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INFLUENCE OF ALLELIC VARIANTS OF POLYMORPHIC GENES OF INTERLEUKINS, NITRIC OXIDE INDUCED SYNTHASE AND THEIR RECEPTORS IN THE COURSE OF HELICOBACTER-ASSOCIATED PEPTIC ULCER DISEASE IN CHILDREN

Key words: *children, peptic ulcer disease, allelic polymorphisms of immunoregulative cytokines, induced NO-synthase.*

Abstract. *This paper presents the results of the analysis of the distribution of allelic polymorphism of interleukin-1beta gene, -8, receptor antagonist and interleukin-1-induced NO-synthase in children of Chernivtsi region suffering from stomach ulcer and duodenal ulcer and healthy ones. It has been established that susceptibility to peptic ulcer disease is associated with the presence of the following genotypes: IL-1β-511C/C, R4/R4 IL-1Ra, IL-8-251A/T and C/C G954C in the promoter area of the gene induced NO-synthase.*

Introduction

Diseases of the digestive system have always been in the focus of doctors because of high prevalence, course and feature of a high risk of early disability. Peptic ulcer disease (PUD) due to the increasing frequency and its leading place in the structure of diseases of gastroduodenal region is of the most social and health value among them [12].

Modern understanding of the formation and development of PUD are based on generalizing the concept of an imbalance between factors of aggression and defense, and are discussed in the context of the persistence of H. pylori [2]. A key element in the development of ulcer is not only lasted H. pylori infection in the organism, but also intimately involved in the process cells of immune system [3].

Disregulation mechanisms of the immune response of the macroorganism are associated with the direct cytopathic effect of H. pylori that potentiates the synthesis of proinflammatory cytokines - important factors of mucosa membrane damage (MM) of the stomach and duodenum (DU) and causes reorganization of its cells, manifested by expression of genes responsible for the synthesis of interleukin [4]. Effects of cytokines on the one hand, is inappropriate activation of the immune system (increased apoptosis of immune cells with subsequent elimination), and on the other hand - the imbalance between the processes of programmed death of epithelial cells and their proliferation [7]. Increased cell death contributes to the development of ulcers of the stomach and duodenum [2].

Infection of the stomach and duodenum H. pylori enhances expression induced NO-synthase (i-NOS), which regulates the action of one of the most important biological mediators parietal cells of the stomach - nitric oxide (NO) [9]. The latter belong to the most important factors of protection of the stomach and duodenum, which supports an active vasodilation, regulates basal blood flow and blood pressure [1, 13].

A large amount of data as to involvement of different polymorphic genes of interleukins and i-NOS and susceptibility to the formation of many socially significant diseases associated with long-term persistence of the pathogen, that determines the course of the disease and the occurrence of complications [7, 10].

Taking into account non mono semantic data the role of specific gene polymorphisms of interleukins and i-NOS in the development of helicobacter-positive ulcer, the priority is to study association of the disease with a number of allelic variants of polymorphic genes of interleukins, i-NOS and their receptors in children.

Objective

To analyze the significance of allelic polymorphism of interleukin-1beta gene, -8, receptor antagonist and interleukin-1 gene, induced NO-synthase in the development and course of peptic ulcer disease in children.

Material and methods

The study involved 120 children aged 7-18 years, patients with ulcer (group under study) and 100 healthy children of appropriate age (comparative group). The average age of the children was 12,6 ± 3,2 years. Children in both groups are Ukrainians, living in Chernivtsi and Chernivtsi region. Verification of

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clinical diagnosis of PUD was conducted in accordance with protocol of the specialty "Pediatric Gastroenterology" (Ministry of Health Care of Ukraine № 59 of 29.01.2013 year) on the basis of clinical, endoscopic (using endoscopic "Pentax FG-24P", according to "the Sydney system" (1990) taking into account the specifics of the study in children (S.Ya. Doletskyy, 1984) and morphological (holding MM biopsy under general rules on assessment of morphological changes of MM according to the "Sydney-Houston system" in sections, hematoxylin and eosin painted using a visual analogue scale semi-quantitative assessment of morphological changes (M.F. Dixon, 1996) in points (0-3 degrees of severity) according five criteria: the severity of chronic inflammation, its activity, sowing H. pylori, the presence of atrophy and intestinal metaplasia). Studies of acid production functions of the stomach were performed using a pH-meter "IKSH 2". H. pylori infection confirmed by brush biopsy ("the Sydney-Houston system", 1996) with the preparation of smears for cytoscopic study to determine H. pylori and its degree of sowing by L.I. Aruin (1998); ELISA for the conventional method using diagnostic test kits "HelikoBest antibodies" (set of reagents for "Vector BEST" (Russian Federation)) with the detection of antibodies to CagA H. pylori antigen in serum.

Study of gene polymorphism of interleukin IL-1β-511C/T, IL-8-251A/T, IL-1Ra VNTR and i-NOS was performed by restriction analysis of amplification products specific areas of the genome. Analysis of amplificative products was performed by electrophoresis in 3% agarose gel with etydium-bromide and visualized under UV light using computer video. The distribution of genotypes of polymorphic loci studied by checking for compliance with Hardy-Weinberg equilibrium using the criterion $\chi 2$. To test the significance of the overall extent of communication nonparametric test of Pearson ($\chi 2$) and the figure odds ratio (OR) were used. Statistical analysis of the data was performed using computer software package "Statistica 6.0".

Results and discussion

Estimation of the distribution of polymorphism gene IL-1β-511C/T showed that risk of ulcer is associated with the presence of low productive allele IL-1β-511*C and homozygous genotype IL-1β-511C/C (OR = 2,90, [1,99-4,24] and OR = 6,47, [3,53-11,84], respectively) in children in the population of Chernivtsi region. In its turn high productive allele IL-1β-511*T heterozygous genotype and IL-1β-511C/T and exhibit protective effect associated with a reduced risk of developing the disease (OR = 0,34, [0,24-0,50] and OR = 0,26 [0,15-0,44], respectively). Analysis of the distribution of genotypes and alleles of the gene IL-1β-511C/T in children of the basic group according to age and course of the disease found no probable associative connections.

Allele R4 IL-1Ra ($\chi 2$ = 28,21, p<0,0001) prevalence was registered among children of the main group whereas allele R2 IL-1Ra (p<0,05) occurred significantly more frequent in children of comparison group. Homozygous genotype R4/R4 IL-1Ra (32,0 % and 47,3 %, $\chi 2$ = 5,82, p<0,01) dominated likely in sick individuals. Instead of, in children of the comparison group homozygous genotype R2/R2 IL-1Ra (34,0 % and 12,7 %, $\chi 2$ = 16,35, p<0,0001) was diagnosed more often.

Thus, allele R4 IL-1Ra (OR = 2,21, [1,32-3,71]) and genotype R4/R4 IL-1Ra (OR = 1,91, [1,12-3,24]) can serve as predictors of the risk of duodenum ulcer disease in children. Allele R2 IL-1Ra (OR = 0,44, [0,26-0,75]) and the genotype of R2/R2 IL-1Ra (OR = 0,28, [0,15-0,53]) proved to be protective factors.

It should be noted that the indirect reaction as to IL-1β and IL-1Ra, which cause destructive processes depend on the combination of genotypes of polymorphic loci persons relevant genes [4]. In this connection, it has been set the frequency of detection of haplotypes different combinations in patients and healthy children.

It has been established that the risk of ulcer increase 5,52 times in the presence of combinations of genotypes IL-1β-511C/C and R4/R4 IL-1Ra (OR = 5,52, [2,65-11,47]) and 3,48 times in the registration haplotype IL-1β-511C/C and R2/R4 IL-1Ra (OR = 3,48, [1,15-10,56]). Haplotype IL-1β-511C/T and R2/R2 IL-1Ra can serve as a marker of resistance to disease development in children (OR = 0,09, [0,03-0,24]).

Significant difference in the distribution of haplotype frequencies of genes IL-1β-511C/T and IL-1Ra, depending on the age and course of the disease we have not found.

Role of allelic variants of the gene polymorphisms IL-8-251A/T in the pathogenesis of ulcer is not ambiguous. The allele IL-8-251*A is considered to be associated with progression of atrophic changes in MM, which increases the risk of ulcer in the Korean population [8], other researchers have shown a relationship between carrier genotype IL-8-251T/T and development of duodenal ulcers [11].

In this study it has been found that in children of the main group frequency of "mutant" allele IL-8-251*A is significantly higher and frequency of "wild" allele of IL-8-251*T is significantly lower than in the comparison group (50,3 % and 24,0 % and 49,7 % and 76,0 %, respectively, $\chi 2$ = 34,73, p <0,0001). Heterozygous genotype of IL-8-251A/T is 2,3 times

more often found in children with UD, which was significantly higher with respect to its frequency in the healthy group ($\chi 2 = 28,96$, $p<0,001$). However, homozygous for the "wild" allele genotype of IL-8-251T/T was diagnosed in patients of the main group 3,3 times less likely than in children of the comparison group ($\chi 2 = 49,19$, $p <0,001$).

Thus, "mutant" allele of IL-8-251*A (OR = 3,21 [2,16-4,77]) and heterozygous genotype of IL-8-251A/T (OR = 4,39 [2,52 -7,64]) are prognostic marker of risk of ulcer and the presence of "wild" allele of IL-8-251*T (OR = 0,31 [0,21-0,46]) and homozygous genotype IL-8-251T/T (OR = 0,14 [0,08-0,25]) may serve as protective markers of the disease development.

"Mutant" allele of IL-8-251*A and homozygous genotype IL-8-251A/A are adverse prognostic features, which can increase the risk of ulcer in boys (OR = 1,72 [1,09-2,72] and OR = 2,75 [1,14-6,67], respectively).

To determine the relationship of different polymorphic variants of the gene with the development of ulcer attention should be paid to systems of intergene interaction.

It has been determined that the combination of IL-1β -511C/C, R4/R4 IL-1Ra, IL-8-251A/T ($\chi 2 = 27,97$, $p <0,001$) risk of the disease will increased 19,1 times (OR = 19,1 [4,49-80,8]); IL-1β-511C/C, R4/R4 IL-1Ra, IL-8-251A/A and IL-1β-511C/C, R2/R4 IL-1Ra, IL-8-251A/T - 6,32 times (OR = 6,32, [0,79-50,7]). The risk will be the smallest in case of combination of the following genotypes: IL-1β-511C/T, R2/R2 IL-1Ra, IL-8-251T/T (OR = 0,02, [0,00-0,18]).

Taking into consideration the importance and relevance of NO significance in the pathology of gastrointestinal tract molecular genetic study of children with UD has been carried out - namely G954C polymorphism i-NOS promoter gene for single nucleotide replacement was identified [10].

"Wild" allele G and genotype GG was met with almost equal frequency in children in both groups ($p>0,05$). Thus the indices of OR were less than one, indicating a possible protective effect of the allele and genotype.

In patients with PUD the mutant allele C occurred 2 times, and GC genotype in 1,83 times more often than in practically healthy children; genotype CC - only in children of the main group. Analysis of epidemiological indices of OR showed that probability of the disease development increases by 1,3 times (95% CI [0,52-3,29], $\chi 2 = 8,41$, $p = 0,012$) in the presence of GC genotype and 3,9 times (95% CI [0,21-73,83], $\chi 2 = 9,6$, $p = 0,0007$) in the case of genotype CC. It should be noted that genotype CC was diagnosed in children with complicated course of ulcer.

The frequency distribution of genotypes according to sex, in general, was almost the same as for the children of the main group and the comparison group of persons.

Thus, the identification of patterns of implementation allelic variants of polymorphism gene of interleukins and induced NO-synthase is an important link disorder of immunoreactivity of macroorganism in the pathogenesis of helicobacter-associated ulcer, whose development is the result of molecular genetic determination of the immune system.

Conclusions

1. Factor increased risk of peptic ulcer disease in children Chernivtsi region is carrier genotypes genes IL-1β-511C/C, R4/R4 IL-1Ra, IL-8-251A/T R4 and CC i-NOS G954C. The highest risk of disease observed in the combination of the above genotypes of interleukin genes.

2. Genotype genes IL-1β-511C/T, R2/R2 IL-1Ra, IL-8-251T/T genotype and their combination CC G954C i-NOS may serve as protectors of development of peptic ulcer in children of Chernivtsi region.

Prospects for further research

Prospects for future research are to develop a unified algorithm for diagnosis and prognosis of the development and course of peptic ulcer disease in children, that in its turn will allow to carry out differentiated genotype-specific treatment of nosology.

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ВПЛИВ АЛЕЛЬНИХ ВАРІАНТІВ ПОЛІМОРФНИХ ГЕНІВ ІНТЕРЛЕЙКІНІВ, ІНДУКОВАНОЇ СИНТАЗИ МОНООКСИДУ НІТРОГЕНУ ТА ЇХ РЕЦЕПТОРІВ НА ПЕРЕБІГ ГЕЛІКОБАКТЕР-АСОЦІЙОВАНОЇ ВИРАЗКОВОЇ ХВОРОБИ У ДІТЕЙ

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Резюме. В статті наведено результати аналізу розподілу алельного поліморфізму генів інтерлейкінів-1бета, -8, рецепторного антагоніста інтерлейкіна-1 та індукованої NO-синтази в дітей Чернівецької області, хворих на виразкову хворобу шлунка та дванадцятипалої кишки, та здорових. Встановлено, що схильність до виразкової хвороби асоціюється з наявністю наступних генотипів: IL-1β-511C/C, R4/R4 IL-1Ra, IL-8-251A/T та C/C G954C у промоторній зоні гена індукованої NO-синтази.

Ключові слова: діти, виразкова хвороба, алельний поліморфізм імунорегуляторних цитокінів, індукована NO-синтаза.

ВЛИЯНИЕ АЛЛЕЛЬНЫХ ВАРИАНТОВ ПОЛИМОРФНЫХ ГЕНОВ ИНТЕРЛЕЙКИНОВ, ИНДУЦИБЕЛЬНОЙ СИНТАЗИ МОНООКСИДА НІТРОГЕНА И ИХ РЕЦЕПТОРОВ НА ТЕЧЕНИЕ ХЕЛИКОБАКТЕР-АССОЦИИРОВАНОЙ ЯЗВЕННОЙ БОЛЕЗНИ У ДЕТЕЙ

Т.В. Сорокман, С.В. Сокольник, Д.Р. Андрейчук

Резюме. В статье описаны результаты анализа распределения аллельного полиморфизма генов интерлейкинов-1бета, -8, рецепторного антагониста интерлейкина-1 и индуцибельной NO-синтазы у детей Черновицкой области, больных язвенной болезнью желудка и двенадцатиперстной кишки, и здоровых. Установлено, что предрасположенность к язвенной болезни ассоциируется с наличием следующих генотипов: IL-1β-511C/C, R4/R4 IL-1Ra, IL-8-251A/T и C/C G954C в промоторной зоне гена индуцибельной NO-синтазы.

Ключевые слова: дети, язвенная болезнь, аллельный полиморфизм иммунорегуляторных цитокинов, индуцибельная NO-синтаза.

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