Original Study

MMP20, KLK4, and ENAM Genes Prognostic Significance in The Dental Erosion Development

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ABSTRACT

Background: The importance of polymorphic variants and combinations of MMP20, KLK4, and ENAM genes, isolated from the buccal epithelium, in the development of dental erosion and caries against the background of periodontal disease has not been fully understood.

Aim: The aim was to study the prognostic significance of MMP20, KLK4, and ENAM genes in the development of dental erosion and caries against the background of gingivitis in young people.

Methods: An instrumental dental examination was carried out on 274 students aged 18-25 years, among whom 60 students of both sexes were selected for molecular-genetic research, who were divided into three groups: I (20) - caries was diagnosed on the background of gingivitis, II (21) - dental erosion was diagnosed on the background of gingivitis, III (19) - against the background of a healthy periodontium - dental erosion. To carry out the molecular-genetic study, the buccal epithelium was collected, from which genomic DNA was isolated by extraction using the DNA-sorb-AM nucleic acid extraction kit.

Results: As a result, it was found that a higher frequency of detecting a combination of haplotypes G/G_A/A of KLK4 (T/G) and KLK4 (G/A) genes, T/C_C/C, C/C_A/C, C/C_C/C of MMP20 (T/C) and MMP20 (A/C) genes, A/G_G/G of ENAM and KLK4 (T/G) genes, A/G_A/A of ENAM and KLK4 (G/A) genes indicates an increased risk of developing dental erosion on the background of gingivitis, while the significant prevalence of haplotype combinations T/T_G/G of KLK4 (T/G) and KLK4 (G/A) genes, A/A_G/G of ENAM and KLK4 (G/A) genes reflects an increased risk of caries development.

Conclusion: The identification of certain polymorphic variants and combinations of MMP20, KLK4, and ENAM genes in the buccal epithelium of young people has a prognostic significance in the development of caries and dental erosion against the background of gingivitis even at the preclinical stage, which makes it possible to timely form risk groups to carry out preventive interventions.

Key words: KLK4, MMP20, ENAM, buccal epithelium, dental erosion, caries.

Introduction

Tooth decay [1] is the most frequent chronic human disease [2-4]. The high dental morbidity among young people (18-25 years old) in Ukraine determines the relevance of research aimed at studying the mechanisms and risk factors for the development of the most common diseases: gingivitis, caries, non-carious lesions. By now, a fairly large amount of data has been accumulated on the contribution of genetic factors to the development of periodontal disease and caries [5].

The significance of certain gene markers, isolated from the buccal epithelium, in the initiation of periodontal tissue diseases has been shown by our works, from which it follows that the risk of developing of generalized periodontitis (GP) at a young age (18-25 years old) increases almost 10 times in the presence of a polymorphic variant T894T of eNOS gene and almost 6 times in the presence of the polymorphic variant G308A of TNF-a gene, while polymorphic variants G894G of eNOS gene and G308G of TNF-α gene predominate in persons with healthy periodontium, which indicates their protective effect against the development of GP. The obtained results of a molecular-genetic study of the buccal epithelium with information on the polymorphism of eNOS and TNF-α genes became the basis for the formation of a new protocol for medical examination of young people (18–25 years old) with periodontal tissue diseases, based on a molecular genetic profile [6-8].

Many genes, which encode matrix proteins and proteinases, necessary to control the processes of mineralization and crystallization of maturing enamel, are responsible for the formation of the structure of tooth enamel. Currently, polymorphisms of genes, encoding the main triad of

amelogenetic proteins (amelogenin, enamelin, ameloblastin), have been almost completely identified [9]. Protein breakdown products accumulate in the enamel in the spaces between hydroxyapatite crystals, helping to maintain their structure. Two types of proteases are distinguished in the tooth enamel matrix: enamelisin (MMP20) and kallikrein-4 (KLK-4).

Kallikrein-4 is the main enzyme of the enamel maturation stage and is responsible for the replacement of the protein matrix with minerals and the formation of the correct crystal organization [10]. Peptidase KLK-4 is a kind of evacuator of residual fragments of matrix proteins; when they are replaced by minerals, it regulates the processing of the organic matrix of the enamel, which ultimately determines the structure and composition of the enamel [11]. The polymorphism of the kallikrein-4 (KLK-4) gene determines the presence of a high or low mass fraction of protein, as well as amino acids, such as aspartic, glutamic, histidine, glycine, leucine, lysine, and cystine, comparable to an increase in kallikrein-translated peptidase of the KLK-gene promoter 4, regulating amelogenesis.

Amelogenesis imperfecta can have different inheritance patterns depending on the gene that is altered. Mutations in the ENAM gene are the most commonly known causes of impaired amelogenesis, and are most often inherited in an autosomal dominant manner. This type of inheritance means that one copy of the altered gene in each cell is enough to cause this disorder [12]. Amelogenesis imperfecta can also be inherited in an autosomal recessive manner - this form of the disorder can result from mutations in ENAM, MMP20, KLK4, FAM20A, C4orf26, or SLC24A4 genes [10-12]. With the pathological activity of KLK-4, thinner enamel crystals are formed [13].

Gene MMP-20 encodes the formation of an enzyme - calcium-dependent proteinase, and affects the formation of the organic matrix of enamel. MMP-20 breaks down accumulated enamel proteins layer by layer, therefore their concentration decreases with depth, and the activity of this enzyme is of great importance for enamel formation [14]. As a result of the immunohistochemical analysis, MMP-20 was found in the dentinal tubules of carious lesions; therefore, MMP-20 embedded in dentin during primary dentinogenesis can be released and activated during the development of carious and non-carious lesions of the teeth [15].

Mutations in the genes MMP20, KLK4, ENAM lead to the clinical manifestation of soft (in terms of physical characteristics), porous enamel, which is unable to withstand functional loads due to the residual amount of protein [16]. However, if the role of individual genes in the origin of imperfect amelogenesis and its role in the development of the carious process is presented in the literature quite fully, then the question of the prognostic significance of polymorphic variants and combinations of MMP20, KLK4, and ENAM genes isolated from the buccal epithelium in the development of both dental caries and non-carious lesions, which include enamel erosion, especially often occurring at a young age, is not fully understood.

In this regard, this study aims to study the prognostic significance of the MMP20, KLK4, and ENAM genes, isolated from the buccal epithelium in the development of dental erosion and caries against the background of gingivitis in young people.

Materials and Methods

Dental examination of 274 students aged 18-25 years was carried out, which included the examination of hard tissues of teeth and assessment of periodontal status. For the diagnosis of non-carious lesions, the classification of Yu. A. Fedorov [16] was used, for carious lesions - International Classification of Dental Diseases (ICD-DA, 1995) [17]. Periodontal status was assessed using traditional objective periodontal indices as well as additional radiological examination. The final diagnosis was made based on the classification of periodontal and peri-implant diseases and conditions [18].

After obtaining informed consent, 60 students of both sexes were selected for the molecular genetic study, among whom 41 had gingivitis induced by the microflora of the biofilm, and 19 had a healthy periodontium. Some of the students were diagnosed with caries against the background of gingivitis (G), and some - with dental erosion. Depending on the revealed dental pathology, students were divided into 3 groups: Group I (n=20) - caries was diagnosed against the background of gingivitis; Group II (n=21) - dental erosion against the background of gingivitis; Group III (n=19) - dental erosion against the background of a healthy periodontium.

For molecular-genetic research, the buccal epithelium was obtained from students of all three groups using buccal brushes. The received samples were frozen and stored at -20°C. Isolation of genomic DNA from the buccal epithelium was carried out by the DNA-sorb-AM nucleic

acid extraction kit in accordance with the manufacturer's protocol [19].

To isolate DNA, 300 μL of lysis solution was added to a sterile test tube containing 1.5 μL of the test material, after which the tubes were thoroughly mixed on a vortex mixer and heated at 65°C for 5 min. After lysis of the cells, proteins were removed by centrifugation at 5000 rpm for 1 minute. 20 μL of a silica gel sorbent was added to the test tubes with samples and thoroughly mixed on a vortex mixer for 5 sec, followed by centrifugation at 5000 rpm for 1 min. After removing the supernatant by vacuum suction, 1000 μL of a washing solution was added, then centrifuged at 10000 rpm for 1 minute (the procedure was repeated twice). After the last washing, the samples were placed in a thermostat at 65°C for 5 min until complete drying. To dissolve the DNA, 50 μL of TE-buffer was added, vortexed, and centrifuged at 14000 rpm for 2 min. The resulting supernatant containing purified DNA was used to conduct an allele-specific polymerase chain reaction (PCR).

To identify the polymorphism rs2664152 T/G and rs2664153 G/A of KLK4 gene, rs2245803 A/C and rs1784423 T/C of MMP20 gene, the polymorphic variant of rs12640848 A/G of ENAM gene, amplification of the corresponding regions by the allele-specific polymerase chain reaction was performed using “SNP-EXPRESS” produced by "LITEX". The tubes with the ready amplification mixture were placed in a Perkin Elmer Gene Amp 2007 thermocycler (USA) to ensure the appropriate temperature regime for the polymerase chain reaction.

Statistical processing of the obtained data was carried out using the statistical program "Statistica 6.0". The reliability of the results obtained for different groups was assessed using the Student's t-test. The processing of the research results was carried out using the odds ratio (OR) criterion with the calculation of a 95% confidence interval (CI) for it. In a pairwise comparison of genotype frequencies in groups, the χ^2 test was used [20]. To establish correlations between the indicators, the pairwise Spearman correlation coefficient was calculated [20].

Results

Comparative analysis of the obtained data in patients with caries (group I) and dental erosion (II) against the background of gingivitis showed that the G/G genotype of the KLK4 gene (rs2664152 T/G) was significantly more frequent ($\chi2$=8.14, p=0.004, OR=12.95%, CI 2.20-65.52) among patients of group II with a 12-fold increase in the risk of dental erosion formation, which indicates a significant role of this genotype in the development of dental erosions. The T/T genotype, even though not significant (p>0.05), prevailed in patients with caries and gingivitis (Table 1). Analysis of the alleles of the KLK4 gene showed that in patients with erosions against the background of gingivitis (group II), the mutant allele G was significantly predominant ($\chi2$=9.61, p=0.002, OR=4.67, 95CI%: 1.84-11. 85), whereas for patients with caries against the background of gingivitis (group I), an increase in the T allele (OR=0.21, 95CI%: 0.08-0.54) was characteristic, which may indicate its importance in the development of carious lesions.

	Polymorphic variants of KLK4 gene (rs2664152 T/G)		
	T/T	T/G	G/G
χ^2	2,01	1,28	8,14
p	0,157	0,258	0,004
OR	3,2	2,83	12,00
95%CI	0,84-12,13	0,69-11,60	2,20-65,52

Table 1. Comparative characteristics of polymorphic variants of KLK4 gene (rs2664152 T/G) during the development of caries (group I) or erosions (group II) in patients with gingivitis

A comparative analysis of the obtained data in patients of I (caries against the background of gingivitis) and II (dental erosion against the background of gingivitis) groups, revealed a significant predominance of A/A genotype ($\chi2$=9.24, p=0.002, OR=11.33, 95CI%: 2.46-52.15) in the presence of erosions, with an 11-fold increase in the risk of their formation, which indicates a significant role of this genotype in the development of dental erosions against the background of gingivitis (Table 2). At the same time, in the group of patients with erosions against the background of gingivitis (group II), significant differences were also revealed in individual alleles of the KLK4 gene (rs2664153 G/A); in the presence of allele A, the risk of developing dental erosion increased more than 9 times ($\chi2$=19.49, p=0.001, OR=9.67, 95CI%: 3.51-26.60), and in the presence of G allele, the risk of developing dental erosion, on the contrary, was significantly lower (OR=0.10, 95CI%: 0.04-0.28).

	Polymorphic variants of KLK4 gene (rs2664153 G/A)		
	G/G	G/A	A/A
χ^2	9,47	0,08	9,24
p	0,002	0,783	0,002
OR	0,07	0,94	11,33
95%CI	0,01-0,39	0,23-3,90	2,46-52,15

Table 2. Comparative characteristics of polymorphic variants of KLK4 gene (rs2664153 G/A) during the development of caries (group I) or erosions (group II) in patients with gingivitis

As a result of the analysis of the obtained data in patients with dental erosions against the background of gingivitis (II) and erosions against the background of a healthy periodontium (III), it was found that there were no significant differences between genotypes (Table 3) and alleles (Table 4) for KLK4 gene (rs2664152 T/G). With regard to the KLK4 (G/A) gene during the development of dental erosions, both against the background of gingivitis and against the background of a healthy periodontium, no significant differences were found (p> 0.05).

	Polymorphic variants of KLK4 gene (rs2664152 T/G)		
	T/T	T/G	G/G
χ2	0,03	2,47	2,72
P	0,855	0,116	0,099
OR	0,88	0,26	3,73
95%CI	0,21-3,66	0,06-1,07	0,98-14,23

Table 3. Comparative characteristics of polymorphic variants of KLK4 gene (rs2664152 T/G) with the development of erosions in patients against the background of gingivitis (group II) and healthy periodontium (group III)

	Alleles of KLK4 gene (rs2664152 T/G)	
	T	G
χ^2	1,65	
P	0,199	
OR	0,5	2,00
95%CI	0,20-1,23	0,81-4,94

Table 4. Distribution of alleles of KLK4 gene (rs2664152 T/G) during the development of erosions in patients against the background of gingivitis (group II) and healthy periodontium (group III)

With regard to MMP20 (A/C) gene, a comparative analysis of the obtained data in patients of groups I (caries against the background of gingivitis) and II (dental erosion against the background of gingivitis), revealed the presence of a protective effect of A/A genotype on the development of dental erosion on the background of gingivitis (χ2=4.88, p=0.027, OR=0.13, 95CI%: 0.02-0.71) (Table 5).

	Polymorphic variants of MMP20 gene (rs2245803 A/C)		
	A/A	A/C	C/C
χ2	4,88	1,19	0,08
p	0,027	0,158	0,783
OR	0,13	3,02	1,60
95%CI	0,02-0,71	0,85-10,78	0,38-6,81

Table 5. Comparative characteristics of polymorphic variants of MMP20 gene (rs2245803 A/C) during the development of caries (group I) or erosions (group II) in patients with gingivitis

At the same time, in the presence of the pathological allele C of the MMP20 gene, an increase in the risk of developing dental erosion against the background of gingivitis was found almost 3 times (χ2=3.99, p=0.048, OR=2.45, 95CI%: 1.01-5,96), while in the presence of the A allele of MMP20 gene, there was a significant reduction in the risk of developing of dental erosion (χ2=3.99, p=0.048, OR=0.41 95CI%: 0.17-0.99).

Analysis of data on the rs1784423 T/C polymorphism of MMP20 gene in patients of groups I (caries against the background of gingivitis) and II (dental erosion against the background of gingivitis) showed that it was the presence of C/C genotype that led to the development of dental erosions, while in patients of group I (caries against the background of gingivitis), this genotype was not detected at all (χ2=4.60, p=0.032). In turn, the presence of T/T genotype led to a decrease in the risk of developing dental erosion by almost 6 times (χ2=5.48, p=0.019, OR=0.17, 95CI%: 0.04-0.66), and the presence of C allele led to an increase in the risk of developing dental erosion by 5 times (χ2=9.43, p=0.002, OR=5.19, 95CI%: 1.88-14.32), while allele T led to reduction of this risk (OR=0.19, 95CI%: 0.07-0.53).

Thus, the above data made it possible to establish the role of individual genes identified in the buccal epithelium in the formation of dental erosion and carious lesions in young people; therefore, at the next stage of the study, it was of interest to analyze the influence of possible combinations of these genes on the development of erosion and caries.

In a comparative analysis of the obtained data in patients of groups I (caries against the background of gingivitis) and II (dental erosion against the background of gingivitis), the combination of G/G_A/A haplotypes of the KLK4 gene turned out to be significant, which contributed to a significant increase in the risk of formation of dental erosion by almost 7 times ($\chi2$=4.08, p=0.043, OR=6.75, 95CI%: 1.24-36.85), while the combination of T/T_G/G haplotypes of the same gene led to a decrease in the risk of developing of dental erosions in 8 times ($\chi2$=4.88, p=0.027; OR=0.13, 95CI%: 0.02-0.71).

Analysis of MMP20 haplotype combinations in group II (dental erosion against the background of gingivitis) revealed tendencies towards an increase in the risk of dental erosion against the background of gingivitis in the presence of T/C_C/C, C/C_A/C, C/C_C/C combinations in the examined patients. In addition, the same group was characterized by an increase in the risk of developing dental erosions in the presence of a combination of genotypes A/G_G/G of ENAM and KLK4 rs2664152 T/G genes, which may indicate their combined effect, potentiating each other.

Comparison of the obtained data in patients of groups I (caries against the background of gingivitis) and II (dental erosion against the background of gingivitis) revealed the significance of the combinations of ENAM rs12640848 A/G and KLK4 rs2664153 G/A genes. At the same time, it turned out that genotypes A/A (ENAM) and G/G (KLK4) were significantly more frequent ($\chi2$=5.97, p=0.015) among patients of group I (caries against the background of gingivitis), which indicates a decrease in the risk of developing dental erosion in the presence of this combination and the possible risk of developing carious lesions, while genotypes A/G (ENAM) and A/A (KLK4) were found significantly more frequent ($\chi2$=5.86, p=0.016) among the patients of group II (dental erosion against the background of gingivitis), which led to an increased risk of the formation of dental erosion.

Comparative analysis of the obtained data in patients with dental erosions against the background of gingivitis (group II) and erosions against the background of a healthy periodontium (group III) did not reveal significant differences in the MMP20 gene (Table 6). There were no statistical differences between the data of these groups for the MMP20 rs1784423 T/C polymorphism (p>0.05) (Table 7). Thus, based on the obtained results, it follows that the risk of developing of erosions against the background of gingivitis (group II) significantly increased in the presence in the buccal epithelium of pathological genotype G/G of KLK4 (T/G) gene by 12 times, pathological genotype A/A of KLK4 (G/A) gene by 11.33 times, the combination of G/G_A/A haplotypes of KLK4 (T/G) and KLK4 (G/A) genes by 6.75 times, the pathological allele C of MMP20 gene (A/C) by 2.45 times, the pathological allele C of MMP20 (T/C) gene by 5.19 times, the combination of the haplotypes of KLK4 (T/G) and ENAM genes - A/G_G/G by 4.15 times and with a combination of haplotypes A/G_A/A of ENAM and KLK4 genes (G/A) by 8.04 times. Consequently, the obtained data demonstrate the significant importance of the influence of the genetic component on the risk of developing both erosions and caries in young people against the background of gingivitis.

	Polymorphic variants of MMP20 gene (rs2245803 A/C)		
	A/A	A/C	C/C
$\chi2$	0,02	0,01	0,05
p	0,928	0,948	0,826
OR	1,89	1,18	0,69
95%CI	0,16-22,75	0,33-4,20	0,18-2,59

Table 6. Distribution of genotypes for MMP20 rs2245803 A/C gene during the development of erosions in patients against the background of gingivitis (group II) and healthy periodontium (group III)

	Polymorphic variants of MMP20 gene (rs1784423 T/C)		
	T/T	T/C	C/C
χ^2	0,04	0,01	0,35
p	0,845	0,975	0,557
OR	0,68	0,82	2,12
95%CI	0,17-2,73	0,24-2,84	0,45-10,10

Table 7. Distribution of genotypes for MMP20 rs1784423 T/C gene during the development of erosions in patients against the background of gingivitis (group II) and healthy periodontium (group III)

As a result of the performed correlation analysis, a statistically significant negative relationship of average strength was established in patients of group I [r=-0.475; p=0.0001] between the diagnosed gingivitis and carious lesions. These data coincide with the works of well-known authors [21], which describe the role of cariogenic microorganisms in the initiation of plaque-induced gingivitis. The formation of dental erosion in the absence of a cariogenic microbial factor keeps the periodontal tissues healthy in patients of group III.

Results confirming this assumption were obtained during the analysis of the obtained data in patients with erosions against the background of gingivitis (group II) and against the background of the healthy periodontium (group III), depending on various polymorphic variants of the studied genes.

Thus, if in our previous studies, on the basis of a comprehensive clinical and molecular genetic examination of young people (18-25 years old), the prognostic significance in the development of gingivitis and generalized periodontitis, identified in the buccal epithelium of polymorphic variants of ACE, eNOS and TNF-α [22] genes, has been proven, then based on the results obtained in this study, it is obvious that polymorphic variants of KLK4, ENAM, and MMP20 genes isolated from the buccal epithelium, affect only the formation of dental erosions and caries in young people (18- 25 years), while not affecting the state of the periodontal tissues. Thus, as a result of the comparative analysis of the obtained data in the group of patients with caries against the background of gingivitis (I) and erosions against the background of gingivitis (group II), it was revealed that polymorphic variants G/G of KLK4 gene (T/G), A/A of KLK4 gene (G/A), as well as C/C of MMP20 gene (T/C) were significantly more often ($p<0.05$) among patients of group II, which indicates an increased risk of developing dental erosions against the background of gingivitis in the presence of these genotypes, while the polymorphic variant G/G of KLK4 gene (G/A), A/A of MMP20 gene (A/C) and T/T of MMP20 gene (T/C) were significantly more frequent ($p<0.05$) among patients of group I, which indicates their importance in the development of caries and a protective effect on the development of dental erosion. Higher frequency of detecting a combination of G/G_A/A haplotypes of the KLK4 (T/G) and KLK4 (G/A), T/C_C/C, C/C_A/C, C/C_C/C of MMP20 (T/C) and MMP20 (A/C) genes, A/G_G/G of ENAM and KLK4 (T/G) genes, A/G_A/A of ENAM and KLK4 (G/A) genes reflect an increased risk of developing dental erosion in patients with gingivitis, while the significant prevalence of combinations of T/T_G/G haplotypes of KLK4 (T/G) and KLK4 (G/A) genes, A/A_G/G of ENAM and KLK4 (T/G) genes indicates an increased risk of developing carious lesions in this category of patients.

Consequently, the significance of the individual characteristics of the organism associated with its genetic variability and, consequently, hereditary predisposition, in the violation of the formation of the structure of enamel, is quite obvious. We have shown the significance of the polymorphism of KLK4, MMP20, and ENAM genes, isolated from the buccal epithelium, in the development of caries and non-carious lesions of the teeth, among which dental erosion has taken the leading place in recent years. The obtained results formed the basis for the method of early diagnosis and prediction of the development of dental erosions against the background of periodontal tissue diseases, which allows revealing a genetic tendency to the development of non-carious lesions and timely applying an adequate set of preventive measures, taking into account the individual characteristics of the organism [23].

Conclusions

1. For early diagnosis of caries and dental erosion against the background of gingivitis in young people (18-25 years old), it is necessary to conduct a genetic study of the buccal epithelium.
2. The performed genetic analysis with the determination in the buccal epithelium of young people of polymorphism of KLK4 (rs2664152 T/G and rs2664153 G/A), MMP20 (rs2245803 A/C and rs1784423 T/C), ENAM (rs12640848 A/G) genes, revealed the possibility of early diagnosis of dental erosions developing both against the background of gingivitis and with a healthy periodontium, taking into account the presence of A allele of KLK4 (G/A) gene, the presence of A/G genotype of ENAM gene, the presence of C/C genotype of MMP20 gene as predictors of the development of dental erosion.
3. Revealing in the buccal epithelium of young people (18-25 years old) with gingivitis of polymorphic variants G/G of KLK4 gene (G/A), A/A of MMP20 gene (A/C), and T/T of MMP20 gene (T/C) with the presence of combinations of T/T_G/G haplotypes of KLK4 gene (T/G), A/A_G/G of ENAM and KLK4 genes indicates their prognostic significance ($p<0.05$) for the risk of development of dental caries.
4. Identification of certain polymorphic variants and combinations of MMP20, KLK4, and ENAM genes in the buccal epithelium of young people has a prognostic value in the development of caries and dental erosion against the background of gingivitis, even at the preclinical stage, which allows the timely formation of risk groups to carry out preventive interventions.

References

1. Parandeh Shirvan H, Talebi M, Parisay I, Al-Shuhayeb M. The Effects of Topical Fluoride Therapy on Microleakage of Fissure Sealants in

Permanent Teeth. Int. J. Pharm. Phytopharm. Res. 2020; 10(4): 44-48.
2. Trishala A, Thangavelu L. Physicochemical Profile of Acacia Catechu Bark Extract-An in Vitro Study. Int. J. Pharm. Phytopharm. Res. 2018; 8(5): 84-87.
3. Alavi Enderajemi S A, Hovakhti A. Study of nutritional pattern and degree of dental caries in patients referred to the dental department of Tasu'a clinic during 2017-2018 in Babol. J. Adv. Pharm. Edu. Res. 2018; 8(2): 12-16.
4. Hamedi R, Niaghiha F, Ebrahimi Bazzaz A, Yazdi Z, Ahmadzadeh H, Pachenari F. Malocclusion & occlusal traits among Iranian female elementary students: an epidemiologic study. J. Adv. Pharm. Edu. Res. 2019; 9(2): 24-35.
5. Grigorovich ESh., Pomorgailo EG, Khomutova EYu, Stepanov SS. Clinical variants of chronic generalized periodontitis, genetic polymorphism and systemic production of inflammatory cytokines. Stomatologiya. 2015; (5): 11-16. (In Russ.).
6. Biloklytska GF, Gorgol KO. New protocol of clinical examination of young people (18–25 years old) with periodontal diseases, based on molecular genetic profile. Suchasna stomatologiya. 2020; (1): 52-57. (In Russ.).
7. Biloklytska GF, Gorgol KO, Kir'yachenko SP. Evaluation of the prognostic significance of G894T polymorphism of eNOS gene in young people (18-25 years old) in the occurrence of periodontal diseases. Visn. stomatologiï. 2018; 27(1): 36-41. (In Russ.).
8. Biloklytska GF, Gorgol KO, Kir'yachenko SP. Influence of G308A polymorphism of TNF-α gene in young people (18-25 years old) on the occurrence of periodontal diseases. Visn. stomatologiï. 2018; 28(2): 23-8. (In Russ.).
9. Lukashevich IK. A method for assessing the prognosis of dental caries by analyzing the polymorphism of the kalekrein-4 gene at mutation points g2664153a and g2142a in blood serum. I. K. Lukashevich, I. R. Abramyan, I. L. Gorbunova. EDRID. 2017. Ssylka aktivna na 13.08.2019. (In Russ.). URL:https://scholar.google.com.ua/
10. YLu. Functions of KLK4 and MMP-20 in dental enamel formation. Y. Lu [et al.]. Biol Chem. 2008; 389 (6): 695-700. URL: https://www.degruyter.com/view/journals/bchm/389/6/article-p695.xm https://doi.org/10.1515/BC.2008.080
11. Nagano T, Kakegawa A, Yamakoshi Y, Tsuchiya S, Hu JC, Gomi K, Arai T, Bartlett JD, Simmer JP. Mmp-20 and Klk4 cleavage site preferences for amelogenin sequences. Journal of dental research. 2009;88(9):823-8. URL: https://www.ncbi.nlm.nih.gov/pubmed/19767579 https://doi.org/10.1177/0022034509342694
12. Gorbunova IL. Genetic determination of tissue resistance of periodontium and tooth enamel in the population of the Omsk region. IL. Gorbunova, IK. Lukashevich, AV. Efimenko. Sovremennye problemy nauki i obrazovaniya. 2016; (3). Ssylka aktivna na 14.08.2019 (In Russ.). URL: http://www.science-education.ru/ru/article/view?id=24546
13. T. Nagano. Mmp-20 and Klk4 cleavage site preferences for amelogenin sequences. T. Nagano [et al.]. J Dent Res. 2009; 88 (9): 823-828. URL: https://www.ncbi.nlm.nih.gov/pubmed/19767579
14. Degtyareva YuV., Korenovskii YuV., Oreshaka OV., Atabaeva OA. The role of matrix metalloproteinases in the development of inflammatory periodontal diseases. Parodontologiya. 2018; 2(87) (In Russ.). https://doi.org/10.25636/PMP.1.2018.2.2
15. Yarmolinskaya MI., Molotkov AS., Denisova VM. Matrix metalloproteinases and inhibitors: classification, mechanism of action. Zhurnal akusherstva i zhenskikh boleznej. 2012; LXI (1):120 (In Russ.).
16. Tkachenko IM. Determination of the genetic predisposition of patients to excessive abrasion of the hard tissues of the teeth. Ukraïns'kii stomatologichnii al'manakh. 2015; 2: 41-47 (In Ukr.).
17. Fedorov YA, Drozhzhina VA. Clinic, diagnosis and treatment of non-carious dental lesions. New data on the prevalence, clinic and features of the treatment of non-carious dental lesions. Novoe v stomatologii. 1997; (10): 145. (In Russ.).
18. Maurizio S. Tonetti. Proceedings of the World Workshop on the Classification of Periodontal and Peri Implant Diseases and Conditions. Mauzizio S. Tonetti, Kenneth S. Kornman. Journal of Clinical Periodontology. 2018; 45: 1-8.
19. AmpliSens® DNA-sorb-AM Nabor dlya izvlecheniya nukleinovykh kislot Rukovodstvo po ekspluatatsii (versiya 06.03.2018, obnovlenie: 12.03.2018) – 2018. Ssylka aktivna na 27.11.2019 (In Russ.). URL: https://www.interlabservice.ru › catalog.

20. Metodichni vkazivki do praktichnikh zanyat' iz distsiplini «medichna statistika» -2014. Ssylka aktivna na 19.06.2020 (In Ukr.). URL: http://www.dnu.dp.ua/metodi/fbio/Laboratorna_diagnostika/5Kurs/Medichna%20statistika.pdf
21. Moskovskii AV, Shumskii, AV, Urukov YuN. Study of the combined incidence of caries and its complications with periodontal inflammation. Vestnik Chuvashskogo universiteta. 2007; (2): 89-96. Ssylka aktivna na 19.06.2020 (In Russ.). URL: https://cyberleninka.ru/article/n/issledovanie-sochetannoy-zabolevaemosti-kariesom-i-ego-oslozhneniyami-s-vospaleniem-parodonta
22. Biloklytska G, Gorgol K. Evaluation of the prognostic significance of G894T polymorphism of eNOS gene, G308A of TNF-α gene and I/D of ACE gene in young people (18-25 years) in the onset of periodontal disease. Stomatologia Współczesna. 2018; (2): 8–17. URL: http://medtourpress.pl/stomatologia-wspolczesna-nr-2-2018/ (date of appeal: 19.06.2020).
23. Ukraine patent for utility model number 136781 / 08.27.19 Byul. №16. Biloklytska GF, Turyanska NI. Method for early diagnosis of dental erosions on the background of periodontal tissue diseases in young people. Ssylka aktivna na 07.08.2020. (In Ukr.). URL: http://www.base.uipv.org.

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