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POLYMORPHISM OF GROWTH FACTOR GENES IN CHILDREN WITH CHRONIC DISEASES OF THE GASTROINTESTINAL TRACT AND DENTAL PATHOLOGY

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The study is devoted to determining the effect of polymorphism of endothelin-1 "EDN1", epidermal growth factor receptor "EGFR", transforming growth factor β1 "TGF-β1" in children with chronic gastroenterological diseases on indicators of hard tissues of permanent teeth, periodontal indices and hygiene indices of oral cavity. 20 children with chronic gastroenterological diseases participated in the research. All patients underwent a comprehensive examination of the main disease and dental status according to a single scheme. The dental examination was carried out in a dental office. As a result of the research, it was established that the data on the influence of genetic polymorphisms of genes on dental indicators, presented in the work, allow to develop effective methods of forecasting and treatment of the main dental diseases in children with chronic gastroenterological diseases.

Key words: hygiene indices, hard tissues of teeth, oral health, polymerase chain reaction, children.

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ПОЛІМОРФІЗМ ГЕНІВ ФАКТОРІВ РОСТУ У ДІТЕЙ З ХРОНІЧНИМИ ЗАХВОРЮВАННЯМИ ШЛУНКОВО-КИШКОВОГО ТРАКТУ ТА СТОМАТОЛОГІЧНОЮ ПАТОЛОГІЄЮ

Дослідження було присвячено визначення впливу поліморфізму ендотеліну-1 «EDN1», рецептора епідермального фактора росту «EGFR», трансформуючого фактора росту β1 «TGF-β1» у дітей з хронічними гастроентерологічними захворюваннями на показники твердих тканин постійних зубів, пародонтальних індексів та індексів гігієни порожнини рота. У дослідженнях брали участь 20 дітей із хронічними гастроентерологічними захворюваннями. Усі пацієнти проходили комплексне обстеження основного захворювання та стоматологічного статусу за єдиною схемою. Стоматологічний огляд було проведено в умовах стоматологічного кабінету. В результаті проведених досліджень було встановлено, що дані про вплив генетичних поліморфізмів генів на стоматологічні показники, представлені в роботі, дозволяють розробляти ефективні методи прогнозування та лікування основних стоматологічних захворювань у дітей із хронічними гастроентерологічними захворюваннями.

Ключові слова: індекси гігієни, тверді тканини зубів, здоров'я порожнини рота, полімеразна ланцюгова реакція, діти.

The work is a fragment of the research project "Correction of pathogenetic mechanisms of disorders of carbohydrate and lipid metabolism in the body and tissues of the oral cavity in patients depending on environmental and nutritional factors affecting carbohydrate and lipid metabolism", state registration No. 0118U006966.

The anatomical and physiological proximity of the organs of the oral cavity and the digestive tract, the common innervation and humoral regulation create prerequisites for the involvement of the organs of the oral cavity in the pathological process with various disorders in the organs of the gastrointestinal tract [1]. Chronic diseases of the stomach and intestines cause various degrees of impaired absorption of food substances, which lead to metabolic diseases, which in turn adversely affect the periodontal condition, the formation and structure of hard tissues of teeth in children.

Various factors, such as cellular, molecular, and genetic factors, can influence the severity of digestive diseases. Several studies have suggested that genetic factors largely explain, in particular, the pathogenesis of gastroesophageal reflux disease, its complications, and the phenotypic variance in the severity of some symptoms, with the contribution of heredity to the development of the disease estimated at 31 % [7]. Mechanisms of regulation of restorative processes in the gastroduodenal mucosa are carried out under the control of cytokines belonging to the group of growth factors. Growth factor genes such as transforming growth factor-β1 (TGF-β1), epidermal growth factor (EGF) deserve special attention. The effect of endothelin (EDN1) on inflammatory mediators is of great importance in the development of the pathological process. Data from the literature show that cytokines of the TGF-β group in the gastrointestinal tract perform the functions of regulators of cell growth and differentiation, metabolism of extracellular matrix proteins, immunological homeostasis, and inflammatory reactions [4]. Epidermal growth factor (EGF) induces cell proliferation, participates in the regulation of their differentiation and promotes the formation of blood vessels [11].

The study of genetic polymorphisms and their associations in children with diseases of the gastrointestinal tract with dental pathology is becoming important today, because it allows to take preventive measures in time, preventing the development or easing the course of the disease, and to apply therapy taking into account the individual characteristics of the patient.

The purpose of the study was to determine the effect of polymorphism of endothelin-1 "EDN1", epidermal growth factor receptor "EGFR", transforming growth factor β1 "TGF-β1" in children with chronic gastroenterological diseases on indicators of hard tissues of permanent teeth, periodontal indices and hygiene indices of oral cavity.

Materials and methods. Genomic DNA samples of 20 children aged 13–17 years with chronic diseases of the gastrointestinal tract and dental pathology were used for molecular genetic analysis. All patients underwent a comprehensive examination of the main disease and dental status according to a single scheme.

The intensity of the carious process in permanent teeth was determined by the DMFS index.

The hygienic condition of the oral cavity in children was determined according to the Stallard index (1969). According to this index, the area of dental plaque was taken into account.

Using the PMA index (Parma), we assessed the prevalence of the inflammatory process in periodontal tissues and determined the severity of gingivitis: up to 25 % – mild, from 25 % to 50 % – moderate, and above 50 % – severe. The degree of the inflammatory process was determined by the intensity of staining of gum tissue with an iodine-containing solution using the Schiller-Pysarev (S-P) test. Bleeding was determined by the method of probing the gingival furrow Muhnlemann, Son (1971) [10, 13].

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").

Isolation of DNA from cells of the buccal epithelium was carried out using a modified Chelex method [14]. Allelic variants of genes EDN1(Lys198Asn), EGFR (A2073T), TGF-β1 (Arg25Pro) were evaluated by the method of allele-specific polymerase chain reaction (PCR). Amplification of the studied regions of the genes was carried out in parallel in two eppendorfs for the normal and mutant variant of the gene in 20 μl of a buffer solution and 100 nm of each oligonucleotide primer, 100. As a negative control sample, a diluent was introduced in vol. add 5 μl to both types of reaction mixture. Amplification was performed on an "Analytik Jena" (Flex Cycler, Germany) thermal cycler. Amplicons were visualized by electrophoresis in a 2 % agarose gel.

During the statistical processing of the obtained results, the computer program STATISTICA 6.1 was used to assess their reliability and measurement errors. Statistical processing of the experimental study results was carried out by the methods of variation analysis using the Student's test. The difference was considered statistically significant at $p<0.01$ [3].

Results of the study and their discussion. The frequency of alleles and genotypes of genes EDN1 (Lys198Asn(G/T5665), EGFR (A2073T), TGF-β1(Arg25Pro(C915T)) in children with chronic diseases of the gastrointestinal tract and dental pathology is presented in Table 1.

Table 1

The frequency of occurrence of alleles and genotypes of the studied genes in children with chronic diseases of the gastrointestinal tract and dental pathology

Allele, genotype EDN1	EDN1 Lys198Asn (G/T5665), n=20 n, %	Allele, genotype EGFR	EGFR A2073T, n=20 n, %	Allele, genotype TGF-β1	TGF-β1 Arg25Pro C915T, n=20 n, %
G	36(90)	A	20(50)	C915	32(80)
T	4(10)	T	20(50)	915T	8(20)
G/G	16(80)	A/A	5(25)	C/C	14(70)
G/T	4(20)	A/T	10(50)	C/T	4(20)
T/T	0	T/T	5(25)	T/T	2(10)

One of the biomarkers and potent vasoconstrictors synthesized in the endothelium is endothelin-1 (EDN1). In this work, the Lys198Asn(G/T5665) polymorphism of the EDN1 gene was studied in a group of children with chronic diseases of the gastrointestinal tract. A functionally complete allele G was detected in 90 % of children, the genotype G/G – in 80 %. Carriers of the heterozygous G/T genotype make up 20 %. The minor T genotype was not detected in the studied group of children with chronic diseases of the gastrointestinal tract. The G/T substitution in the fifth exon at the 5665th position of the nucleotide sequence leads to the replacement of the amino acid lysine (Lys) with asparagine (Asn) at the 198th position

of the amino acid sequence, changing the protein structure and activity of the enzyme. Homozygous carrier of the Asn allele of this polymorphic marker is associated with a high level of endothelin-1.

Epidermal growth factor (EGF) is involved in cell proliferation and differentiation. EGF is an important growth factor for the regulation of normal esophageal tissue through binding to EGFR. The activity of growth factors depends on the expression of the receptor apparatus. EGFR is found in various body tissues. EGFR activity is important for most of its functions, including altering cell motility and initiating DNA synthesis. The epidermal growth factor receptor (EGFR), found in the epithelium of the digestive tract, plays an important role in epithelial repair and shows increased expression in various neoplasms, including esophageal tumors. The epidermal growth factor receptor (EGFR) gene polymorphism A2073T was studied in children with chronic diseases of the gastrointestinal tract. The study showed the following prevalence of the genotypes of the A2073T polymorphism of the EGFR gene in the sample of children under study: The functionally complete A/A genotype is 25.0 %. Mutant genotype T/T among the studied patients was also found in 25.0 %. The most common was the heterozygous variant of the A2073T gene – 50.0 %. Alleles A and T are equally represented – 50.0 % each.

EGFR binds not only epidermal growth factor, but also TGF-β1, the transforming growth factor-β1 gene, thus stimulating the processes of cell proliferation and repair. This cytokine regulates the expression of genes encoding fibrillar collagen types I and III. TGF-β1, produced by mast cells, eosinophils, and esophageal epithelial cells, is a key cytokine for epithelial fibrosis and epithelial cell transformation. A study of the C915T(Arg25Pro) gene TGF-β1 polymorphism in a group of children with chronic diseases of the gastrointestinal tract showed that 80 % of children are carriers of the homozygous C/C genotype. The heterozygous variant of the C915T gene (Arg25Pro) was found in 18.4 %. The minor T/T (Pro25Pro) genotype among the studied patients is present in 1.6 %.

Table 2 shows the assessment of the influence of polymorphic variants of the studied genes EDN1 (Lys198Asn(G/T5665), EGFR (A2073T) and TGF-β1(C915T, Arg25Pro) on indicators of hard tissues of permanent teeth, periodontal indices and oral hygiene index in children with chronic gastroenterological diseases.

Table 2

Assessment of the impact of polymorphic variants of the studied genes on indicators of hard tissues of permanent teeth, periodontal indices and oral hygiene index in children with chronic gastroenterological diseases, M±m

	EDN1 Lys198Asn (G/T5665)		EGFR A2073T			TGF-β1 Arg25Pro C915T	
Genotype	G/G	G/T	A/A	A/T	T/T	C/C	C/T+T/T
n	16	4	5	10	5	14	6
%	80	20	25	50	25	70	30
DMFS	5.0±3.0	5.3±3.5 p>0.05	5.8±4.2	3.8±3.4 p>0.05	2.8±1.5 p>0.05	5.0±3.4	1.8±3.6 p>0.05
PMA, %	43.6±19.1	42.6±21.1 p>0.05	53.1±12.2	47.3±17.0 p>0.05	17.8±16.4 p>0.05	42.3±19.2	36.2±28.3 p>0.05
S-P test	1.75±0.30	1.7±0.36 p>0.05	1.8±0.20	1.7±0.3 p>0.05	1.3±0.8 p>0.05	1.64±0.5	1.7±0.43 p>0.05
Bleeding index	0.79±0.32	0.7±0.25 p>0.05	0.62/0.27	0.77±0.38 p>0.05	0.5±0.40 p>0.05	0.72±0.41	0.63±0.34 p>0.05
Stallard	2.1±0.55	1.7±1.1 p>0.05	2.0±0.08	2.1±0.8 p>0.05	1.9±0.8 p>0.05	2.1±0.6	2.0±0.8 p>0.05

Note. p – reliability indicator of differences of clinical indicators of a group of children with functionally complete genotypes from a group of children with polymorphic genotypes.

The study of the effect of polymorphism of the endothelin-1 (EDN1) gene in children with chronic gastroenterological diseases on indicators of hard tissues of permanent teeth, periodontal indices and oral hygiene indices showed that this polymorphism does not affect the indicators of the studied dental indices.

When comparing the average values of the hard tissues of permanent teeth in the examined children, it was found that the DMFS index is 1.5 times higher in the presence of the functionally complete AA genotype of the EGFR gene compared to children carrying the heterozygous AT genotype, and 2 times higher in the presence of the mutant TT genotype. The same trend is observed for the TGF-β1 gene: the presence of the A2073T mutant T allele reduces the DMFS index by 2.8 times in children with chronic gastroenterological diseases.

The study of periodontal indices showed that the degree of inflammation according to the PMA index was 53.1±12.2 in children with a functionally complete genotype of the EGFR gene, and in children with a homozygous mutant gene this value was 17.8±16.4, i.e. 3 times less. This regularity is also found in the presence of the mutant allele in the heterozygous form, but to a lesser extent (12 % decrease). Intensity

of inflammation (Schiller-Pysarev test) decreases by 1.4 times in carriers of the homozygous mutant genotype, and bleeding (bleeding index) decreases by 1.2 times.

The presence of the mutant allele 915T of the TGF-β1 gene also helps to reduce the values of periodontal indices by 15–20 % in children with chronic gastroenterological diseases compared to children of the same group without this gene polymorphism.

Polymorphism of EGFR and TGF-β1 genes does not affect Stallard oral hygiene index.

A polymorphic variant of the EGFR gene, resulting from an A/T substitution at position 2073, leads to the splitting of the "wild" fragment, resulting in a truncated form of EGFR, which forms a shortened transcript without a transmembrane domain, which cannot bind inside the membrane and cannot act as a signal transmitter. The -2073T/T genotype of the EGFR gene contributes to a lower expression of the epidermal growth factor EGF.

It was shown that the variant TT genotype of the EGFR c.2073A>T polymorphism was associated with a significant reduction in the risk of astrocytoma compared with the AA genotype.

The TGF-β1 gene is highly polymorphic. A polymorphism at position +915 of the signal sequence that changes codon 25 (arginine→proline) is associated with individual variation in TGF-β1 production levels. The homozygous C915C(Arg25Arg) genotype is associated with a higher level of TGF-β1 than the heterozygous C915T(Arg25Pro) genotype, which leads to suppression of the inflammatory response and dysregulation of the above processes. The C915T(Arg25Pro) polymorphism of the TGF-β1 gene can influence the modulation of the inflammatory process in periodontitis. It was demonstrated that 72.5 % of patients with chronic periodontitis had the 915C genotype. In healthy people, this genotype was found in 52.5 % of cases. Most likely, children with the C/C (Arg/Arg) genotype of the TGF-β1 gene compared to carriers of the T/T (ProPro) genotype will have more significant changes in the structure of the esophagus tissues, which can lead to a more severe course of the underlying disease and periodontitis.

Endothelial dysfunction affects regional blood circulation, causing tissue hypoxia, thereby reducing the resistance of the esophageal tissue and its ability to resist acid reflux. Tissue hypoxia leads to higher levels of peroxidation products and decreased esophageal tone and motility, supporting increased production of hydrochloric acid. On the other hand, in patients with chronic diseases of the gastrointestinal tract, endothelial dysfunction can develop as a secondary pathology, increasing the clinical course of the disease [12]. TGF-β1 can exert a profibrotic effect on esophageal fibroblasts and directly induce the expression of profibrotic genes such as fibronectin, collagen I, periostin, and smooth muscle actin in fibroblasts [8, 9]. At the same time, it enhances the synthesis of intercellular matrix proteins, promotes wound healing, and has an anabolic effect. Endothelin, affecting cytokines and other mediators of inflammation, leads to the development of a pathological process in the periodontium [5], however, as our study showed, these changes are not critical. The presence of the minor T allele in the heterozygous genotype in the studied group of children may affect the development of endothelial dysfunction. Heterozygous AT genotypes also have protective properties [2]. In an experimental mouse model of periodontitis, it was demonstrated that EGFR inhibition provides a significant reduction in periodontal inflammation and alveolar bone loss [6]. Thus, inhibition of EGFR is important for the restoration of the epithelium of the digestive tract and is potentially an effective treatment of periodontitis. Lys198Asn(G/T5665) polymorphism of the endothelin-1 (EDN1) gene in children with chronic gastroenterological diseases does not affect the indicators of hard tissues of permanent teeth, periodontal indices and oral hygiene index. The obtained data on the influence of genetic polymorphisms of genes on dental indicators allow for the timely development of effective methods of forecasting and treatment of both hard tissues of teeth and inflammatory-dystrophic changes in periodontal tissues.

Conclusions

1. EDN1 gene polymorphism in children with chronic gastroenterological diseases does not affect indicators of hard tissues of permanent teeth, periodontal indices and oral hygiene index.

2. The study of the effect of EGFR and TGF-β1 gene polymorphisms in children with chronic gastroenterological diseases on indicators of hard tissues of permanent teeth, periodontal indices and oral hygiene indices showed that the presence of mutant alleles of the studied genes is a protective factor. EGFR, TGF-β1 gene polymorphisms do not affect the "Stallard" oral hygiene index.

3. The data on the influence of genetic polymorphisms of genes on dental indicators presented in the work allow for the development of effective methods of forecasting and treatment of the main dental diseases in children with chronic gastroenterological diseases.

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MANIFESTATIONS OF DYSTROPHY IN THE PERIODONTIUM. CLINICAL AND ULTRASTRUCTURAL STUDY

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Dystrophic changes of all periodontal structures and teeth in patients with periodontitis and generalized periodontitis were investigated in the study. Characteristic signs of the gums' pathological contour, as well as other clinical and radiological features of dystrophy differ in patients with periodontitis and generalized periodontitis and contribute to the improvement of their differential diagnosis. Ultrastructural examination of the patients' gums with periodontitis revealed disseminated microthrombosis, mucoid edema and fibrinoid transformation of intermediate connective tissue, and coagulation-dystrophic changes in periodontal tissues and cells.
Key words: periodontitis, parodontosis, gingival ultrastructure, coagulation dystrophy

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ПРОЯВИ ДИСТРОФІЇ В ПАРОДОНТІ. КЛІНІКО-УЛЬТРАСТРУКТУРНЕ ДОСЛІДЖЕННЯ

У роботі досліджувалися дистрофічні зміни всіх структур пародонту та зубів у хворих на пародонтоз та генералізований пародонтит. Характерні ознаки патологічного контуру ясен, а також інші клініко-рентгенологічні особливості дистрофії відрізняються у хворих на пародонтоз та генералізований пародонтит і сприяють покращенню їх диференційної діагностики. При ультраструктурному дослідженні ясен пацієнтів із пародонтозом виявлено дисемінований мікротромбоз, мукоїдний набряк та фібриноїдну трансформацію проміжної сполучної тканини та коагуляційно-дистрофічні зміни тканин і клітин пародонту.
Ключові слова: пародонтит, пародонтоз, ультраструктура ясен, коагуляційна дистрофія

The work is a fragment of the research project "Development and improvement of methods for diagnosis, prevention and treatment of periodontal diseases, caries and its complications", state registration No. 0120U002139.

In the development of diffuse periodontal lesions, such as generalized periodontitis (GP) and parodontosis, in addition to inflammation, dystrophic processes play an important role. According to the definition [7, 11], dystrophy is any disorder resulting from a metabolic disorder. Dystrophy can affect all components of the periodontium and occur in patients of various ages, including young people [7, 11]. This process is represented by the clinical manifestation of two main diseases – generalized periodontitis and