBM is believed to be a developmental anomaly rather than a pathologi-

cal variety, as we assume that a varied age could not be a delineating factor.

Morphological types of CBM

In the present CBCT study, we found the superior recess type of CBM to be more predominant followed by inferior recess type and both the females who exhibited CBM were of the superior recess type. Bayrak et al. [17] reported superior recess type to be more prevalent among females and Serindere et al. [14] reported inferior recess type to be more prevalent among males, which was in conformity with our study even though there was no statistical significance observed among the different types of CBM. Pasalkar et al. [13] in their study reported superior recess type to be predominant among the Indian population, which is similar to our finding. We also observed the complete variants such as superior and inferior types of CBM, but other types of CBM were not found in our study population. Despite the scarcity of literature on prevalence studies evaluating the CBM, our study was one among the few to assess the gender-based differences among the morphological forms of CBM.

Clinical significance of CBM

The clinical importance of CBM may be due to its diagnostic concern as these variations act as a potential pathway for the progression of intra-cranial infections. This anomaly of the skull base is present in the region of the sphenoid sinus, nasopharyngeal and clivus region, which are all considered as anatomical structures of clinical significance [18]. Therefore, this anatomical variant of the clivus should be considered as one of the differential diagnoses of conditions, such as iatrogenic fractures of the clivus postneurosurgical procedures and enterogenous cyst, such as meningocele. Various pathological conditions have been reported to be associated with CBM. Recurrent meningitis secondary to atypical bacterial infection has been documented to be associated with a canal type of CBM and surgical repair of the canal by grafting was considered to remove the potential passage of the infection for the prevention of recurrence of meningitis [6, 19]. CBM is known to be associated with cysts such as Tornwaldt's cyst [20] and meningocele [21]. Khairy et al. described the presence of CBM to be related with a case of cerebrospinal fluid (CSF) leak in a twenty-two old male patient who complained occurrence of chronic manifestations such as running nose and headache associated with the frontal region [22]. Previous reports suggesting the presence of an incomplete inferior recess in the basi-occipital region have also been described as an incidental finding on skull base radiographs in cases of Apert's syndrome and neurofibromatosis in children [6]. However, there is a lack of clear consensus regarding the coexistence and detrimental effects of the anatomical variant over the pathology.

Limitations and future prospects

Our study was an attempt to evaluate the prevalence of CBM among Indian population and we have found significant difference in the prevalence among males and females. However, the study has certain inherent limitations owing to its retrospective nature, as there is a lack of clinical correlation with the presence of CBM. Further prospective, large-scale clinico-radiographic studies should be advocated to facilitate a better understanding of the pathological relationship of CBM in disorders of the cranial cavity in addition to ascertaining the true prevalence of this anatomical variant of the clivus region.

CONCLUSION

In conclusion, the present study evaluated the frequency of CBM along with its morphological types which showed an increased male predilection. It is essential for maxillofacial radiologists to have a solid understanding of both normal and variant skull-base anatomy within the imaged field, such as CBM, in order to recognize the associated anomalies and to facilitate proper decisions by the clinicians especially in surgical procedures. The morphological study of the skull and its foramina can also serve as a way to compare and understand the changes in human evolution.

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Conflicts of interest: *The authors report no conflicts of interest.*

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ORIGINAL ARTICLE

EXPANSION OF PSYCHOSOCIAL DISTRESS IN NURSES WORKING IN HEMODIALYSIS DURING EXPOSURE TO COVID-19 PANDEMIC IN BULGARIA

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Abstract. *A new type of virus, SARS-CoV-2, has spread globally throughout the world. The COVID-19 epidemic rapidly spread worldwide. The health care system, society and medical professionals have prepared to adapt, train and resist the increased psychosocial pressure. In this situation, the role and place of medical professionals has become noticeably more prominent, and the psychosocial stress of the work environment has increased its impact – progressing to high levels of harmful intensity towards the medical professionals. Focusing within the borders of Bulgaria, it can be argued that the initial two waves of high levels of COVID-19 prevalence were difficult and critical to overcome. The aim of our study is to investigate the impact of psychosocial stress on the functional status examined with the response of arterial blood pressure of nurses, practicing long-term care in hemodialysis clinics and centers during exposure to COVID-19 pandemic. A study was conducted on the subjective assessment of psychological and social risk factors, and arterial systolic and diastolic blood pressure in 2018-2019 (pre-COVID-19 period) and 2020 (COVID-19 period), respectively. The methods used were the NIOSH questionnaire – to assess psychosocial stress at work and to measure blood pressure with a sphygmomanometer. The results showed that the expansion of psychosocial risks from the work environment impacted on the functional status of nurses, with an increase in the level of social support and systolic blood pressure during the COVID-19 compared to the pre-COVID-19 period, and a decrease in the level of control when comparing the same periods. We will monitor and observe these effects with concern in order to respond in a timely manner with effective and practical strategies, policies and programs to counter and prevent psychosocial stress in the workplace. One of the outlined guiding goals and trends for this will be to both preserve and prolong the work life of nurses and to reposition them as a key and significant group for the healthcare system.*

Key words: *COVID-19 pandemic, psychosocial stress, nurses, hemodialysis, arterial blood pressure*

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INTRODUCTION

The unexpected pandemic of COVID-19 has dramatically transformed our modern world. The standard and accustomed social, economic and societal order has had to stressfully adapt and synchronize with the global dynamics of a pandemic of COVID-19. A significant part of the burden and responsibility was born by nurses who worked continuously in high-risk inpatient and outpatient environments. The uncertainty, expansive dynamics, tensions and technological innovations in this pandemic environment sharply increased both individual and professional demands. Consequently, the burden of stress exposure was palpably felt and increased. Nurses were exposed to and continued to give professional care to indigent patients in an environment with a high personal health risk, with rising morbidity. They are exposed to the detrimental influence of psychosocial factors of their professional lives, which are reflected in areas such as: mental, psychological and physical health. Medical care – both qualitatively and quantitatively – has been negatively affected by persistent workplace stress and the burden of social responsibility. The significant factors for the expansion of psychosocial stress among the nursing group are numerous, but most prominent are - job strain, high intensity work and work dynamics, uncertainty and risky environment, external social pressure and perceived limitations to influence to the external factor COVID-19. As a key conclusion, it is possible to confirm in this case the theory of the increase of the harmful impact of stress from an external factor for the individuals and the society, where the possibility of impact is limited and creates an insurmountable conflict [1-3]. Herein lie the underlying sources – expansion in the scope of influence and negative impact of psychosocial stressors in the work environment, which have emerged as key among nurses practicing long-term care and life sustaining healing activities.

The aim of our study is to investigate and assess the effect of psychosocial risk factors on the functional status examined with arterial blood pressure of nurses working in hemodialysis during exposure to the COVID-19 pandemic. Two periods were compared: pre-COVID-19 (before the pandemic COVID-19: 2018 and 2019) and COVID-19 (during the pandemic situation: 2020).

MATERIALS AND METHODS

Materials

The study is observational. A study of psychosocial risk factors in medical, nephrology nurses from six hemodialysis centers and clinics was conducted from November 2018 to February 2019 – Pre-COVID-19 period. The study included 68 nurses with mean age of 42.2 (42.2 ± 10.81) from different hemodialysis centers in Bulgaria. All the 68 above-mentioned nurses from the centers situated in the cities of Burgas, Pleven, Plovdiv and Sofia took part in the study during the pre-COVID period, and 45 of them participated during the pandemic of COVID-19. In the period before the COVID-19 pandemic, 68 nurses who completed occupational stress assessment questionnaires between November 2018 and February 2019 were studied to investigate psychosocial risk factors in the work environment. During the period of the COVID-19 pandemic, the 45 nurses completed the occupational stress assessment questionnaires between May and November 2020. After examining the psychosocial factors, a study of the functional status was conducted by studying the response of arterial systolic and diastolic pressure using a sphygmomanometer. The nature of the nurses' work activity consists of permanent, specific, long-term and continuous care of patients with Chronic Kidney Failure (CKD). The psychological burden of hearing about the patient's personal experience with the illness and providing logistical support for the diseased is enormous for the nurses.

Methods

NIOSH methodology for the assessment of occupational psychosocial stress

The method for the assessment of occupational psychosocial stress was applied using the NIOSH questionnaire, which was validated and adapted for Bulgarian conditions and language [4]. The Bulgarian version of the questionnaire meets all scientific requirements and is in line with studies conducted in the field. With its help and using Karasek's model [5, 6] four groups of work situations can be identified by combining the significant psychosocial factors: job demands and control over the work process. The following scales of the psychosocial stress questionnaire were investigated: workload; job demands; control over the work process; cognitive skills; extra-work activities; social support from immediate supervisor, colleagues and family; self-esteem in relation to work; psychosomatic complaints.

The method used to assess occupational psychosocial stress is through the NIOSH questionnaire [7, 8], which analyzes and evaluates those factors in the individual's professional (work) environment, that are subjectively perceived and experienced as stressful. The methodology reflects the individual's self-assessment of the characteristics of psychosocial factors in the worker's work environment. The tool includes the following six groups of scales: work stressors; factors outside of work; individual factors;

buffering factors; short-term psychological, physiological and behavioral reactions; long-lasting psychological, physiological and behavioral reactions.

The NIOSH questionnaire is divided thematically into 8 (eight) parts, examining psychological and psychosocial factors from the working environment: 1. Assessment of control capabilities – 16 questions; 2. Assessment of social support – 4 questions with 3 directions each; 3. Assessment of job requirements – 11 questions; 4. Assessment of workload and responsibilities – 11 questions; 5. Assessment of mental requirements – 5 questions; 6. Assessment of self-esteem in relation to work – 10 questions; 7. Assessment of general health status – 17 questions; 8. Extracurricular activity – 7 questions

Self-assessment in different ratings for individual questions varies on a scale from 1 – „very little“ to 5 – „too much“. The thematic division of the NIOSH questionnaire is related to the need for a comprehensive analysis and subsequent assessment of workplace stress and related psychosocial risk factors. Subjective assessment by medical professionals under study is important and constructive in terms of statistical analysis and reliability to detect and characterize objective stressogenic catalysts.

The amount of questions and their individual and subjective assessment is pre-selected to be able to compile and analyze a personal assessment by categories to build the statistical analysis for the study of stressors.

Arterial systolic and diastolic blood pressure measurement using a sphygmomanometer

Systolic and diastolic blood pressure were measured using a sphygmomanometer [9]. The measurement of systolic and diastolic arterial pressure was in accordance with the European Classification for the Study and Reference of Arterial Pressure and the Definition of Arterial Hypertension Categories [10].

Data Analysis

The shapes of the frequency distributions were verified using the Kolmogorov-Smirnov one-sample test. Comparison between two independent groups was performed using t-test or Mann-Whitney test. The relationship between two categorical variables was examined using the Chi-square test. The level of significance assumed was $\alpha = 0.05$. The corresponding null hypothesis was rejected when the p-value is less than α. SPSS version 13.0, a specialized statistical package, was used to process the study data.

RESULTS

This article focuses on the study of the effect of psychosocial stress on functional status examined with the response of arterial blood pressure in nurses practicing long-term care in hemodialysis clinics and centers in the context of COVID-19 pandemic in Bulgaria. To accomplish the aim, a study was conducted to assess psychosocial risk factors, and arterial systolic and diastolic blood pressure in nurses from different hemodialysis centers and clinics.

This article does not focus on the full multifaceted and extensive analysis of the research conducted on the impact of psychosocial stress. The data available are in the period before the pandemic (pre-COVID-19) and are compared with data during the pandemic (COVID-19 period) in the same nurses. It is interesting to note that all nurses were female and their positive and voluntary attitude towards the present study was higher before the pandemic setting. This can be explained by high workload, increased stress in the work environment, pressure of social factors and finally low levels of positivity. Of the 68 nurses who participated in the study during the pre-pandemic period, 66% or 45 participated during the COVID-19 period. A t-test for linked samples was used to compare the mean values of the various indicators between 2018 and 2019, and 2020. The results of the analysis of psychosocial factors and arterial blood pressure are presented in Table 1.

Table 1. Mean values of psychosocial factors, and arterial systolic and diastolic pressure

Indicator	N	Mean	SD	P
Control 2018 and 2019	45	3,21	0,69	< 0,001
Control 2020	45	2,35	0,37	
Social Support 2018 and 2019	45	3,55	0,85	< 0,001
Social Support 2020	45	3,98	0,53	
Job Requirements 2018 and 2019	45	3,74	0,56	0,716
Job Requirements 2020	45	3,70	0,52	
Workload and Responsibilities 2018 and 2019	45	3,26	0,50	0,804
Workload and Responsibilities 2020	45	3,28	0,33	
Mental Requirements 2018 and 2019	45	3,27	0,69	0,050
Mental Requirements 2020	45	3,12	0,50	
Confidence 2018 and 2019	45	2,93	0,40	0,674
Confidence 2020	45	2,90	0,33	
General Health 2018 and 2019	45	1,82	0,59	0,169
General Health 2020	45	1,68	0,41	
Blood Pressure Systolic – 2018 and 2019	45	124,87	17,02	< 0,001
Blood Pressure Systolic – 2020	45	129,40	12,89	
Blood Pressure Diastolic – 2018 and 2019	45	82,42	12,31	0,799
Blood Pressure Diastolic – 2020	45	82,73	9,70	

The table shows that nurse's control had significantly lower average levels during the COVID-19 period compared to the pre-COVID-19 period (2.35 versus 3.21), $p < 0.001$. Levels of social support increased significantly in the COVID-19 period, compared to the pre-COVID time (3.98 versus 3.55), $p < 0.001$. The COVID-19 period is characterized by significantly higher systolic blood pressure levels on average in comparison to the pre-COVID-19 period (129.4 vs 124.87), $p < 0.001$. For the remaining parameters, no significant difference was demonstrated between the two periods compared ($p > 0.05$).

DISCUSSION

Exposure to COVID-19 in Bulgarian medical professionals including the nurses in our study, who practice long-term care in hemodialysis clinics and wards, has become a powerful stressor affecting and altering the functional physiological and psychological state of the individual. The results indicated that there is an expansion of the level of psychosocial stress in the COVID-19 period compared to the pre-COVID-19 period. Our findings show a significant increase in systolic blood pressure and impact on psychosocial risk factors as social support increased and at the same time the level of control decreased due to the impact of COVID-19 situation during the COVID-19 period compared to the pre-COVID-19 period. In nurses, the COVID-19 period was characterized by significantly higher systolic blood pressure levels on average. The diastolic blood pressure does not change its level in the COVID-19 period, but along with the systolic blood pressure value forms the category of normal blood pressure and normal hypertension – one degree but still higher than the optimal blood pressure and hypertension according to the European guidelines for reference values of blood pressure and hypertension level. Psychosocial risk factors also respond sensitively to exposure to the stressor risk factor, COVID-19. The nursing profession has relatively high and stable levels of control. But our study shows that nurses working in hemodialysis centers have significantly lower average levels of control during the COVID-19 period compared to the pre-COVID-19 period, which is a serious predictor of increased psychosocial strain and stress in the workplace. Levels of social support increased significantly during the COVID-19 period relative to the pre-COVID-19 one, indicating that increased levels of social support have a beneficial buffering effect on the functional state of the nurses' body by reducing and neutralizing the effects of stress inducted by COVID-19.

Understanding the importance and value of nurses' work has increased and taken its necessary place in the risk environment. Trust and support for nurses has increased and improved. Job requirements and responsibilities – by their nature have not changed, except that new and higher hygiene requirements have been added that are relevant to increasing the quality of nursing work and care. The workload and responsibilities maintain their standard levels in hemodialysis centers due to the nature of the illness of patients with CKD who require care for their underlying chronic illness. Cognitive mental demands demonstrate that the pandemic setting has not affected the level of mental demands required, according to the nurses interviewed. Despite their increased knowledge and new hygiene standards, no progression was reported on this index. Self-esteem, another psychological factor, did not show a significant change in the levels during the two periods considered, which is viewed as not a surprise, due to the traditional modesty of the Bulgarian nurse. The nurses' general state of health was subjectively evaluated as close to the levels of the pre-COVID-19 period and with no apparent change.

The strain on nurses at all levels has changed in intensity and they have had to work under new psychosocial stresses such as the COVID-19 situation. Researchers from around the world have turned their attention to this new psychosocial risk environment and results of studies conducted have shown that new high rates of burnout, post-traumatic stress syndrome and impaired mental and psychological health [11-13]. Other author teams from almost all countries have started active studies in this area because the impact and effect of this new stressor is visible and obvious. For example, Chinese research team stated that psychological stress among health care workers during the active part of the pandemic was significantly increased by 51%, and study participants possessed depression, anxiety and fear [14]. The result of our study show that the effects of workplace stress not only affect the quality of nursing work, but also are a prerequisite for strong psychological pressure on nurses who experience deep emotional conflict from the inability to respond adequately and quickly against this new external stressor and impact the functional physiological state of the body. Different authors, using different approaches, have identified profound and intense psychological and emotional changes in the health and social adequacy of nurses in the face of a pandemic [15-17].

Naturally, the focus of public opinion has concentrated on the nursing professionals at all levels of the healthcare system, where this interest and curiosity

has greatly increased stressogenic levels in individual and institutional aspects. Until now, no such interest and no such sustained strain has been observed on the peacetime nursing workforce. On the other hand, workplace and non-work-related stress peaked and maintained high levels for the second year, which is a prerequisite for a strong and negative impact on the health status of the individual. When compared with similar studies, the results are like ours, but differ in terms of status and self-esteem, which is indicative of Bulgarian medics and their worldview [18, 19]. In line with the WHO recommendations on psychosocial strain and mental health of physicians during a COVID-19 pandemic [20], it is necessary for nurses themselves to pay attention to their personal mental and physical health, but we as a society also have a duty to help and preserve this valuable resource for a long time.

On 13.3.2020, the government of Bulgaria declared a state emergency and the growth of the infected with the virus rose sharply. Our health system faced a new challenge of treating the sick and providing beds, doctors, nurses and ventilators. The main efforts were twofold – protecting the health of citizens and chronically ill patients, preventing the spread of the virus and actively treating the infected. The COVID-19 pandemic has largely disrupted the established way and stereotype of living. The deleterious effects of this insidious disease include high levels of illness and spread, significantly high and unexpected mortality of those infected, economic hardship and financial disruption for the individual as well as for organizations and the society as a whole, stress on the entire eco system of health and social development, high levels of stress associated with partial knowledge and partial credibility of information about and from the disease and a dominant fear of varying intensity of the impact of the virus and the behavior of health care institutions.

The dynamics of modern society affected the people by accelerating the work processes and increasing the adaptation, work, cognitive and communication demands on workers. A logical negative effect of the rapid dynamics of professional and social living is the increasingly rapid erosion of the functional state and attrition of workers. Therefore, work factors, psychological risks of the work environment and occupational safety have taken a leading role in research and integrated management practices and strategic imperatives for sustainable collective development. Changes in the very structure and intensity of living are at the heart of the changed nature of work activity [21]. This calls for a new attitude and a new, different and up to date reading of the psychosocial risk factors of the working environment. The new understanding and contemporary assessment of the psychosocial factors of the work environment and their associated health risks have attracted the attention of researchers. One of the reasons for this is their key importance to health and this has clearly emerged in our new technology-based way of communication, COVID-19 regulated world [22]. According to the WHO, health is defined as a state of physical, mental and social well-being [23]. Following this postulate, it implies the creation of prerequisites and working conditions in which it is impossible to detect the presence of somatic and mental disorders and/or diseases. Moreover, the key requirement is to establish sustainable rules, norms and programs to prevent the harmful effects of the nature of the work process on the health of employees and workers. The main objective is not only to preserve and prolong an active working life, but also to enhance performance and satisfaction – a sense of significance and mental health. Therefore, an essential part of the WHO definition of health is also providing the opportunity to recover and to enhance cultural and intellectual levels. In summary, it is possible to argue that the state of health is a continues process of full adaptation of the organism to changing environmental and working conditions [9, 10]. Paying attention to the working capacity and the continuous efficiency of working life, the factors of working environment and work have been identified, which have the main influence with different strength, exposure and intensity on mental and physical health. The etymology of these factors as well as their characteristics and effects on health has been the subject of various studies and research. Researchers seek not only to systematize and expose them from all possible sides, but also to find ways of prevention and conditioned awareness of their harmful effects. It is difficult to summarize or rank in order of value and importance which are the leading factors, because as technology and ways of working have advanced, safety and health have been at the forefront and subject not only to scrutiny but also to social evaluation.

Research and management interest has recently been attracted by psychosocial risk factors of the work environment. On the one hand, this is a logical consequence of the established and nurtured focus on the individual and his or her comprehensive, multifaceted and holistic health as a key to society and a measure of European belonging. On the other hand, it is an expected effect of the natural evolution of social, labor and economic life with all the dynamics of its multi- segmental comprehensiveness. There is an opinion of a diverse range of authors [24-26]

that psychosocial risk factors have a more significant and deeper effect on the development of diseases compared to physical factors. The relevance of their thesis applies largely to the next generations of workers, the main reason being the dynamic lifestyle and the resulting stress in the new style of living [27]. In the dynamics of what is happening and in the presence of a pandemic with a new biological agent, their visionary thesis is a reality among the leading and relevant today, the highest stress profession of medical professionals. The new extremely key role with intense health significance of psychosocial factors can be found in various spheres of the social, cultural and economic life of our modern information dependent COVID dynamic society. Therefore, psychosocial risk factors and their intense health impact are counted among the direct factors of work activity [4]. Evidence of the increased interest of researchers, theorists and practitioners are the increasing number of studies, articles and monographs [28-30], where the object of research is precisely the effects of their severity on health, the root causes and consequences of the disposition by and of psychosocial factors.

CONCLUSION/IMPLICATIONS

The results of our study discussed the comparison of data from the two periods help to form the conclusion that the COVID-19 pandemic in nurses practicing long term care in hemodialysis clinics and wards represents a powerful stressor, significantly affecting the functional, physiological and psychological state of the studied individuals.

The analysis of the survey data on the impact of psychosocial stress among nurses practicing long term care in hemodialysis clinics and wards is indicative of the nature of the stress – before and during the COVID-19 pandemic, as well as the nature of the profession and the overall psychological attitude of the Bulgarian nurses. There is a need for more research and the embedding of psychosocial stress prevention policies where the main objective will be not only to preserve and prolong the working life of this social group of nurses, but also to reposition it as a leading, risky and significant one for public health and the economy. It appears that the COVID-19 “situation’’ will continue – it is not a sprint, but a marathon in which the fittest, the most prepared and the most supported finish.

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CASE SERIES

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BILAYER TECHNIQUE FOR ALVEOLAR RIDGE AUGMENTATION IN PRE-PROSTHETIC IMPLANT SURGERY: INDICATIONS AND PROBLEMS

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Abstract. Background: *Prosthetic rehabilitation of missing teeth with dental implant-supported restorations has recently become a predictable treatment option in contemporary dentistry with a highly successful rate. Due to different factors, vertical and horizontal bone loss could present, and the available alveolar bone may not be sufficient for optimum implant position. Ridge augmentation procedures could be applied to increase the volume of the deficient sites for accurate prosthetic implant placement, which assures functional and esthetic stability of tissues around the implants, essential for long-term success.* ***Our*** *study aims to evaluate the efficiency of the bilayer technique of guided bone regeneration for alveolar ridge augmentation procedure in cases of bone deficiency for optimum implant placement and long-term success.* ***Materials and methods:*** *We present several cases of alveolar bone deficiency treated with the bilayer technique – the combination of allo- and xenograft, covered by collagen membrane, with long-term follow-up. The defects were filled with allograft, and a layer of xenograft and barrier membrane was placed above it. This technique combines the benefits of all xenografts and barrier membranes.* ***Results:*** *The bilayer technique with allo- and xenograft and collagen membranes is predictable, with a high success rate and lower morbidity. We have a 100% survival rate of the implants placed in a grafted area with long-term follow-up with excellent aesthetic and functional results.* ***Conclusions:*** *The bilayer technique uses the benefits of two bone graft materials, is associated with less morbidity for the patients, and has excellent long-term results if performed accurately according to indications and technique.*

Key words: *guided bone regeneration, bone substitute materials, dental implants, bilayer technique*

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INTRODUCTION

Alveolar ridge atrophy after tooth loss is very common and may compromise the optimal placement of implants. Afunctional atrophy, trauma, oncologic diseases, odontogenic infections, and congenitally missing teeth may cause bone deficiency. A wide range of oral surgical procedures, such as guided bone regeneration (GBR) (through the use of bone graft materials and resorbable and non-resorbable membranes), tent screw (umbrella technique), auto-block grafting, natural bone regeneration (NBR- using PRP and PRF +/- bone graft) and distraction osteogenesis, can be applied for reconstruction of alveolar ridge deficiencies [1-4].

Guided bone regeneration prevents the migration of epithelial and gingival connective tissue cells, provides space, and stabilizes the coagulum in the defect in the postoperative period. The GBR procedure allows entry into the desired site/s of cells capable of new bone formation.

Important for the success of GBR are membrane stability, primary wound healing, absence of infection, and good postoperative care. Tension-free primary closure is a critical factor in preventing wound dehiscence, and a barrier membrane should be fixed without mobility to ensure good and sufficient bone regeneration [3, 4].

This bilayer bone graft procedure combines the benefits of xenografts and allografts. Xenograft gives mechanical support for prolonged space maintenance and has osteoconductive properties. Demineralized freeze-dried bone allograft (DA) contains bone morphogenic proteins and osteoinductive properties, stimulating new bone formation. DA is highly biodegradable and has osteoconductive properties but less structural support than xenografts and mineralized allografts. It could be modified using sticky bone (DA with A-PRF and a layer of xenograft above it covered by collagen or A-PRF membrane) [6, 7].

The bone graft procedure could be performed in two stages (as a delayed approach) or one stage (as a simultaneous approach – GBR combined with at the same time implant placement). In case of minimal bone deficiency and good implant stability can be achieved, the one-stage approach can be applied. This technique is appropriate for horizontal alveolar ridge augmentation, bone dehiscence, or fenestrations and not so efficient for vertical alveolar ridge augmentation.

Intrabony defects are much more amenable and easier for regeneration due to facilely maintaining space and stabilizing the bone graft and membrane. With proper suturing technique easily can be achieved primary soft tissue closure. Other defects can be more challenging in pre-prosthetic surgery cases, such as lateral and vertical bone augmentation procedures [5].

The success of the bone grafting procedure thoroughly depends on the exclusion of epithelial cells during new bone formation from osteoblasts and fibroblasts [8]. Aghaloo et al. assessed the success of different augmentation techniques, such as GBR, block auto grafting, distraction osteogenesis, ridge splitting, etc., based on implant survival [9]. They conclude from their systemic review that GBR is one of the best techniques for successful ridge augmentation according to implant survival [8, 9].

PURPOSE

Our study aims to evaluate the efficiency of the bilayer technique of GBR for alveolar ridge augmentation in cases of bone deficiency for optimum implant placement and long-term success.

MATERIAL AND METHODS

We present several cases of alveolar bone deficiency treated with the bilayer technique, combined with allo- and xenograft, covered by collagen membrane, with long-term follow-up.

The defects were filled with allograft above a layer of xenograft and barrier membrane. This technique combines the benefits of allo- and xenografts and barrier membranes. Xenograft gives mechanical support for prolonged space maintenance. Demineralized freeze-dried allograft (DA) provides a structural framework (osteoconductive capabilities) and contains bone morphogenic proteins that stimulate osteoinduction (osteoinductive capabilities). DA is biodegradable and provides less structural support than xenografts and mineralized allografts. Gamma irradiation and ethylene oxide are used as sterilization techniques. It significantly decreases the risk of transmitting infection but decreases the osteoinductive properties of the graft, mainly morphogenetic proteins.

The GBR technique can be applied in two stages (delayed approach) or one stage (simultaneous approach with implant placement). If the bone deficiency is low and implant stability can be achieved, the one-stage approach can be applied.

Case 1. GBR In Horizontal Bone Loss

We present a case of a 65-year-old female with performed GBR-bilayer technique. Five months after augmentation, the implant placement was made. The implants were with very good initial stability. The two-stage approach is preferable if more bone must be regenerated, and the risk of postoperative complication will be reduced (Fig. 1).

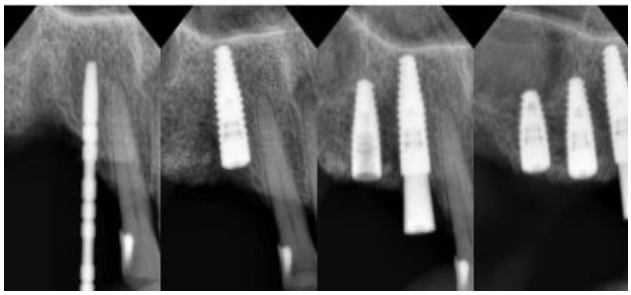


Fig. 1. A Implant placement

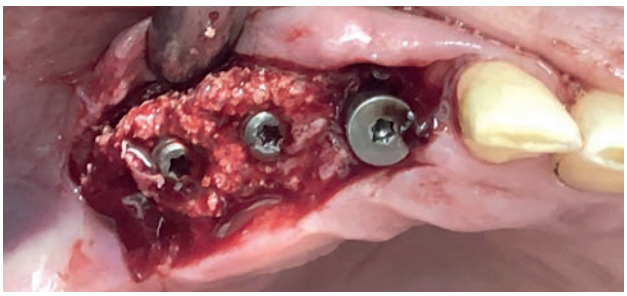


Fig. 1. B Intraoperative picture

Case 2. GBR In Immediate Implantation

We present a case of a 32-year-old male patient with a horizontal fracture in the root of 11 teeth after an accident. After several endodontic treatments, the fistula vestibular persists, and the patient was referred for dental implant treatment. After CBCT evaluation, tooth extraction and immediate implant placement were scheduled. The bilayer technique for GBR was performed covered over by collagen membrane. This technique could be used for the treatment of dehiscence-type defects around implants (Fig. 2).

GBR in case of alveolar bone deficiency/preprosthetic surgical procedure

We present three more cases with horizontal and vertical bone deficiency treated with GBR bilayer technique (Fig. 3, 4, 5).

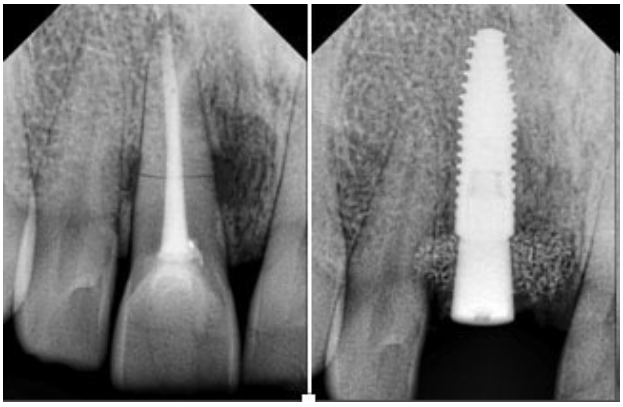


Fig. 2. A Preoperative and postoperative x-ray

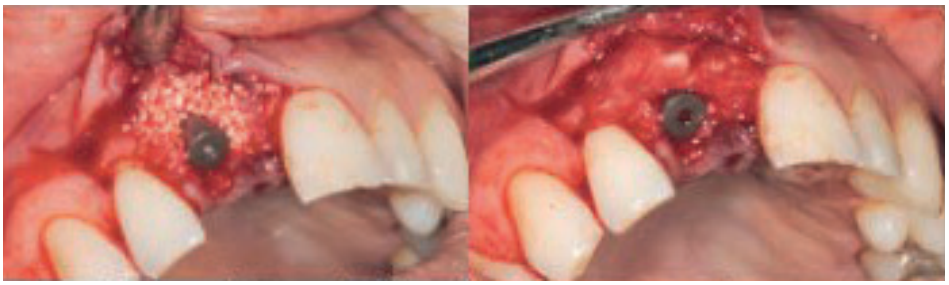


Fig. 2. B Bilayer technique intraoperative

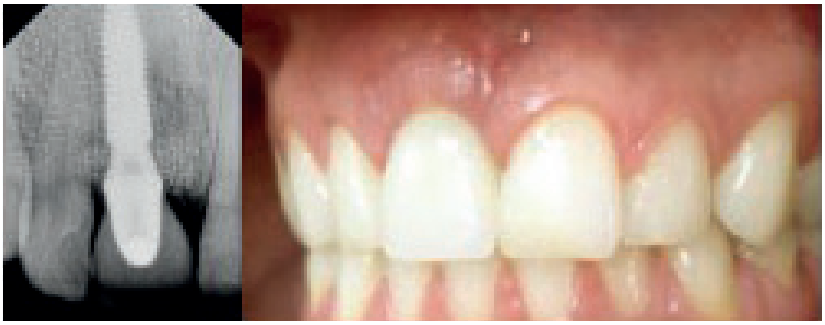
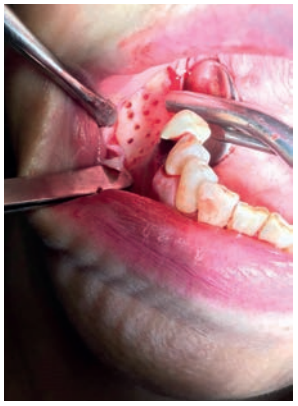


Fig. 2. C Prosthetic restoration with a crown six weeks after implantation.



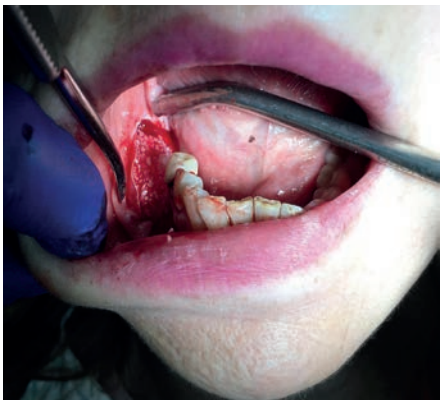


Fig. 3. A Preoperative alveolar bone deficiency – clinical

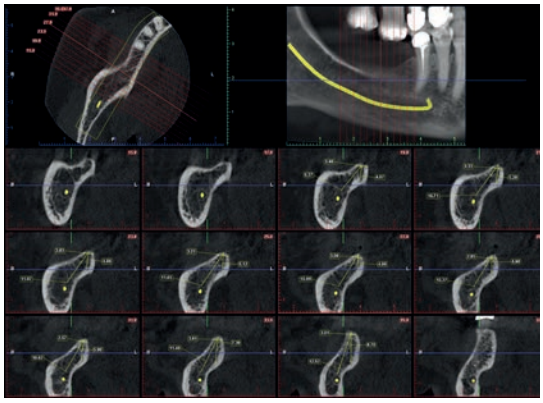
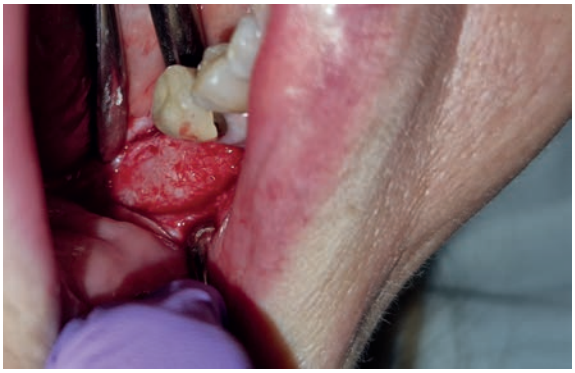


Fig. 3. B Preoperative alveolar bone deficiency – CBCT



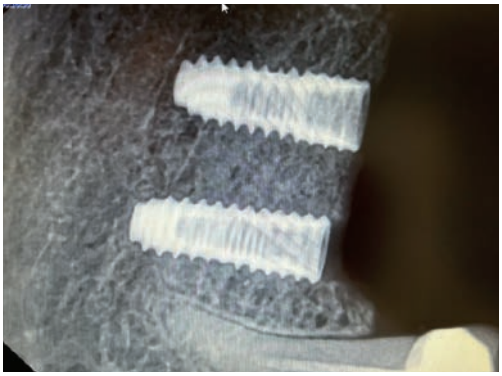
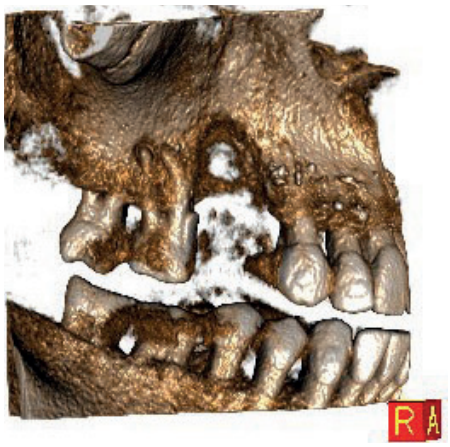
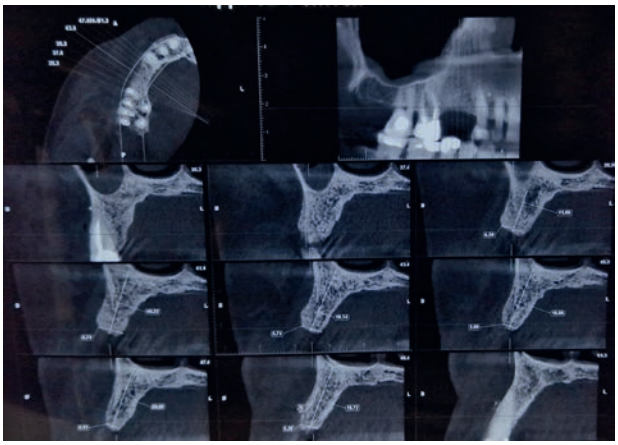


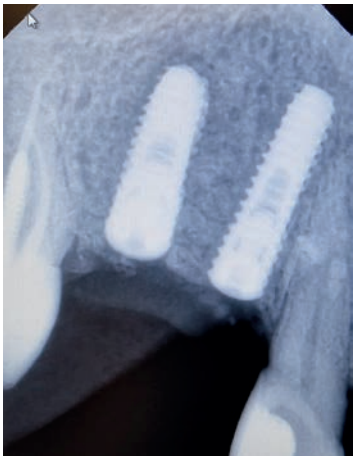
Fig. 3 C Five months after the operation – GBR bilayer technique



A) before operation

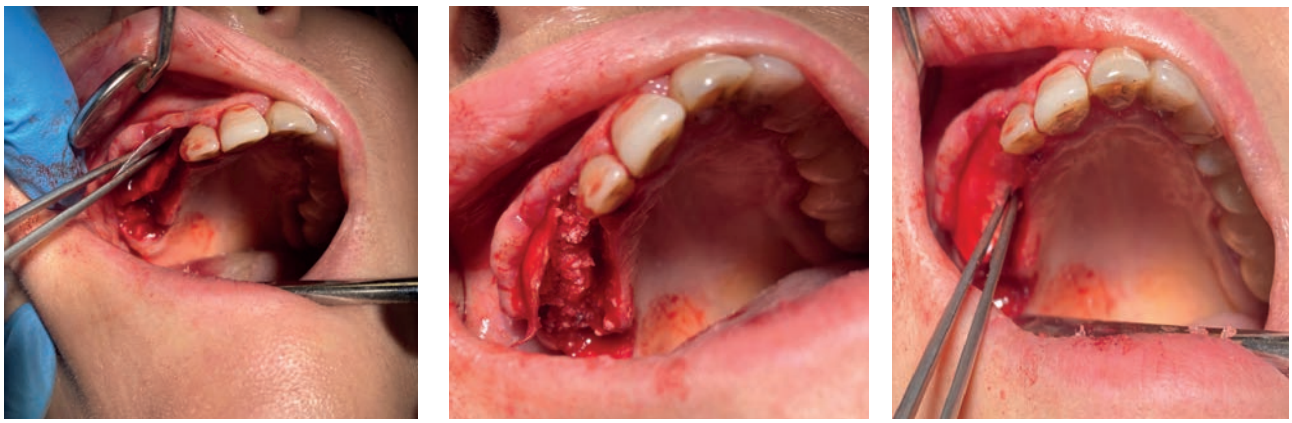


B) 5 months after operation

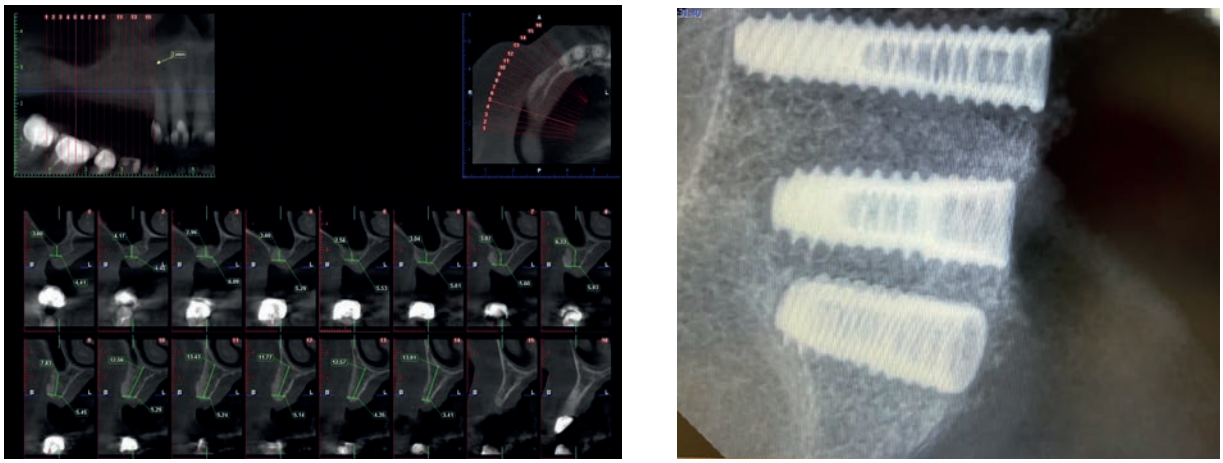


C) After dental implant procedure

Fig. 4. Management of horizontal and vertical alveolar bone deficiency



A) intaoperative



B) 5 months postoperative

C) after dental implant procedure

Fig. 5. Surgical management of deficient alveolar ridge by GBR- bilayer technique

The post operative period in all cases was uneventful. And all the cases were completed with prosthetic restorations.

RESULTS

The bilayer technique that we described and use with allo-, xenograft, and collagen membranes is predictable clinical protocol, with a high success rate and lower morbidity in mainly horizontal bone deficiency. We have 100% a survival rate and success rate of the implants placed in a grafted area with long-term follow-up.

DISCUSSION

The predictability and success of bone graft procedures are based on several principles and conditions: space maintenance, prevention of local trauma, stability of bone graft and membrane, nutrition, and primary wound closure [5, 10].

Space maintenance

Providing space maintenance is an obligatory condition and can be challenging for clinicians depending on the characteristics of the defect site, which has to be grafted. If significant bone augmentation is required in a severely resorbed alveolar ridge, creating space is critical for the success of GBR.

Different bone substitutes can be used as autogenous, xenografts, allografts, and alloplastic materials [4]. An ideal biomaterial for bone regeneration should be able to form or stimulate new bone formation. The processes and speed of resorption of bone graft and apposition of new bone must be balanced [4, 6].

GBR is very popular among clinicians because of its advantages as the unlimited availability of biomaterial, less morbidity (no donor site), reduced operation time, and less risk of postoperative complications [9, 10, 11].

Xenografts are bone grafts from animals such as cows, horses, or species other than humans [12]. Deproteinized bovine bone (DBB) is the most popular xenograft material frequently used in GBR procedures. DBB has osteoconductive capability that serves as a framework due to the interconnecting pore system favorably for the migration of osteopro-

genitor cells. Over time, DBB particles are incorporated within the bone. DBB has low substitution rates because of slow resorption therefore, it can provide space maintenance over the long term [4, 6]. It was shown in the literature that DBB graft particles remain in living bone even after ten years postoperatively [12]. Graft materials with low substitution rates are a good framework for host bone regeneration during the healing period and decrease resorption of the augmented bone [4, 6]. Residual graft particles can affect negatively the healing process of the augmented zone and decrease the regenerating rate, especially in the area of integrated implant surface [12]. In cases that require a greater amount of ridge augmentation – vertical, horizontal, or composite defects, DBB can be mixed with autogenous particulate bone and applied as a mixture which increases possible osteogenic factors and pluripotential cells at defect site [2] or, as we perform the bilayer technique. Most authors recommend allowing 6-9 months to heal augmentation regions before the procedure of implant placement. During the healing process, DBB graft material maintains the space of the augmented site, and autogenous particles encourage the migration of pluripotent cells and the incorporation of this framework with the living bone.

Allografts are bone grafts harvested from the same species but are genetically not similar donor to the recipient [4, 6]. Allograft donors are meticulously screened, specimens are carefully sterilized to reduce the possibility of disease transmission and are freeze-dried. Mineralized allografts (MAs) provide good stability and space maintenance because of their physical properties. [4, 6, 13] Osteoconductive scaffolds of MAs ensure volume preservation and new bone formation [14]. It can be composed of cortical and cancellous particles or both. Mineralized cortical particles with slow resorption rates offer a scaffold and enhance the volume of the augmented site. The cancellous particles have faster resorption rates and cannot ensure a space for a long time but encourage the ingrowth of bone cells and angiogenesis. Less resorption can be expected if the amount of cortical graft particles is increased in the composite graft. [15]. Demineralized allograft (DA) contains bone morphogenic proteins and, therefore has an excellent osteoinduction capability.

In the literature are described different techniques of grafting procedures, often applied DA mixed with other slowly resorbed graft materials to maintain the space for a long period after surgery [16]. The most common indications for the use of demineralized grafts are envelope-type defects and socket preservation. Implants can be placed safely after four months of surgery [16]. Some authors do not recommend using DA in vertical and horizontal augmentation because of expected bone loss after long-term healing [15].

In clinical practice, barrier membranes are routinely used in GBR. There are two barrier membranes: resorbable and non-resorbable [4, 6, 10].

Resorbable membranes, made of native collagen (non-cross-linking) have high biocompatibility, good tissue integration, and ensure rapid vascularization [15].

The most important benefits of resorbable membranes are no need for membrane removal after healing, resulting in decreased morbidity, easy manipulation, and a lower rate of postoperative complications. In achieving space maintenance, resorbable membranes are less successful than non-resorbable membranes. These membranes could be used with bone graft materials and additional tools such as tenting screws (umbrella technique) or titanium plates for space maintenance. Still, they may lose their barrier function early due to rapid biodegradation [16, 17, 18]. The resorption time depends on the membrane's material, thickness, vascularization, cellular activity, and exposure in the oral cavity [19]. One of the advantages of non-cross-linked collagen membranes is the spontaneous closure and epithelization over the membrane if exposure occurs during the healing period [20]. Epithelization of the exposed membrane occurs within a week after suture dehiscence, but the grafting volume may be negatively affected during the new bone formation, and some bone loss may be expected [4, 6]. Some clinicians recommend using a double non-cross-linked membrane over the augmented site to prolong the resorption time [6]. For prolonged degradation time, cross-linking resorbable collagen membranes are indicated [8].

Several essential factors may influence the success of GBR: regeneration time, resorption rate at the augmented site, and space maintenance and it is very important the choice of graft (depends on its properties – type of sterilization, viscoelasticity, hydrophilic), primary closure of the grafted area, membrane choice, surgical technique, absence of dead space, availability of autogenous bone, composition of the graft, vascularization, regeneration potential of the host bone [22].

Stability

The stability of the augmented site in the GBR procedure during healing is an important factor for successful GBR. Stabilization of graft material is obligatory in the prevention of local trauma [5]. Barrier membranes are used to cover the augmented site, which protects epithelial and connective tissue cell

migration in the regenerating bone. Sometimes, additional tools are used to ensure stability and prevent local trauma as pressure of lip and mastication force pressure [20].

Membrane fixation can be achieved by pins, sutures which prevent migration of the graft, which is essential for the success of the bone graft procedure [23].

Nutrition

Some clinicians make perforations of the cortical bone before bone grafting for better migrating vessels to the augmented site. Several benefits of decortication of the recipient site have been demonstrated [23]: revascularization is increased after decortication, particularly in the mandible, the release of growth factors can improve healing, and the perforated encourages integration and stability of the graft [23, 24]. There are different studies in the literature suggesting that decortication is not necessary for better regeneration [23, 24].

The goal is to create conditions for restoration of the prosthetic field and for subsequent prosthetic treatment with a decrease of the risk of atrophy and functional disorders [25, 26, 27, 28].

Primary closure

Protection of the grafted site is an important factor. Primary closure is essential, and complications are strongly associated with the grafting volume needed [4, 6].

To achieve successful GBR, the condition of soft tissue should be evaluated meticulously before treatment planning such as the gingival biotype, the amount of keratinized mucosa, the vestibular depth, and previous surgical interventions [6]. For the protection of the augmented site and primary healing, several factors should be considered: flap design is important for tension-free flap closure, primary tension-free flap closure, suturing technique and suturing materials (the clinician should be aware and familiar with different suturing techniques to reduce the pressure on the edges of the flap), accurate postsurgical medications and postoperative care, the bilayer technique with allo- and xenograft and collagen membrane is predictable, with a high success rate and lower morbidity procedure.

CONCLUSION

Many surgical techniques, approaches, and biomaterials have been discussed in the literature that clinicians have the choice to use in reconstructive procedures of alveolar bone deficiencies. The success of these procedures mainly depends on the clinician's experience and skill. The surgeon and patient should carefully evaluate the benefits and risks of the chosen procedure and methods and graft material related to indication in every single case and consider the ideal treatment option. The technique we described is easy, and prosthetic-driven augmentation is recommended for a better outcome.

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CASE REPORT

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OPERATIVE TREATMENT OF IDIOPATHIC ISOLATED CLITOROMEGALY – A CLINICAL CASE

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Abstract. *Idiopathic clitoromegaly is a relatively rare disease. A case of isolated idiopathic clitoromegaly is presented. It concerns a 31-year-old woman, in whom the clitoris has showed a tendency for progressive enlargement in the last 3-4 years. The patient had no gynecological and systemic diseases and disorders. Karyotype and hormone levels were normal. No cystic changes in the ovaries and other changes in the abdominal organs were detected during ultrasound. Computerized axial tomographic (CAT) scan of the adrenal glands was performed and showed normal appearance. Method of selection in such cases is operative treatment – clitoroplasty with storage of neurovascular bundle of the clitoris.*

Key words: *clitoromegaly, clitoroplasty*

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INTRODUCTION

Clitoromegaly is a common anomaly, but acquired isolated clitoral elongation is rare [1] and its causes can be: hormonal, non-hormonal, pseudoclitoromegaly, idiopathic clitoromegaly [2, 3].

The degree of clitoromegaly is most often determined according to the classification of Von Prader A., 1975 [2], which, depending on the intensity of hyperandrogenism, differs in several forms: clitoral hypertrophy with reduced vaginal passage; with a narrowed vaginal entrance; marked clitoral hypertrophy, male external genitalia with hypospadias and hypertrophied penis-like clitoris and labia majora like scrotum. Endocrinopathies, virilizing tumors, and androgen use are the most common hormonal causes of clitoromegaly [4].

A case of a patient with isolated idiopathic clitoromegaly treated surgically is presented.

CASE DESCRIPTION

A 31-year-old female, 0-gravida, complained that in the last 3-4 years, the clitoris has shown a tendency for progressive growth. The patient associated the onset of complaints with the beginning of regular sexual intercourse, which became the cause of negative psycho-emotional feelings and physical sensations (a feeling of worry at the thought of the presence of this problem), physical (discomfort with certain movements) and sexual (discomfort and awkwardness during sexual intercourse) discomfort. The woman denied the use of drugs, psychotropic and hormonal medications. No family history was reported – there are no other cases of clitoral enlargement in the family. Appearances of menarche when she was at the age of 15, the menstrual cycle was regular – every 30-32 days, lasts 3-4 days, settled suddenly. From the objective research – the patient was with normal female phenotype. From the gynecological

examination - in a calm state, the clitoris was 20 mm long (according to the patient's data, it increases to 30 mm during sexual excitement) (Figure 1). Vaginal entrance – was with normal anatomy structure. The external opening of the urethra was located 1.5 cm above the entrance to the vagina. The woman has normal secondary female genitalia with female pubic hair. There was no evidence of obesity – height 172 cm, weight 65 kg.

From the analysis of hormonal tests such as: estradiol, progesterone, FSH, LH, DHEA-S, testosterone, Prolactin, ACTH, cortisol, TSH – were in normal referent value, and tumor marker CEA were in normal referent value also. The values in Table 1 showed the hormonal status of the patient.

Table 1. hormonal status of the patient

FSH (follicular phase)	4.18 mIU/ml
LH (follicular phase)	5,03 mIU/ml
Estradiol (follicular phase)	314,6 pmol/l
Progesteron (luteal phase)	25 nmol/l
DHEA-S	9,7 nmol/l
Prolactin	312,3 mU/ml
ACTH	26,3 pg/ml
Cortisol	218 nmol/l
TSH	2,6 mUI/l
testosterone	2,46 nmol/l

Ultrasound of the small pelvis and abdominal organs showed that there was no data for polycystic changes in the ovaries, no tumor formations were visualized in the pelvis.

From the obtained results, no connection could be made between clitoromegaly and the results from the paraclinical and instrumental examinations. The patient underwent clitoroplasty with preservation of the neurovascular bundle (Figure 2 and Figure 3).

OPERATIVE TECHNIQUE

Under general anesthesia, a semilunar incision was made over the dorsal surface of the clitoris. The dorsal surface was prepared to the pubic fascia without cutting the suspensory ligaments. 5 superficial ligatures were applied (the neurovascular bundle was preserved) on the pubic fascia – at 10, 11, 12, 1 and 2 o'clock. They were tied so that there is no sharp angle when tightening them, which makes erection difficult. Excess skin was cut away. With single resorbable sutures, the normal anatomy was restored – the skin was sewn to the foreskin. The patient was followed for a period of 6 months. The postoperative period went smoothly, without early and late complications (Figure 4).

At the follow-up examination 6 months after the intervention, the patient said that the sensitivity of the clitoral area was normal. The woman was satisfied with the aesthetic and functional result (Figure 5).

DISCUSSION

Clitoroplasty is the method of choice in the treatment of clitoromegaly [4,5]. It aims to improve the psychoemotional state of the woman. At the same time, it is necessary to preserve sexual function and sensitivity with a mandatory cosmetic effect.

For the first time, Young performed a similar plastic correction on a child with congenital adrenal hyperplasia in 1937 [6]. Depending on the intensity of

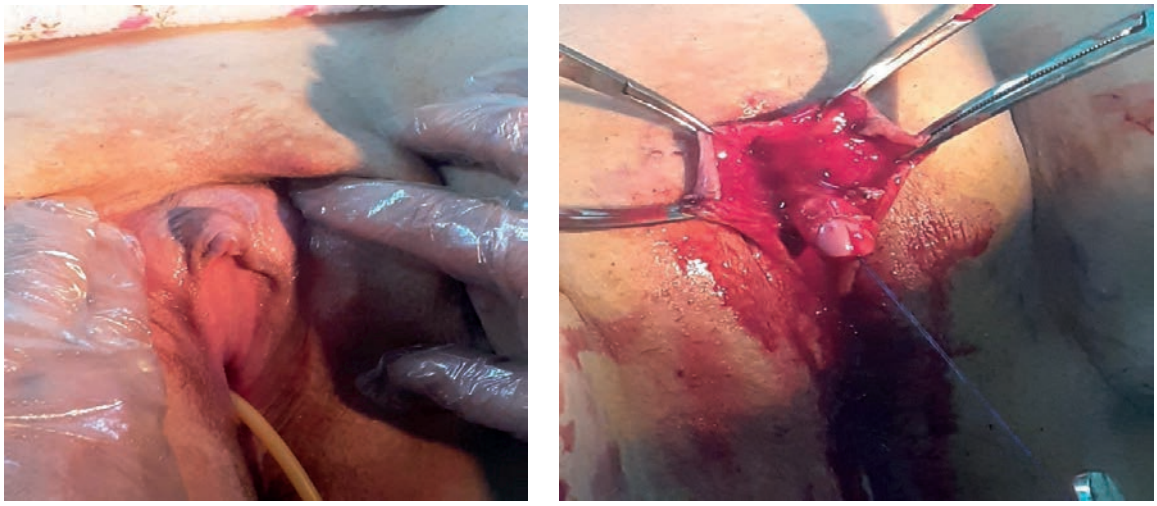

Fig. 1, 2. Clitoris, labia minora, urethral entrance and vaginal entrance before surgical correction (left); Dissection of the foreskin from the dorsal surface of the clitoris (right)

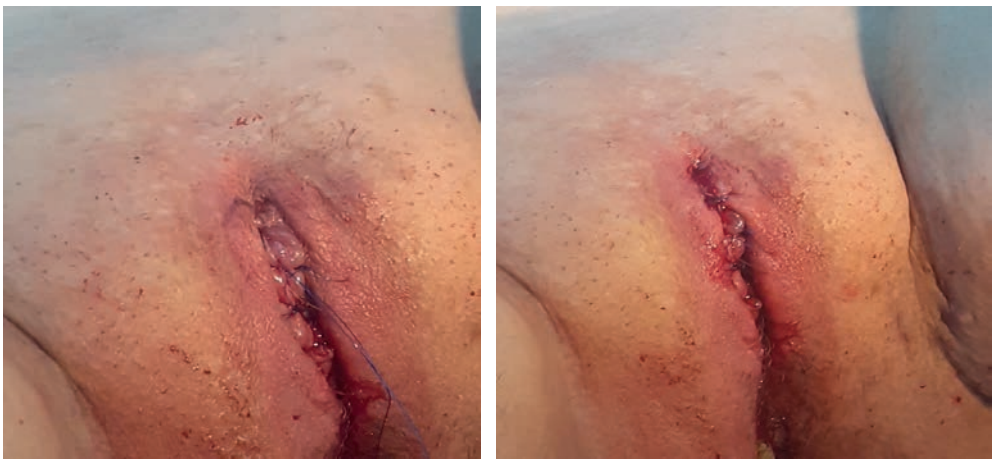

Fig. 3, 4. Reconstructed anatomy and shaped foreskin (left); Clitoroplasty completed (right)

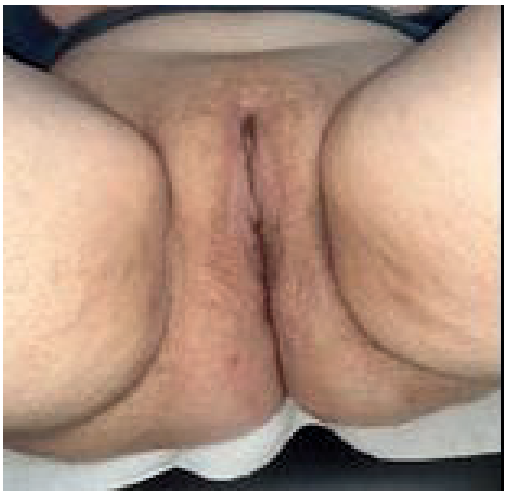

Fig. 5. Status at 6 months after surgery

hyperandrogenism, Prader distinguishes 5 stages in girls:

1. Clitoral hypertrophy, normal vaginal entrance.

2. Clitoral hypertrophy, narrowed vaginal entrance due to partial fusion of the labia minora.

3. Emphasized clitoral hypertrophy, persistent urogenital sinus is observed.

4. The hypertrophied clitoris resembles a penis and the labia majora imitates the scrotum, the vagina opens into the urethra and at the base of the clitoris.

5. Male external genitalia, possibly with hypospadias, without testes in the scrotum.

Endocrinopathies, virilizing tumors, and androgen use are the most common hormonal causes of clitoromegaly.

The most common cause of clitoromegaly is congenital hyperplasia of the adrenal cortex [3]. Adrenal hyperplasia due to 21-hydroxylase deficiency is a rare, congenital disorder presenting with hyperplasia of the adrenal glands in utero due to lack of cortisol due to 21-hydroxylase enzyme deficiency associated with hyperandrogenism. Severe cases occur in 1 in 10-14,000 newborns. Mild cases without classic symptoms are much more common, 1:1000.

Hormone-producing ovarian tumors that can cause clitoromegaly are androblastomas. They originate from the Sertoli-Leydig cells of the hilus of the ovary. They produce androgens that lead to menstrual disorders – amenorrhea and to virilization of women - enlargement of the clitoris and increased hair growth. Very rarely, an androblastoma is hormonally inactive or secretes estrogens.

Non-tumor causes of clitoromegaly can be neurofibromatosis and epidermoid cysts of the clitoris.

Neurofibromatosis (benign) or the so-called von Recklinghausen's disease has two separate, genetically and clinically different forms – type 1 and type 2. The disease is characterized by a wide variability of the clinical picture – from mild asymptomatic forms to very severe manifestations of the clinical picture and a high risk of life-threatening malignant diseases of the patient. Type 1 is more common (1:3,000 – 1:4,000). It is inherited in an autosomal dominant manner. Women suffering from this disease develop benign and malignant tumors of the central and peripheral nervous system. In girls, it can rarely lead to enlargement of the clitoris and labia [4]. Epidermoid cysts of the clitoris are one of the rare non-hormonal causes of clitoromegaly. In such cases, a mobile, soft, non-fluctuating mass protrudes from the clitoral region. There is often evidence of trauma in this area in the woman's history [5].

Clitoroplasty is the method of choice in the treatment of clitoromegaly. It aims to improve the psycho-

emotional state of the woman. At the same time, it is necessary to preserve sexual function and sensitivity with a mandatory cosmetic effect.

For the first time, Young performed a similar plastic correction on a child with congenital adrenal hyperplasia in 1937 [6]. Later J.G. Hampson and J. Money suggest in similar cases to amputate the clitoris [7]. Various operative methods have been described, but the cases of operative correction in sexually mature women in which the dorsal and ventricular vascular-nerve bundles are preserved are few [8]. Clitoroplasty with preservation of neurovascular pedicles, which was performed in the described case, is an optimal volume of surgery in women with clitoromegaly of first degree. In this case, correction of the labia minora and plastic surgery of the introitus vagina is not necessary. Some authors also suggest resection of the corpora cavernosa of the clitoris [6]. In this case, correction of the labia minora and plastic surgery of the introitus vagina is not necessary.

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REVIEW

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EPIDEMIOLOGICAL SURVEILLANCE OF ACUTE FLACCID PARALYSIS FOR ERADICATION OF POLIOMYELITIS (A BRIEF REVIEW)

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Abstract. *In May 1988 the World Health Assembly set to World Health Organization (WHO) the goal to achieve a global eradication of poliomyelitis by the year 2000. Surveillance of the acute flaccid paralysis (AFP) remains the 'gold standard' for the detection of polio. The criterion of sensitivity of the surveillance system is the incidence from non-polio related AFP, in children under 15 years of age. The aim is to detect more than 1 case of AFP, per 100,000 children. In 2019, WHO announced the eradication of wild poliovirus 3, and poliovirus 2 was eradicated in 2015. Wild poliovirus 1 continues to circulate. The main goals of the WHO Polio Eradication Strategy for the period 2022-2026 are: permanently interrupt all poliovirus transmission in endemic countries (Afghanistan and Pakistan), stop cVDPV (circulating vaccine-derived poliovirus) transmission and prevent outbreaks in non-endemic countries.*

Key words: *poliomyelitis, polio eradication, acute flaccid paralysis (AFP), inactivated polio vaccine (IPV), oral polio vaccine (OPV), circulating vaccine-derived poliovirus (cVDPV)*

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INTRODUCTION

In May 1988, the World Health Assembly set the World Health Organization (WHO) the goal of achieving a global eradication of polio by 2000. Many strategies were developed. For the 2019-2023 period, including the eradication, integration and safe storage of available viable polioviruses. WHO's last strategy concerns the 2022-2026 period.

The main aims of WHO Polio Eradication Strategies are: achieving a high level of commitment to the provision of qualified personnel; cooperation between health services and humanitarian programs; administration of a polio vaccine, in the most effective way, in order to stop the transmission of wild polioviruses; reaching a high immunization coverage; application of immediate anti-epidemic actions in all possible cases of poliomyelitis and acute flaccid paralysis (AFP). The two goals of Polio Eradication Strategy 2022–2026 are to permanently interrupt all poliovirus transmission in endemic countries in order to stop cVDPV (vaccine-derived poliovirus) transmission and prevent outbreaks in non-endemic coun-

tries. These goals are to be achieved by investigating any suspected case of polio, isolating the virus from fecal samples and conducting emergency immunization in the event of outbreak; a more comprehensive and systematic approach to the presentation and risk management at all levels of polio eradication; raising the level of sensitivity of AFP oversight systems, community engagement, and research of case studies in the Afghanistan and Pakistan regions [1, 2, 3].

The sensitivity of the surveillance system is the criterion of incidence from non-polio-related AFP, in children under 15 years of age. The aim is to detect more than 1 case of AFP, per 100,000 children under 15 years of age.

An AFP case is defined as any person up to 15 years of age with a disease including Guillain-Barré syndrome and transverse myelitis, which is characterized by a varying severity, acute onset with weakness or paralysis of a muscle group. [4].

AFP had appeared in medical textbooks since 1789. In 1840 an epidemic was registered in Sweden followed by an epidemic in 1881 in the United States. Early epidemics were called “polio”, from the Greek “inflammation of the gray matter.” [5].

Etiology and modes of transmission

Enterovirus infections are acute infectious diseases with different clinical features. They are transmitted by fecal-oral and airborne routes of transmission, through food, water and contaminated hands. The group of enterovirus infections is characterized by significant polymorphism in the clinical picture, as well as with different severity and variables in the course of the disease. The incubation period is 2-18 days, most often 3-6 days. The pathogenesis is similar to that of poliomyelitis. The entrance doors are the digestive system or nasopharynx.

In the 1970s, the so called “new enteroviruses” were discovered, with neurological symptoms- types 70 and 71. In 1975 in Bulgaria, an epidemic of enterovirus 71 was registered with a high case-fatality rate, especially in young children [6]. Respiratory and cardiac paralysis occurred in bulbar forms. During that epidemic, 89 paralytic cases with 29% case fatality rate in ages 1-3 years were observed [7].

Enteroviruses belong to the genus Enterovirus (EV) from the family Picornaviridae. They were classified into twelve species according to the International Committee on the Taxonomy of Viruses (ICTV). Although five species infect only animals (EVE – J), the remaining seven species are known to infect humans: EV A – D and rhinovirus A – C (RV A – C). These species include hundreds of serotypes, such as polioviruses (PV), and coxsackievirus A and B (CV-A and -B), ECHO viruses and human rhinovirus (HRVs), including several human pathogens such as PV, CV-A16, CV-B3, EV-A71, EV-D68 and HRV [8].

AFP is not a diagnosis. AFP is a clinical syndrome – i.e., a collection of signs and symptoms. There are many infectious and non-infectious causes of AFP. Polio, caused by the wild polio virus (the natural circulating strain of polio) is one of the causes of AFP. As a part of the world-wide campaign to eradicate polio, all countries in the world do surveillance for polio by looking for clinical cases of AFP and investigating each one thoroughly in order to assure that it is not caused by wild polio virus. The clinical syndrome of AFP is defined as the acute onset of weakness or paralysis, with reduced muscles tone in children < 15 years. Persons over the age of 15, who develop a paralytic illness and in whom polio is suspected, are also classified as AFP cases. AFP usually has a progressive and acute onset, but it may become chronic. AFP can be fatal if the paralysis affects the diaphragm, as persons may develop respiratory failure [9].

The detection of at least one case of AFP per 100,000 children under 15 serves as an indicator for the country’s ability to identify polio even in its absence. When the initiative of the World Health Assembly (for global eradication of poliomyelitis) was launched there were more than 350,000 new cases of paralytic polio worldwide, in 125 countries altogether. In 2015, WHO announced the eradication of the wild poliovirus 2, followed by an eradication of the wild poliovirus 3 in 2019. Wild poliovirus 1 continues to circulate, however. In 2018, the incidence has decreased to 33 cases in the region of Afghanistan and Pakistan (poliovirus type 1), while in 2019 the detected cases reached 71. Since the eradication initiative of 2000 did not achieve its goals, hopes are being postponed for 2026. More than 2.5 billion children have been vaccinated worldwide as part of the global polio eradication strategy [10, 11].

Epidemiological studies of outbreaks of AFP in the world

Data supports the emergence of AFP in 2014 in the United States. The detected cases were seasonal and occurred between August-December of 2014. 120 cases of AFP were reported. Patients who have developed AFP were aged 2 to 5 years and displayed a pronounced clinical syndrome, with an acute onset and flaccid paresis and lesions of the gray matter of the spinal cord. Poliomyelitis-like viruses are targeted as etiological agents: enteroviruses, flavoviruses, herpes viruses, adenoviruses. The etiological study

revealed enterovirus D68, in 54% of cases enterovirus A71 [12].

Cases of aseptic meningitis, encephalitis; hand, foot, and mouth disease have been studied. AFP is a widespread syndrome worldwide. Between 2008-2017, 2666 cases have been studied in Cyprus. In 26.1% of the cases echovirus 30 has been proved; echovirus 6 – in 14.2%, and coxsackievirus A6- in 10.9%. In addition to paresis, they cause myocarditis, neonatal sepsis, conjunctivitis, and hepatitis. The infectious agents are unstable in the external environment. EV-71 circulates in the region of Southeast Asia, while Coxsackie A6 and A16 in Europe [13].

Russia was certified as a polio-free country in 2002, (CDC, 2002). From 1998 to 2014, 146 cases of polio were reported, 19 of which were caused by the wild type 1poliovirus imported into Russia in 2010. The remaining 127 were associated with the VAPP (vaccine-associated paralytic poliomyelitis) due to immune disorders in children [14].

In Germany 72 cases of aseptic meningitis and AFP – with proven etiological agents ECHO 18 and ECHO 30 – were studied in 2013. Their seasonality is in late summer and early autumn. Young patients are affected and the mechanism of transmission is fecal-oral or airborne. The disease is benign, with fever, nausea and vomiting being the most common symptoms [15].

According to the WHO, AFP affects children under the age of 15, with a sudden onset of muscle weakness in one or more limbs. In a study carried out in Australia between April 2015 and March 2017, in 24 of the AFP cases, enterovirus D-68 was identified as the causative agent [16].

Polio was an important medical problem before the immunization era. Nowadays, patients with polio (PP) suffer from polio sequelae or have developed post-polio syndrome (PPS) with increasing paresis, pain and fatigue. A total of 65 hospitalized patients were studied in the Malcesine hospital in Northern Italy. The following data were summarized in a telephone interview – the presence of post-polio syndrome, concomitant diseases, hypertension, cardiovascular disease, diabetes mellitus [17].

A study conducted by the Taiwanese Centers for Disease Control (July 2015 to August 2016) has shown that 23 out of 74 cases exhibit neurological symptoms and has proved EV D-68 as a new associated pathogen associated with acute flaccid paralysis [18].

A retrospective study between 2014-2018 was performed in Iran, in which two stool specimens were collected from each AFP patient up to 14 days from the onset of paralysis within 4 days with confirmation in Atlanta, Georgia, U.S.A. Patients with severe immunodeficiency – agammaglobulinemia associated with the X chromosome, neutropenia, chronic granulomatous disease, complement deficiency, have proven to hold the highest risk for asymptomatic infection. The reason for polioviruses societal reintroduction could be linked to the so-called immunodeficiency-associated vaccine-derived poliovirus (iVDPV) after the polioeradication [19].

During the COVID-19 pandemic, in August 2020, WHO announced that all 47 countries in its African Region were certified free of wild poliovirus. Currently endemic Afghanistan and Pakistan are the places where wild poliovirus remains circulating in the environment. In 2022, cVDPV type 2 and type 3 have been found in unvaccinated patients in the U.K. (and in external environment such as in sewage samples collected from north and east London), U.S.A., Ukraine and Israel [20].

Epidemiological surveillance of AFP

WHO recommends monitoring cases of AFP and polio, as well as poliovirus circulation in order to achieve polio eradication. Polio is considered to be eradicated if absent for 3 consecutive years. Pakistan and Afghanistan remain endemic areas. They should be monitored and the migration processes related to them should be followed, in view of the immunization status of the children. Two Italian regional reference centers conducted a pilot study in 1996 and they reported and registered the following diagnoses: Guillain-Barré syndrome, polyradiculoneuritis, traumatic neuritis and neoplastic neuritis. An active surveillance system – established in 1997 – was introduced. The objectives of the system include the processing of the results of the control examination after the 60th day, as well as the introduction of the terms “probable” and “confirmed” case. The results of the fecal samples are also important. The examination materials include serum, cerebrospinal fluid, nasopharyngeal lavage, PCR [12, 21].

In order for the epidemiological surveillance to be effective, it is important to detect enteroviruses in clinical materials, environmental samples, trace circulating strains, and typing those. Completeness of reporting is important – up to 80% of weekly alert information. The correct and targeted epidemiological diagnosis is a way to timely and correct clinical diagnosis. Reporting the “zero case” is also important. Importantly, 80% of the cases must be investigated within 48 hours of the initial notification. All fecal samples must be taken in two consecutive days up to 14 days from the onset of paralysis, stored below 80C temperature, and submitted for examination to

the National Reference Laboratory within 3 days of their collection. Of note, 80% of the AFP cases must undergo a follow-up examination 60 days after the onset of paralysis.

Up to 100,000 "suspected cases" are reported each year to rule out polio. Environmental monitoring is carried out in more than 70 countries worldwide. The tests have to be send to the Global Polio Laboratory Network, which includes the samples of wastewater in accordance with Resolution WHA71.16, on the restriction of poliovirus adopted in 2018 by the 71st World Assembly. All countries in the world are committed to minimizing the places determined for the retention of poliovirus [1, 22, 23].

Local surveys for Stara Zagora region, Bulgaria

As of April 26, 1991, in the children's ward of Kazanlak, Bulgaria, there were eight patients with polio. Four of the children were without immunization – outside the immunization age, one of the patients had one dose, two with three doses and one not covered due to temporary contraindications. It was considered to be a nosocomial outbreak of polio during the winter season with a probable source of infection the first hospitalized child by a fecal-oral or air-borne mode of transmission of infection [24].

Between 1999-2010, 1585 at the age ≤ 15 years old with facial paralysis and 2 patients with paralysis of nervi oculomotorius were studied in Bulgaria. From fecal samples were isolated three vaccine polioviruses and 46 NPEV (non-polio enteroviruses): 1 Coxsacki A9; 9 Coxsacki B and 36 ECHO viruses. The largest numbers of NPEV were isolated in the months between June-November, which coincides with the period of increased seasonal activity of enteroviruses. 11 cases of acute flaccid paralysis for the period 2012-2021 (5 cases – Guillain-Barré syndrome, 1 – encephalomyelopolyradiculoneuritis, 1 – meningopolyradiculoneuritis, 1 – damage to the lumbosacral plexus, 2 – damage to the nervus fibularis sinistra, 1 – damage to the nervus suralis dextra), other than facial paralysis (81 patients), were registered for Stara Zagora region, in children up to 15 years of age [23, 25].

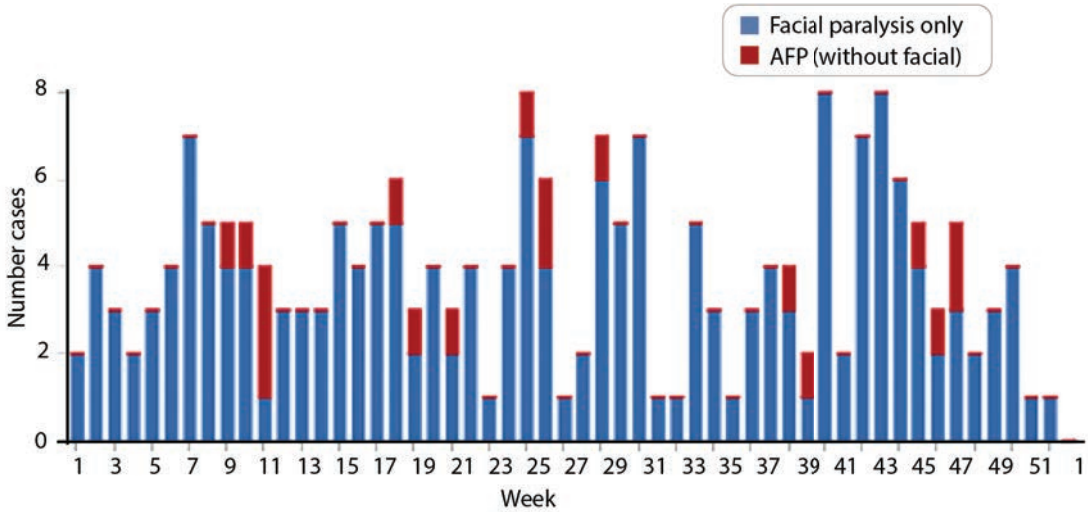

Fig. 1. Registered AFP cases in Bulgaria by weeks in 2022 and 2023 (22)

CONCLUSION

Epidemiological surveillance for AFP with timely epidemiological anamnesis, proper diagnosis and monitoring of the taken clinical materials are key to monitoring progress toward polioeradication. Completeness of the conducted control examinations is required after the 60th day. Monitoring of waste and sewage waters is recommended, twice during the year, each 6 months. Of critical importance is compliance with the mandatory Immunization Schedule, strict periodic control of poliomyelitis immunizations and maintenance of a high immunization coverage. The activation of inter-institutional contacts – Health system, medical facilities, pre-hospital structures, NGOs, volunteers, mediators in the work on immunization coverage is of key importance. Surveillance of acute flaccid paralysis remains the "gold standard" for the detection of poliomyelitis.

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REVIEW

PREVALENCE OF INCIDENTAL FINDINGS IN ORAL AND MAXILLOFACIAL CONE-BEAM COMPUTED TOMOGRAPHY: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Abstract. Background: *As the use of cone-beam computed tomography (CBCT) is increasing and the quality of images is enhanced, the likelihood of incidental findings detection in CBCT radiographs is raised. Variable frequency of incidental findings on oral and maxillofacial CBCT scans has been reported.* ***Aims:*** *To perform a systematic review and meta-analysis to resolve the conflicting results about the overall prevalence of incidental findings in oral and maxillofacial CBCT scans.* ***Methods:*** *We searched the literature in PubMed, Embase, and Scopus databases from inception to 31 October 2022 to identify studies that reported the frequency of incidental findings in the subjects undergoing CBCT imaging. We pooled the extracted data and reported the estimates as a percent with a 95% confidence interval (CI).* ***Results:*** *A total of 21 eligible studies were included, comprising 9,788 patients (54.2% women) and 10,625 CBCT scans. Analysis showed that the incidental findings were present in 69.1% (95% CI: 55.6-80.0) of the CBCT scans. There were 1.48 incidental findings per CBCT scan. Pooled prevalence of incidental findings in men was 50.2% (95% CI: 23.1-77.3), which was higher than in women (41.8% [95% CI: 16.5-72.2]).* ***Conclusion:*** *A considerable prevalence of incidental findings was observed in oral and maxillofacial CBCT scans.*

Key words: *incidental findings, oral and maxillofacial radiology, cone-beam computed tomography, systematic review*

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INTRODUCTION

Cone-beam computed tomography (CBCT) is a medical radiographic imaging method with a lower radiation dose than traditional CT, which can provide three-dimensional images with high resolution [1, 2]. This technique has become widely used in dental practice over the past two decades, from orthodontics and pediatric dentistry to implantology and prosthetics [3, 4]. Therefore, the CBCT image data must be analyzed carefully to avoid missing any significant findings.

As the use of CBCT increases and the quality of images is enhanced, the likelihood of incidental findings detection in CBCT radiographs is raised. Incidental findings refer to any unexpected abnormality detected on imaging examinations unrelated to the reasons for requesting the diagnostic tests [5-7].

These findings can be normal without needing clinical/preclinical measures or pathological requiring further assessments [5, 8, 9]. Prior studies mentioned a variable frequency of incidental findings on CBCT scans [10-12]. For example, the study by Lopes et al. [10] interpreting 150 CBCT scans reported that the total number of incidental findings was n=560, which were observed in 138 scans. Also, Barghan et al. [11] stated that 653 incidental findings were identified in 77.3% of the 400 CBCT scans.

Despite different surveys investigating the prevalence of incidental findings in oral and maxillofacial CBCT images, there needs to be a comprehensive study trying to provide a conclusive answer to this issue. In the present study, we performed a systematic review and meta-analysis to resolve the conflicting results about the overall prevalence of incidental findings in CBCT scans.

METHODS

Search strategy and eligibility criteria

The present systematic review and meta-analysis has been reported based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guideline [13]. We searched the literature in PubMed, Embase, and Scopus databases from the inception to 31 October 2022 with no language restrictions using the following keywords: *cone-beam computed tomography* OR *CBCT* AND *incidental*. The search was applied to the Title/Abstract. We included studies published between 2007 and 2022 that reported the frequency of incidental findings in the subjects undergoing CBCT imaging. We also manually searched the references of the retrieved articles. The exclusion criteria included:

1. Reviews, case reports, editorials and letter to the editors
2. Duplicate articles
3. Surveys without extractable data on study outcome
4. Full-texts not being available

Study selection and data extraction

We independently screened titles and abstracts of all publications primarily identified by our search for suitability evaluation using pre-designed eligibility forms. We also retrieved full-texts of the papers that seemed relevant to the study outcome for detailed examination, where a decision could not be made based on title or abstract screening. Any discrepancies were resolved by consensus between the authors. For each eligible study finally included in this systematic review, we collected the following data: first author's name, publication year, study location (country), number of patients, number of men and women (if available), the mean age of the subjects, number of CBCT scans, number of incidental imaging findings. We used Google Translate for translating Non-English reports, where required.

Statistical analysis

We combined the CBCT scans with incidental findings in each study using a random-effects model to give a pooled prevalence for all studies. The estimates were presented as a percent with a 95% confidence interval (CI). The heterogeneity between the studies was investigated by the I-squared index, ranging from 0.0% to 100.0%; a p-value less than 0.10 was considered statistically significant [14]. Subgroup analyses were carried out according to sex and publication date. For the subgroup analysis by publication date, we split the publication year into 2007-2012, 2013-2017, and 2018-2022. The forest plots were used to visually illustrate the effect estimates of the enrolled studies. We also utilized a funnel plot to assess the publication bias. We conducted all statistical analyses using Comprehensive Meta-Analysis V2 software.

RESULTS

Search results and study selection

The search of the online databases initially yielded 471 citations. After removing duplicates and those not meeting the suitability criteria during the title/abstract screening, 34 articles remained, and their full-texts were obtained and assessed. After excluding ineligible papers, 21 studies were finally enrolled [6, 7, 9-12, 15-29]. A flowchart of the studies' identification, exclusion, and inclusion process at each phase is depicted in Fig. 1 as per the PRISMA.

Study characteristics

In total, 21 studies were included in this systematic review and meta-analysis, comprising 9,788 patients (54.2% women) and 10,625 CBCT scans. There were seven studies from the USA, three studies from India, two studies from Turkey, one study from Australia, one study from Brazil, one study from Canada, one study from Germany, one study from Iran, one study from Italy, one study from Korea, one study from Saudi Arabia, one study from the UK. The language of all papers was English. The publication date was from 2007 to 2022. The basic characteristics of the included studies are represented in Table 1.

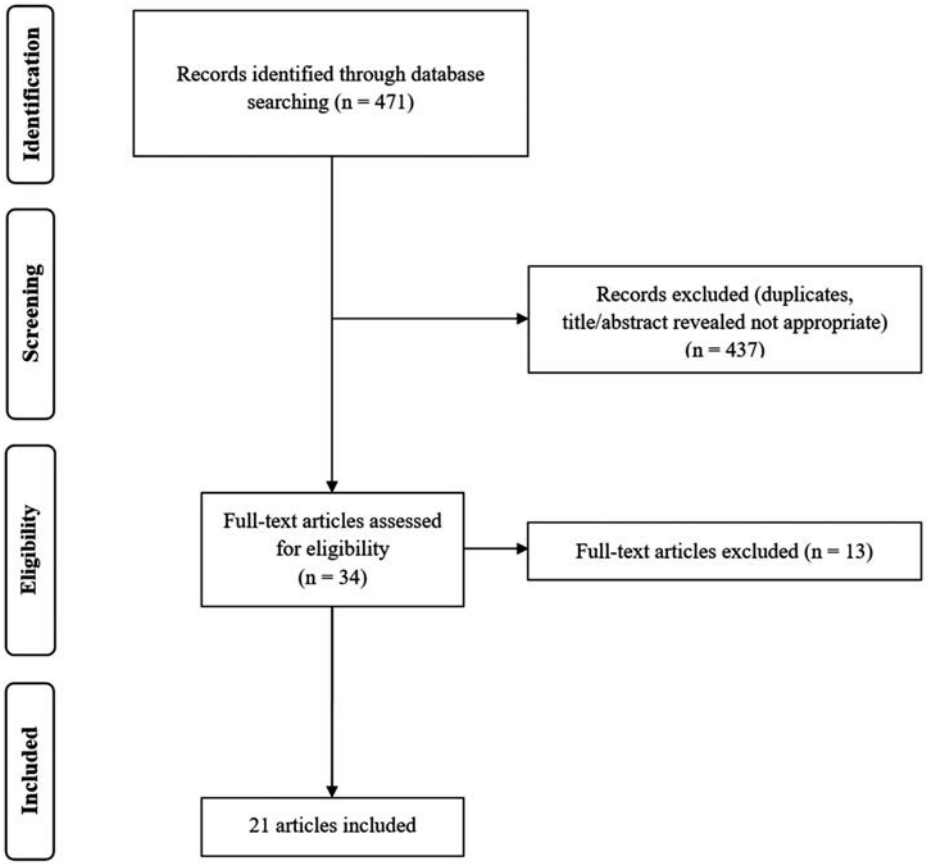

Fig. 1. PRISMA flow diagram

Table 1. Baseline information of the studies included

Study	Country	Subjects (n)	Men (n)	Women (n)	Mean age (years)	CBCT scans (n)
Allareddy, 2012 [6]	USA	1000	382	618	NA	1000
AlSakr, 2021 [15]	USA	208	101	107	62	303
Barghan, 2016 [11]	USA	400	146	254	47.1	400
Binshabaib, 2021 [16]	Saudi Arabia	400	211	189	44.1	400
Braun, 2022 [17]	Germany	374	165	209	50.9	374
Cağlayan, 2012 [18]	Turkey	207	78	129	30.3	207
Cha, 2007 [9]	USA	500	227	273	39.3	500
Choi, 2021 [19]	Korea	1020	400	620	21.7	1020
Doğramacı, 2014 [20]	Australia	183	54	129	18.3	183
Drage, 2013 [21]	UK	329	145	184	14.5	329
Edwards, 2014 [12]	Canada	427	180	247	14.2	427
Giaccaglia, 2022 [7]	Italy	61	32	29	11	61
Kachlan, 2021 [22]	USA	1002	406	596	NA	1002
Kurtuldu, 2020 [23]	Turkey	300	148	152	46.7	300
Lopes, 2017 [10]	Brazil	150	68	82	37	150
Mehdizadeh, 2020 [24]	Iran	384	184	200	36.8	384
Mutalik, 2018 [25]	USA	500	214	286	62	500
Price, 2011 [26]	USA	300	135	165	49.3	300
Singh, 2021 [27]	India	1108	685	423	NA	1850
Ul, 2021 [28]	India	140	67	73	NA	140
Warhekar, 2015 [29]	India	795	451	344	37.2	795

Abbr.: CBCT, cone-beam computed tomography

Prevalence of incidental findings

Analysis of studies showed that the incidental findings were present in 69.1% (95% CI: 55.6-80.0; I-squared = 99.2%, p < 0.001) of the CBCT scans (Fig. 2). The funnel plot was suggestive of publication bias (Fig. 3). The overall estimate indicated that there were 1.48 incidental findings per CBCT scan. The pooled prevalence of incidental findings in men was 50.2% (95% CI: 23.1-77.3; I-squared = 92.0%, p < 0.001), which was higher than in women (41.8% [95% CI: 16.5-72.2; I-squared = 94.5%, p < 0.001]). The pooled prevalence of incidental findings in CBCT images was 83.5% (95% CI: 33.7-98.1; I-squared = 99.5%, p < 0.001) for studies published during 2007-2012, 69.4% (95% CI: 36.7-89.9; I-squared = 99.3%, p < 0.001) for studies published during 2013-2017, and 60.8% (95% CI: 44.0-75.3; I-squared = 99.1%, p < 0.001) for studies published during 2018-2022.

DISCUSSION

Incidental findings could be identified on CBCT imaging examinations in dental practice, with various prevalence rates reported in different studies worldwide [10, 23, 26-28]. In this study, we aimed to systematically review the available data to provide an overall estimate of the prevalence of incidental findings in oral and maxillofacial CBCT images. For this purpose, we screened hundreds of sources initially generated by database search using strict suitability criteria. Finally, a total of 21 studies (containing more than 10 thousand CBCT scans) were eligible for inclusion in this systematic review and meta-analysis. Based on the analyses, more than two-thirds of the CBCT images demonstrated incidental findings (1.48 incidental findings per CBCT scan). In addition, the prevalence of incidental findings was higher in men than in women. Finally, the pooled prevalence was highest for studies published between 2007 and 2012 and least for those published during 2018-2022.

To the best of our knowledge, this is the first systematic review and meta-analysis that endeavored to give an overall estimate for the incidental findings prevalence in maxillofacial CBCT imaging. In the review article by Khalifa and Felemban [30], the authors assessed five studies on the nature and potential clinical significance of incidental CBCT findings. They categorized the incidental findings as seven anatomic regions, including cervical vertebrae, intracranial, dentoalveolar, temporomandibular joint (TMJ), pharyngeal airway, sinonasal, and soft tissue of the neck. The authors also divided the clinical significance of the incidental findings into high (requiring intervention or referral, such as airway issues and carotid atherosclerosis), moderate (requiring monitoring or follow-up, such as TMJ osteophyte and flattening condyle), and low (such as sinonasal polyps and tonsillolith). They finally declared that most of the incidental findings were normal variants or had low clinical significance.

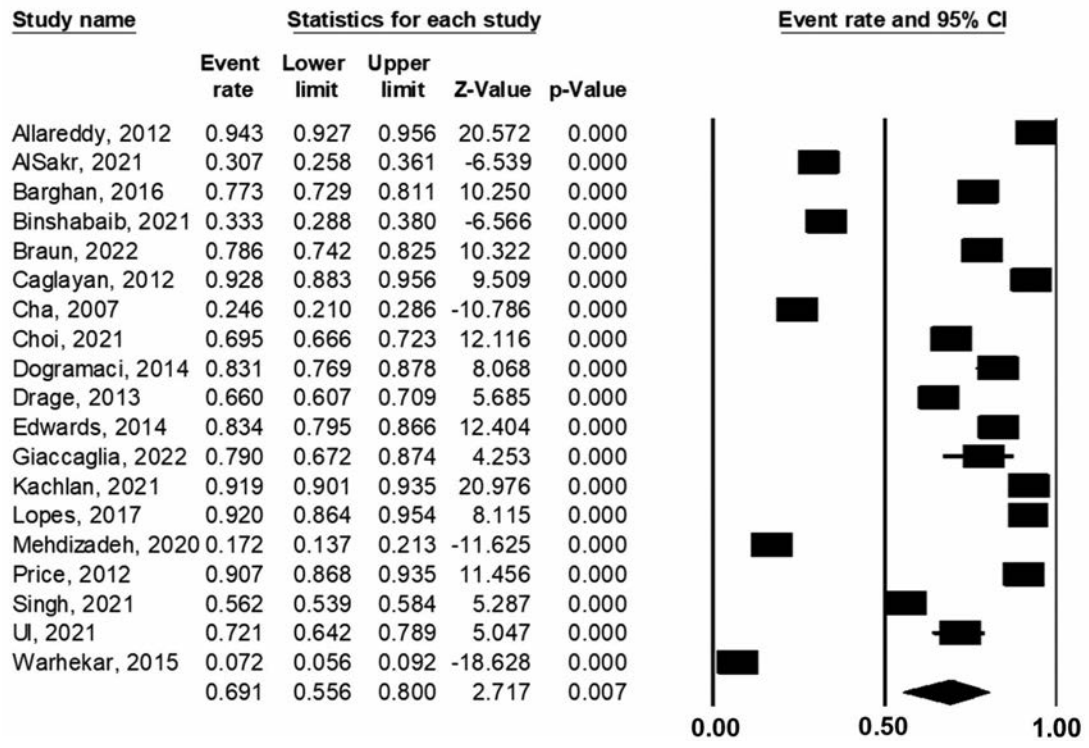

Fig. 2. Pooled prevalence of incidental findings in oral and maxillofacial cone-beam computed tomography

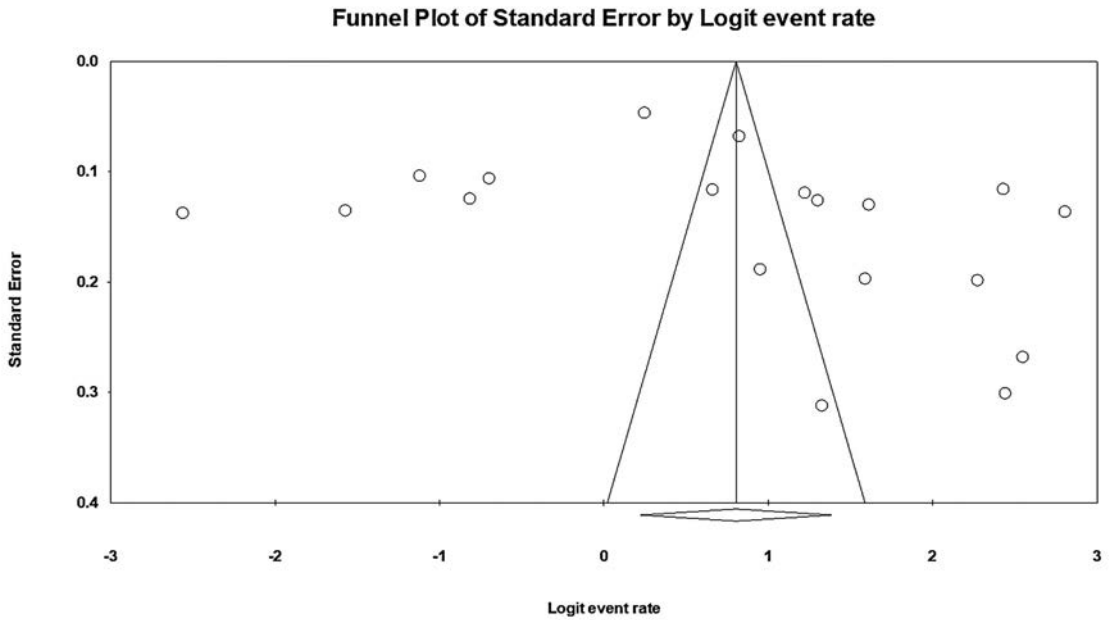

Fig. 3. Funnel plot to assess publication bias across studies assessing incidental findings in oral and maxillofacial cone-beam computed tomography

In the present study, we found a high prevalence of incidental findings reported in CBCT images; however, only some might be notable. Reporting clinically insignificant incidental findings can probably lead to excessive anxiety and stress in the patients, as well as unnecessary clinical/paraclinical procedures. In addition, a lack of sufficient training in CBCT data analysis can result in false-positive detections. On the other hand, according to ethical and legal regulations, clinicians need to report all relevant medical information to patients [31, 32]. Therefore, it is suggested to standardize the threshold of what defines clinically significant incidental findings by professional radiological bodies.

A limitation of the present study was the high heterogeneity between the included surveys, which could be explained by variations in study location, populations, etc. Of course, it should be mentioned that the heterogeneity was not justified by the subgroup analysis according to sex and publications date. On the other hand, publication bias could explain the heterogeneity. It is proposed to perform more homogeneous studies.

CONCLUSION

This systematic review and meta-analysis revealed a considerable prevalence of incidental findings in oral and maxillofacial CBCT scans. Medical and dental specialties need to collaborate to establish professional guidelines on the diagnostic approach, clinical significance, and management of incidental findings in CBCT images.

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