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INFLUENCE OF ALLELIC VARIANTS OF PRO-INFLAMMATORY IL1B, IL17, TNF AND ANTI-INFLAMMATORY IL10 CYTOKINE GENES ON THE SEVERITY OF GINGIVITIS IN CHILDREN UNDERGOING ORTHODONTIC TREATMENT

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The study is devoted to determining the effect of allelic variants of pro-inflammatory IL1B, IL17, TNF and anti-inflammatory IL10 cytokine genes on the severity of gingivitis in children undergoing orthodontic treatment. The study involved 18 children undergoing orthodontic treatment with varying degrees of severity of gingivitis. Dental examination was carried out in a dental office. The data presented in the paper on the effect of genetic polymorphisms of cytokine genes on the severity of gingivitis in children undergoing orthodontic treatment allows timely identification of potential genetic risk groups for inflammatory periodontal diseases for prevention and therapy, taking into account the individual characteristics of the patient.

Key words: gingivitis, oral health, genetic polymorphism, children, polymerase chain reaction.

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ВПЛИВ АЛЕЛЬНИХ ВАРІАНТІВ ГЕНІВ ЦИТОКІНІВ ПРОЗАПАЛЬНИХ IL1B, IL17, TNF ТА ПРОТИЗАПАЛЬНОГО IL10 НА СТУПІНЬ ТЯЖКОСТІ ГІНГІВІТУ У ДІТЕЙ, ЩО ПРОХОДЯТЬ ОРТОДОНТИЧНЕ ЛІКУВАННЯ

Дослідження присвячене визначенню впливу алельних варіантів генів цитокінів прозапальних IL1B, IL17, TNF та протизапального IL10 на ступінь тяжкості гінгівіту у дітей, які проходять ортодонтичне лікування. У дослідженні брали участь 18 дітей, які проходять ортодонтичне лікування, з різним ступенем тяжкості гінгівіту. Стоматологічний огляд було проведено за умов стоматологічного кабінету. Представлені в роботі дані про вплив генетичних поліморфізмів генів цитокінів на ступінь тяжкості гінгівіту дітей, які проходять ортодонтичне лікування, дозволяє своєчасно виявити потенційні групи генетичного ризику запальних захворювань пародонту для профілактики та терапії з урахуванням індивідуальних особливостей пацієнта.

Ключові слова: гінгівіт, здоров'я порожнини рота, генетичний поліморфізм, діти, полімеразна ланцюгова реакція.

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Prevention and treatment of periodontal diseases is one of the urgent problems of modern dentistry. In the progression of periodontal diseases, immune-inflammatory processes with the participation of cytokines responsible for tissue destruction play a key role.

It has been shown that an incorrect bite affects the health of the periodontium [3]. Orthodontic treatment affects gum health, and plaque buildup on orthodontic appliances causes gingivitis. The presence of gingivitis is a very common phenomenon in adolescents and adults undergoing orthodontic treatment. Therefore, orthodontic patients must follow a strict oral hygiene protocol to maintain optimal gum health [1]. However, even after maintaining good oral hygiene, patients usually develop mild to moderate gingivitis within 1–2 months after appliance placement. If it is not timely diagnosed and treated, the development of inflammatory and destructive periodontal diseases is possible. Inflammatory periodontal diseases are one of the most complex problems of dentistry, which is explained both by their multicomponent nature and by the long course due to the insufficient effectiveness of the proposed means and methods of their use.

An important role in the pathogenesis of gingivitis is played by the regulation of the inflammatory response. The degree of expressiveness of inflammatory and destructive processes in periodontal diseases has a genetic component [6]. Gene polymorphisms in the regulatory regions of cytokine-encoding genes that affect the amount of cytokines produced and play a fundamental role in inflammatory diseases. It has been shown that salivary cytokines are correlated with periodontal status and inflammatory load in the oral cavity [8].

The purpose of the study was to determine the influence of allelic variants of pro-inflammatory IL1B, IL17, TNF and anti-inflammatory IL10 cytokine genes on the severity of gingivitis in children undergoing orthodontic treatment.

Materials and methods. Genomic DNA samples of 18 children aged 13–17 years undergoing orthodontic treatment were used for molecular genetic analysis. The children were divided into 2 groups: 7 children with mild gingivitis and 11 with moderate and severe gingivitis.

Dental examination was conducted in the dental office at the Department of Epidemiology and Prevention of Major Dental Diseases, Pediatric Dentistry and Orthodontics of the SE "The Institute of stomatology and maxilla-facial surgery National academy of medical sciences of Ukraine" (SE "ISMFS NAMS").

Using the Parma index (PMA), the prevalence of the inflammatory process in the periodontal tissues was assessed and the severity of gingivitis was determined: up to 25 % – mild, from 25 % to 50 % – medium, and above 50 % – severe.

DNA isolation from buccal epithelial cells was performed according to a modified method using Chelex [10]. Allelic variants of polymorphisms IL1B T-511C rs16944, IL17 G–197A rs2275913, TNF G–308A rs1800629, IL10 G–1082A rs1800896 were assessed by allele-specific PCR method. Amplification of the studied regions of the genes was carried out in parallel in two test tubes (Eppendorf) for the normal and mutant allele of each gene in 20 μl of a buffer solution with the addition of 100 nM of each pair of allele-specific oligonucleotide primers (sets SNP-express-EF NPF "Litekh"). As a negative control sample, diluent in a volume of 5 μl was added to both types of reaction mixture. Amplification was performed on a "Labcycler" thermal cycler (SensQuest, Germany). Amplicons were visualized by electrophoresis in a 2 % agarose gel.

Statistical processing of the obtained results, including the test for deviation from the Hardy-Weinberg equilibrium (HWE) and the assessment of the association of genotypes and alleles with the risk of periodontitis by the Pearson $\chi 2$ method, was carried out using the DeFinetti genetic statistics program on the website of the Institute of Genetics (Munich, Germany). Associations were characterized by odds ratio (OR) with 95 % confidence interval and Pearson's $\chi 2$ test. The difference was considered statistically significant at $p<0.05$ [2].

Results of the study and their discussion. 18 children aged 13–17 years who are undergoing orthodontic treatment were examined. Mild gingivitis was found in 38.8 % (7 patients) of the examined group. Moderate and severe gingivitis (PMA index>30 %) was found in 61 % of the examined (11 patients).

The pro-inflammatory cytokine interleukin-1 (IL-1) is a key mediator of the inflammatory process. The T–511C polymorphism of the interleukin 1 (IL1B) gene was studied in children undergoing orthodontic treatment. The study of the results, presented in table 1, showed that T and C alleles of this gene are represented in the ratio of 80.6 % and 19.4 % (Table 1).

Table 1

Frequency of alleles and genotypes of genes IL1B T–511C rs16944, IL17 G–197A rs2275913, TNF G–308A rs1800629, IL10 G–1082A rs1800896 in children undergoing orthodontic treatment

IL1B T–511C rs16944		**IL17** G–197A rs2275913		**TNF** G–308A rs1800629		**IL10** G–1082A rs1800896	
Allele, genotype	n=18 n (%)	Allele, genotype	n=18 n (%)	Allele, genotype	n=18 n (%)	Allele, genotype	n=18 n (%)
T	29(80.6)	G	21(58.3)	G	22(61.1)	G	15(41.7)
C	7(19.4)	A	15(41.7)	A	14(38.9)	A	21(58.3)
TT	11(61.1)	GG	8(44.4)	GG	9(50)	GG	3(16.7)
TC	7(38.9)	GA	5(27.8)	GA	4(22.2)	GA	9(50.0)
CC	0	AA	5(27.8)	AA	5(27.8)	AA	6(33.3)

The distribution of genotypes of the T-511C polymorphism of the interleukin 1 (IL1B) gene in the studied sample of children is as follows: the functionally complete T/T genotype is 61.1 %, the heterozygous variant of the gene was found in 38.9 % of the examined children. A minor homozygous variant of the C/C gene was not detected.

IL-17 plays a key role in the body's defense against extracellular bacterial and fungal infections. The distribution of different IL-17A G/A genotypes among patients undergoing orthodontic treatment was 44.4 % for the GG genotype, 27.8 % for the AG genotype, and also for the AA genotype. The distribution of the G allele was 58.3 %, and the A allele was 41.7 %.

Tumor necrosis factor (TNF) plays an important role in the protection of periodontal tissues from infection. In its absence, anti-infective resistance is impaired. The tumor necrosis factor gene is activated together with other pro-inflammatory cytokines responsible for protein synthesis. After activation of TLR receptors of innate immunity and their recognition of antigens of microorganisms, as well as endogenous molecules detected during tissue inflammation. A study of the G–308A polymorphism of the TNF-α gene, a cytokine involved in systemic inflammation, was conducted in 18 children undergoing orthodontic treatment. It was established that the functional variant of the TNF-G/G gene (50.0 %) and the G allele (61.1 %) at the promoter position –308 predominate among the examined patients. Heterozygous genotype was found in 22.2 % of patients in the study group. The 308G>A polymorphism of the TNF-α gene in the A/A homozygous state in the studied group of children is 27.8 %.

IL-10 is a key regulator of the immune response. This interleukin suppresses the synthesis of pro-inflammatory cytokines by macrophages. IL-10, an anti-inflammatory cytokine, prevents a protective immune response to pathogens by blocking the production of pro-inflammatory cytokines such as TNF-α and the Th1-polarizing cytokine IL-12 by directly acting on antigen-presenting cells such as macrophages and dendritic cells. The G–1082A rs1800896 polymorphism of the interleukin 10 (IL10) gene was studied in children undergoing orthodontic treatment. The study showed that the functionally complete G/G genotype is 16.7 %. Mutant genotype A/A among the examined patients was found in 33.3 %. The most frequent heterozygous variant of the G –1082A gene was found – 50.0 %. Allele G is 41.7 %, allele A – 58.3 %.

Table 2 revealed a reliable association of the severity of gingivitis with the carrier of the T-511C allele of the IL1B gene (OR 6,53 CI (1.36–31.35)) (Table 2).

Table 2

Comparative analysis of the distribution of IL1B, IL17, TNF, IL10 gene alleles in groups with different degrees of severity of gingivitis

Gene/ polymorphism/allele / Groups		Mild degree of gingivitis	Moderate and severe gingivitis	Odds ratio (OR), Confidence interval 95 % (CI), Рівень значущості (p)
IL1B T–511C rs16944	T	14	15	OR=6.53 (1.36–31.35) p<0.05
	C	0	7	
IL17 G–197A rs2275913	G	10	11	OR=2.50 (0.59–10.44) p>0.05
	A	4	11	
TNF G–308A rs1800629	G	10	12	OR=2.08 (0.49–8.71) p>0.05
	A	4	10	
IL10 G–1082A rs1800896	G	6	9	OR=1.08 (0.27–4.21) p>0.05
	A	8	13	

According to the results of the comparative analysis of polymorphic loci of IL17, TNF, IL10 genes in groups with different degrees of gingivitis, no statistically significant differences were found in the allele frequencies of the studied genes.

Interleukin 1 is a pro-inflammatory cytokine released by monocytes, macrophages and dendritic cells. The T–511C polymorphism of the IL1B gene affects the level of expression and is associated with inflammatory and oncological diseases. The role of interleukin 1 in the development of periodontal diseases consists in the induction of inflammatory mediators. It was shown that in the lines of immortalized human gingival fibroblasts, in the presence of interleukin 1, the level of transcription of genes for inflammatory cytokines, chemokines, metalloproteases, cell adhesion molecules and the transcription factor NF-kβ, which controls the expression of immune, antiapoptotic response and cell cycle genes, increases. IL-17A G –197A (rs2275913) polymorphism is associated with susceptibility to various types of proinflammatory diseases. IL-17 may play an important role in the etiopathogenesis of periodontal diseases, as it increases the inflammatory response in periodontal tissues [5]. IL-17A has been shown to induce the production of inflammatory chemokines and cytokines by macrophages and neutrophils. In the rs22759133 allele, the G nucleotide at position –197 is located in the promoter region of the IL-17A gene near the motifs that bind to two nuclear factors of activated T cells. This region is required for the expression of the IL-17A gene, it has been shown that replacing G at this position with A leads to increased production of the cytokine. In our study, the presence of the mutant allele A increases the severity of gingivitis by 2.5 times compared to the group carrying the functionally complete allele G. Tumor necrosis factor (TNF) plays an important role in the protection of periodontal tissues from infection. G-308A polymorphism of the TNF-α gene is

associated with increased basal and induced production of TNF-α during inflammation. The G/A polymorphism of TNF-α-308 (rs1800629) is localized in the promoter region. Allele-G is associated with a normal level of tumor necrosis factor TNF-α in blood plasma. Allele A is associated with an increased level of TNF-α production, which leads to the activation of local inflammatory reactions and dysregulation of various biochemical processes [9]. The study showed that carriers of the mutant allele A are prone to a more severe course of gingivitis (2 times) compared to a group of children undergoing orthodontic treatment who have a functionally complete allele G. The lack of statistical validity in the study of the G/A polymorphism of TNF in relation to different degrees of gingivitis, as well as for the G-197A polymorphism of the IL17 gene, can be explained by the small sample of patients. IL-10 is a key regulator of the immune response. This interleukin suppresses the synthesis of pro-inflammatory cytokines by macrophages [4]. In addition, IL-10 suppresses the synthesis of active forms of nitrogen and oxygen by macrophages and monocytes. IL-10 slows down the transformation of blood monocytes into tissue macrophages and dendritic cells. IL-10, as a rule, is not detected either in the blood or in the gingival fluid of healthy people [7]. The study of the influence of the G-1082A polymorphism of the IL10 gene in children undergoing orthodontic treatment on the severity of gingivitis showed that this polymorphism does not affect the severity of gingivitis. No statistically significant differences were found between groups of children with different genotypes.

Conclusions

1. A reliable association of the severity of gingivitis in children undergoing orthodontic treatment with the carrier of the T-511C allele of the pro-inflammatory IL1B gene was revealed.

2. The study of IL17G-197A and TNF-α-308G/A gene polymorphism showed that carriers of mutant alleles are prone to a more severe course of gingivitis compared to a group of children undergoing orthodontic treatment who have functionally complete alleles.

3. The study of the influence of the G-1082A polymorphism of the IL10 gene in children undergoing orthodontic treatment on the severity of gingivitis showed that this polymorphism does not affect the severity of the course of gingivitis.

4. The data presented in the work on the influence of genetic polymorphisms of cytokine genes on the severity of gingivitis in children undergoing orthodontic treatment allows timely identification of potential genetic risk groups for inflammatory periodontal diseases for prevention and therapy, taking into account the individual characteristics of the patient.

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