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HELICOBACTER PYLORI AND HOST GENE POLYMORPHISM OF TNF RS1800629 IN PATIENTS WITH GASTRODUODENAL PATHOLOGY

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ABSTRACT
Children with and without H.pylori infection were examined. It was revealed that the presence of the GA TNF-α rs1800629 genotype is an unfavorable prognostic marker in the development of this pathology in the presence of HP infection. At the same, the GGgenotype rs 1800629 is a protective genotype for the underlying pathology, regardless of the presense of HP infection**.**

KEYWORDS: Children, children with H.Pylori, children without H.pylori, polymerase chain reaction, gene polymorphism, TNF-α gene rs1800629

INTRODUCTION

Currently, chronic pathology of the stomach and duodenum in children and adolescents occupies a significant place in the structure of the overall morbidity. The appeal of children to medical institutions for diseases of the digestive system does not reflect the true prevalence of this pathology. According to the results of the All-Russian clinical examination, diseases of the digestive system rank second in the structure of childhood morbidity in adolescents. The incidence of chronic gastritis and chronic duodenitis in Tashkent region 200 per 1000. According to the forecasts of experts from the World Health Organization, (WHO), by the middle of the XXI century, diseases of the digestive system will occupy one of the leading places, due to the lifestyle of a modern person (stress, poor nutrition, physical inactivity, bad habits), environmental pollution, an increase in the diet nutritional share of substandard and genetically modified food products.

Helicobacter pylori (H. pylori) infection is currently considered as the leading etiopathogenetic factor of peptic ulcer disease (PUD) and chronic gastritis (CG) in childhood [1]. The results of large-scale studies have shown that the share of peptic ulcer disease associated with Helicobacter pylori infection accounts for 70-80% of cases of detection of duodenal ulcers and 50-60% of stomach ulcers [2]. There is more and more evidence of the role played by Helicobacter pylori infection in the occurrence and development of stomach cancer [3].

Tumor necrosis factor alpha (TNF-α) is a proinflammatory cytokine synthesized mainly by macrophages and monocytes, which plays an important role in the initiation and enhancement of the immune-inflammatory response to H. pylori infection [4]. At the cellular level, TNF-α stimulates the production of pro-inflammatory cytokines such as interleukin-1, -6, -8. Responsible for the immune and inflammatory response, including necrosis [5, 6]. The genes encoding them are among the main YB candidate genes. Polymorphic variants of cytokine genes and their receptors can have a significant impact on the risk of developing gastric ulcer and DU. In addition, the bacterium, inducing an immune-inflammatory reaction, contributes to the death of the macroorganism's own cells, which causes the development of deep dystrophic and atrophic changes in the gastric mucosa with the phenomena of metaplasia and dysplasia. Further hyperplastic processes lead to the development of gastric cancer [7]. In this regard, early detection, timely diagnosis and treatment of children with precancerous conditions and changes in the gastric mucosa are relevant.

To date, a sufficient number of studies conducted to study the immune response against H. pylori - infection, which are reflected in the foreign literature. Single nucleotide polymorphisms in some genes encoding cytokines alter the expression of cytokines in the coolant and can affect the clinical outcome of H. pylori infection [1]. Polymorphisms that lead to increased levels of IL-1β and TNFα, as well as polymorphisms that cause a decrease in IL-1RA expression, cause more severe inflammation and are associated with an increased risk of developing atrophic gastritis and gastric cancer [8].

Data on the characteristics of the production of proinflammatory cytokines in the coolant in children are scarce in the literature. According to some data, in children, as well as in adults, a predominance of the immune response to H. pylori with the participation of Th1 with the production of the corresponding cytokines is observed [9]. According to

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other data, the cytokine response in the coolant to H. pylori in children may be lower than in adults. This can protect children from developing severe gastro duodenal diseases such as stomach ulcers. Several studies have shown an increase in the local production of IL-1β, TNF-α, IL-8, IL-18 and IFN-γ in children infected with H. pylori compared with uninfected children. At the same time, there was no increase in IL-4 production [1].

H. pylori infection affects the entire coolant due to the presence of various pathogenic factors in this microorganism. H. pylori infection is chronic, in which the production of IFN-γ, IL-12, IL-18, IL-23, TNF-α is observed. The development of inflammatory and immune responses leads to an increase in the production of proinflammatory cytokines, such as IL-1β, IL-8, TNF-α, IFN-γ, the local production of cytokines in the coolant of which can serve as a marker of H. pylori infection, the density of microbial colonization and the severity of the disease. The immune response to H. pylori infection in children is an earlier pathological response and can serve as a kind of model for studying the characteristics of the course of H. pylori. In the conditions of epy Republicof Uzbekistan, such studies have not been carried out, at the same time, the tendency to weight gain, rejuvenation and a high frequency of complications dictate the need to study this issue.

MATERIALS AND METHODS

Determination of polymorphism of the studied genes DNA isolation. The material for DNA isolation was venous blood from the cubital vein with a volume of 3-5 ml (Beckton-Dickinson vacutainers were used for blood sampling) with an anticoagulant / preservative 15% tripotassium EDTA (Ethilendianin-tetraacetic acid). Blood for further processing could be stored for up to 24 hours at a temperature not higher than + 4 ° C.

To obtain genomic DNA, a two-stage blood cell lysis method was used. Lysis of erythrocytes was carried out by double centrifugation of the entire volume of whole blood in RCLB (Red cells lysis buffer) at 1500 rpm for 15-20 minutes. The use of RCLB induces an osmotic shock of erythrocytes, leading to their swelling and further destruction.

The supernatant containing destroyed erythrocytes was carefully decanted from the tube, and the remainder of the supernatant was aspirated. The clot of the leukocyte mixture remaining at the bottom was lysed in leukocyte lysis buffer WCLB (White cells lysis buffer) in an amount depending on the volume of the leukocyte mixture. WCLB is also a preservative for storing leukocyte lysates even at room temperature. In this state, lysates could be stored indefinitely.

Further purification of leukocyte mass lysates is based on the method of S. Miller et al. (1988) in the modification proposed by the Stanford University laboratory.

To 400 µl of leukocyte mass lysate, add 150 µl of 5M NaCl, mix on a shaker and place on ice for 10-20 minutes, then centrifuge at 1200 rpm for 15 minutes. The supernatant is taken into another Eppendorf tube and 100% ice-cold ethanol is added. With gentle shaking, a quaternary chain of the DNA molecule appears in the mixture; the mixture is centrifuged at 1200 rpm for 15 minutes; the supernatant is removed, and the whitish spot remaining on the bottom of the tube is washed again in 80% ethanol at 1200 rpm for 10 minutes. The supernatant is discarded, the residual alcohol is carefully removed, the tube is left open until the alcohol has completely evaporated (for 12 hours at room temperature or in a thermostat at 40-45 ° C for 2 hours).

After evaporation of the alcohol, a solution of TE (Tris-EDTA) diluted with distilled water in a ratio of 1: 3 (TE: water) pH 8.0 is added to the test tube with dried DNA.DNA was stored at –20 ° C.

Methods for identifying allelic gene variations. Polymerase chain reaction (PCR) was performed on a Rotor-Gene-2000 thermal cycler (Corbett Research) usingcorresponding primers and 10 µl of PCR mixture (manufacturer"NPO Lyteh"), containing 2 mM MgCl2, Taq DNA polymerase and "Cresol red".

Polymerase chain reaction (PCR). To detect the studied polymorphisms, amplification of certain regions of the corresponding genes was performed. To determine the polymorphic alleles of the genes under study, the PCR method with allele-specific primers was used on commercial test systems of the Scientific and Production Company "Litekh" (Moscow). Amplification was carried out in 200 µL flat-cap tubes (PCR®TUBES, Axygen), numbered assay samples and negative control. For each sample, 2 tubes were prepared with working amplification mixtures obtained 20-30 minutes before PCR from thoroughly mixed reagent kits thawed and brought to room temperature (Litech) at the rate of 1 sample: 17.5 µl of diluent, 2.5 µl of the reaction mixture, 0.2 µl of Tag-polymerase. After adding Tag polymerase and mixing by pipetting, 20 µl of the corresponding amplification mixture was added to each of the numbered tubes, 1 drop of mineral oil was added to all tubes and 5 µl of the analyzed DNA sample was added under the oil layer. As a negative control sample - diluent in a volume of 5 µl in both controls. The tubes are tightly closed and after centrifugation at 1500-3000 rpm in a vortex for 3-5 seconds (+18 + 25 ° C) they are placed in a heated up to + 94 ° C (steady-state temperature in the “Pause »).

The detection of amplification products were carried out by horizontal electrophoresis in 3% agarose with 1% ethidium bromide in an electrophoretic chamber at an electric field strength of 10-15 V / cm after adding an amplificate (15-20 µl) to the agarose gel wells in the sequence corresponding to the sample numbering. Control over electrophoretic separation was carried out visually by the movement of the dye strip from the start by 1.5–2 cm (distillation time 30 ± 2 min). The results of electrophoresis were visualized with UV irradiation (wavelength 310 nm) in a transilluminator. The presence of a signal is determined by the intensity of the luminescence of the amplicon bands.

Statistical data processing. Statistical processing of the results was carried out using the statistical software packages and a number of formulas.

The following indicators are generally accepted in medical statistics:

1. The calculation of the gene frequency in healthy and sick people is carried out according to the formula of the ratio of the number of a certain allele to twice the total number of individuals in the sample.

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2. The main indicator for haplotypes is linkage disequilibrium (LD).

3. The gene frequency is determined taking into account the Hardy-Weinberg law for the biallelic system. The $\chi 2$ value, exceeding 3.841 (which corresponds to $p < 0.05$), is considered as an indicator of a significance.

RESULTS AND DISCUSSION

The TNFA gene is located on chromosome 6 on its short arm in the p21.3 region and contains 4 exons. There is evidence of the relationship between TNFA gene polymorphism and the amount of its production. Scientists have identified several SNPs of the TNFA gene: -1031T / C, -863C / A and -857C / A, -308G / A and -238G / A. Among them, the most studied are two polymorphic variants of the TNFA gene: -238G / A -308G / A, which have a multidirectional effect on the production of this cytokine: at position -308, the replacement of guanine by adenine increases the production of the TNFA cytokine.

A comparative study of the frequency distribution of alleles and genotypes of polymorphic markers of the TNFα-308G / A gene in the groups of patients with HP and in the control showed a statistically significant increase in the frequency of the A allele in patients compared with the control group (35.16% and 8.42%, respectively; OR = 4.64; 95% CI: 2.573> 4.64> 8.368; $\chi 2 = 29.255$). At the same time, the G allele of the studied polymorphism was much less common compared to the control group (82.42% and 91.58%, respectively; OR = 0.216; 95% CI: 0.119> 0.216> 0.389; $\chi 2 = 29.255$)

Table 1. Distribution of alleles and genotypes of TNF-a rs1800629 in the group of patients with H.pilory

Genotype	Case, n=91	Case, %	Genotype	Control, n=95	Control, %	$\chi 2$	OR (95% CI)
G	150	82,42	G	174	91,58	29.255	0.119 >0.216> 0.389
A	64	35,16	A	16	8,42		2.573 >4.64> 8.368
GG	59	64,84	GG	79	83,16	8,15	0.188 >0.373> 0.743
GA	32	35,16	GA	16	16,84	8,15	1.345 >2.678> 5.33
AA	0	0,00	AA	0	0,00		0.119 >0.216> 0.389

Further, a comparative analysis of TNFα-308 G/A genotypes for the GG genotype revealed significant differences between patients with HP and the control group (64.84% and 83.16%, respectively; OR = 0.373; 95% CI: 0.188> 0.373> 0.743; $\chi 2 = 8.15$). The analysis of the heterozygous GA genotype revealed differences between the frequency of occurrence in patients with ADR and the control group (35.16% and 16.84%, respectively; OR = 2.678; 95% CI: 1.345> 2.678> 5.33; $\chi 2 = 8.15$) As already described above, a significant difference was found in the frequency of occurrence of the A allele, the studied TNFα-308G / A polymorphism, but the genotypic analysis of the homozygous AA genotype was not detected.

Table 2. Distribution of alleles and genotypes of TNF-a rs1800629 in the group of patients without H.pilory

Genotype	Case, n=89	Case, %	Genotype	Control, n=95	Control, %	$\chi 2$	OR (95% CI)
G	159	89,33	G	174	91,58	9.515	0.206 >0.385> 0.717
A	38	21,35	A	16	8,42		1.395 >2.599> 4.843
GG	70	78,65	GG	79	83,16	0.606	0.356 >0.746> 1.562
GA	19	21,35	GA	16	16,84	0.606	0.64 >1.34> 2.80
AA	0	0,00	AA	0	0,00		

A comparative study of the frequency distribution of alleles and genotypes of polymorphic markers of the TNFα-308G / A gene in the groups of patients without AD and in the control showed a statistically significant increase in the frequency of the A allele in patients compared with the control group, as well as in the group of patients with AD (21, 35 % and 8.42%, respectively; OR = 2.599; 95% CI: 1.395> 2.599> 4.843; $\chi 2 = 9.515$). At the same time, the G allele of the studied polymorphism was much less common compared to the control group (89.33% and 91.58%, respectively; OR = 0.385; 95% CI: 0.206> 0.385> 0.717; $\chi 2 = 9.515$).

Further, a comparative analysis of TNFα-308G / A genotypes for the GG genotype did not reveal significant differences between patients without HP and the control group (78.65% and 83.16%, respectively; OR = 0.746; 95% CI: 0.356> 0.746> 1.562 ; $\chi 2 = 0.6$). Analysis of the heterozygous GA genotype also did not reveal differences between

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the frequency of occurrence in patients without ADR and the control group (21.35% and 16.84%, respectively; OR = 1.34; 95% CI: 0.64> 1.34> 2.80; $\chi 2$ = 0.6). As already described above, a significant difference was found in the frequency of occurrence of the A allele, the studied TNFα-308G / A polymorphism, but the genotypic analysis of the homozygous AA genotype was not detected.

After analyzing the distribution of alleles and genotypes in the groups of patients with HP infection and without the presence of HP infection in comparison with the control group, an analysis was performed between the two groups of patients.

Analysis of the frequency distribution of alleles and genotypes of polymorphic markers of the TNFα-308G / A gene in groups of patients revealed a statistically significant increase in the frequency of the A allele in patients with HP compared with patients without HP, (35.16% and 21.35%, respectively; OR = 1.785; 95% CI: 1.128> 1.785> 2.826; $\chi 2$ = 6.197). The G allele of the studied polymorphism was much less common in the group with HP infection compared with the group without concomitant infection (82.42% and 89.33%, respectively; OR = 0.56; 95% CI: 0.354> 0.56> 0.887; $\chi 2$ = 6.197).

Further, a comparative analysis of TNFα-308G / A genotypes for the GG genotype did not reveal significant differences between patients with HP infection and without HP (64.84% and 78.65%, respectively; OR = 0.5; 95% CI: 0.257 > 0.5> 0.973; $\chi 2$ = 4.23). The heterozygous GA genotype was also detected more often in patients with AD, compared with patients without AD and in the control group (35.16% and 21.35%, respectively; OR = 1.99; 95% CI: 1.028> 1.998> 3.885; $\chi 2$ = 4, 23). No homozygous AA genotype was identified.

Table 3. Distribution of alleles and genotypes of TNF-a rs1800629 in the group of patients with H.pilory and without H.pilory

Genotype	Case, n=91	Case, %	Genotype	Control, n=89	Control, %	$\chi 2$	OR (95% CI)
G	150	82,42	G	159	89,33	6.197	0.354 >0.56> 0.887
A	64	35,16	A	38	21,35		1.128 >1.785> 2.826
GG	59	64,84	GG	70	78,65	4,23	0.257 >0.5> 0.973
GA	32	35,16	GA	19	21,35	4,23	1.028 >1.998> 3.885
AA	0	0,00	AA	0	0,00		

Thus, we can conclude that the presence of the GA TNF-a genotype rs1800629 is an unfavorable prognostic marker in the development of this pathology in the presence of HP infection. At the same time, the GG genotype rs1800629 is a protective genotype for the underlying pathology, regardless of the presence of HP infection.

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