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CHANGES OF THE NITROGEN MONOXIDE CONCENTRATION IN CHILDREN WITH PEPTIC ULCER DISEASE

Key words: *ulcer disease, nitrogen monoxide, children, inducible nitrogen monoxide synthase.*

Abstract. *The level of nitrogen monoxide is reliable elevated in the blood and saliva of Helicobacter pylori infected children with ulcer disease, it decreases by duration of disease more than 3 years, and by severe course of disease, does not depend on the age of children and localization of ulcer defect. There was the following distribution of iNOS (G954C) genotype in patients with UD: homozygotes for G-allele – 47,5%, heterozygotes – 38,3%, homozygotes for C-allele – 14,2%. The total prevalence of mutant C-allele of iNOS amounts 52,5%.*

Introduction

In recent years, conceptions of nitrogen monoxide sources (NO) and its metabolism at the molecular and cellular levels significantly increased [1]. New informative methods of determining, experimental models, new synthesized substances – donors and inhibitors of NO allows to evaluate the role of NO in many diseases, including the gastrointestinal tract [3, 5].

We studied correlation between NO secretion of gastric mucosa and the activity of Helicobacter pylori (H. pylori) infection. It has been established that increased production of NO is caused by an active inflammatory response involved by H. pylori, which is able to metabolize urea. NO is a stable compound in acidic conditions and can be used as an indicator of inflammation [5]. A the positive role of NO in inhibition of H. pylori infection progression is known too[7].

We have found that an increased expression of induced NO-synthase (i-NOS) in gastric mucosa infected by H. pylori raises the level of NO [4]. Nevertheless, the question of the role of NO in pathogenesis of ulcer disease (UD) remains unstudied in pediatric patients, in particular in pediatric gastroenterology.

Objective

To increase the effectiveness of prognosis and treatment of UD in children by optimizing the diagnosis and development of complex pathogenetic therapy of the disease.

Material and methods

We conducted a comprehensive clinical, molecular genetic and laboratory-instrumental examination of 120 children with UD, aged 7-18 years (main group) and 100 healthy children of appropriate age (comparison group), who lived in Chernivtsi and Chernivtsi region.

The study was conducted using a simple randomization.

The average age of children with UD was 14,8 ± 2,0 years, and healthy children ---– 14,7 ± 2,5 years ($p>0,05$). 47,5% of patients of the main group and 52% of patients of comparison group were the inhabitans of the rural areas. 53,5% of examined children suffering from UD, were boys. There were almost equal numbers of children with duration of UD up to one and over 3 years.

Verification of clinical diagnosis was carried out in accordance to the treatment of children in "Children's Gastroenterology" (Ministry of Health of Ukraine № 438 of May 26, 2010).

All children were interviewed with studying anamnestic, genetic, social, environmental, household and other characteristics.

Clinical studies were performed by the standard method of patient's examination. Particular attention was paid to children's complaints of pain, its location, seasonality, the nature of the factors that enhanced and relieved the pain.

Paraclinical studies included: complete blood count, blood chemistries, blood test for glucose, urinalysis, fecal presence of helminthes eggs, stool analysis, the study of intestinal microflora.

To verify presence of mucosal ulcers, detection of refluxes and comorbidity conducted fibroesophagogastroduodenoscopy (FEGDS) using fibrogastroduodenoscope "Pentax FG-24P" was carried out determining endoscopic criteria of H. pylori definition and biopsy of stomach (antrum and body) and duodenal

mucosa by generally accepted rules of sampling with the next preparation of smears, staining and bacterioscopy for diagnosis of H. pylori and determination of the degree of sowing. H. pylori contamination was confirmed by solid-phase ELISA test by means of qualitative and quantitative determination of IgG-antibodies to H. pylori in the serum (UBI MAYIWELL ™, USA). Along with FEGDS was ntragastric pH-metry using apparatus "IKSH-2" studing of secretory and alkaline functions of the stomach by the difference of pH in the body and antrum was carried out.

An ultrasound investigation of the abdominal cavity organs performed by apparatus "Aloca SSD-680" according to the conventional method.

Blood for molecular genetic studies and determining NO concentration was taken from the cubital vein fasting in the morning.

Collection of 1 ml of saliva was performed fasting too and after triple rinse of the mouth with distilled water. The concentration of nitrite in plasma and saliva was determined using standard. Sodium nitrite was used as a standard by the method of Golikov P.P., et al., 2004.

Amplification of the necessary area of iNOS promotor was performed by polymerase chain reaction (PCR) using specific primers (iNOS G954C direct and iNOS G954C inverse), which were synthesized by Sigma-Aldrich (Germany). Amount of DNA for PCR was 50 ng on reaction. DNA amplification was performed in a medium of the following composition: 1 Ч PCR buffer (Hot Start PCR-buffer, Fermentas, Lithuania), $MgCl_2$ – 3 mM, a mixture of dNTP – 0,4 mM of each, primers – 1 mM of each, DNA polymerase (Maxima Hot Start Taq DNA Polymerase, Fermentas) – 3 units of activity in the sample. The total volume of the reaction mixture was 50 mkl. PCR was performed using Thermocyclers PTC-100 (MJ Research Inc, USA). Analysis of PCR was carried out by electrophoresis in 2% agarose gel using trysborat buffer. To visualize DNA fragments, gel stained with ethidium bromide and photographed under ultraviolet light on the installation GelDoc 2000 (BioRad, USA). To determine the length of the obtained fragments, their electrophoretic mobility compared with mobility of the DNA-marker Gene Ruler DNA Lader Mix (Fermentas).

For genotyping iNOS, in terms of single nucleotide substitution G954C, obtained PCR products were treated with restriction Eco31 I (Bsa I). Processing of PCR-product restriction was performed according to the enzyme manufacturer's recommendations (Fermentas). These restricted fragments were analyzed by electrophoresis in 2% agarose gel.

The results of investigation were evaluated using descriptive, correlative methods and analysis of variance. We used statistical modules "Fundamentals of Statistics" and "Correlation analysis", methods of biostatistics and clinical epidemiology (computing of the associative links between etiological factors and UD using non-parametric test of Pearson ($ч^2$), odds ratio (OR) with 95% confidence interval (CI).

Results and discussion

The level of NO in plasma of children with UD was (11,62 ± 0,9) mmol/l wich is in 1,36 times lower than in the comparison group of children (15,84 ± 0,8) mmol/l, $p<0,05$. The level of NO in saliva in children of the main group was (41,06 ± 1,9) mmol/l and it's in 1,37 times lower than in comparison group of patients (56,08 ± 1,8) mmol/l, $p<0,05$. The rate of NO reduction in plasma and saliva did not differ significantly, which may indicate a direct equivalent of the NO content in plasma and saliva.

Probable difference between the NO indices according to age and gender in intra analysis in both groups of children was not found ($p>0,05$). This fact allows to use present index regardless of age and gender. Probable strong direct correlation between the NO level in plasma and saliva was found in children of main group aged 7-12 years and 13-18 years ($r = 0,82$ and $r = 0,84$ respectively, $p<0,05$), which indicates the possibility of using of NO levels in saliva as a marker of NO concentration in the blood. It has been established that the NO level is independent of ulcer location.

In cases of H. pylori-negative ulcer the NO level in biological fluids was significantly higher (16,89 ± 0,8 mmol/l in plasma and 50,66 ± 1,6 mmol/l in saliva) as compared with group of H. pylori-positive patients (11,27 ± 0,7 mmol/l in plasma and 38,15 ± 1,4 mmol/l in saliva, $p<0,05$). Probable strong direct correlation between the NO level in plasma and saliva was established in both variants of disease ($r=0,86$ and $r=0,88$ respectively, $p <0,05$).

The NO concentration in biological fluids was significantly higher in children who suffered from UD less than 1 year (11,63 ± 0,4 mmol/l in plasma and 41,23 ± 1,2 mmol/l in saliva) and 1-3 years (11,74 ± 0,3 mmol/l in plasma and 41,67 ± 1,1 mmol/l in saliva) as compared with patients whose disease duration was more than 3 years (9,42 ± 0,6 mmol/l in plasma and 35,40 ± 1,1 mmol/l in saliva); in the acute stage (11,13 ± 0,6 mmol/l in plasma and 42,47 ± 1,0 mmol/l in saliva) compared with remission (9,47 ± 0,5 mmol/l in plasma and 38,04 ± 1,1 mmol/l in saliva); with light and medium courses of disease (12,21 ± 0,4 mmol/l in plasma and 44,58 ± 1,0 mmol/l in saliva and 11,75 ± 0,3 mmol/l in plasma and

Table

Association of alleles and genotypes of iNOS gene G954S with ulcer disease in children, (n=120)

Gene	Allele and genotype	OR	95% CI	χ^2	p
iNOS G954C	G	0,55	0,23-1,30	3,92	0,041
	C	1,83	0,77-4,32	6,84	0,016
	GG	0,62	0,25-1,54	4,28	0,039
	GC	1,30	0,52-3,29	8,41	0,012
	CC	3,90	0,21-73,83	9,60	0,0007

40,17 ± 1,1 mmol/l in saliva, respectively) in comparison with the severe course (9,03 ± 0,5 mmol/l in plasma and 33,46 ± 1,2 mmol/l in saliva); with sizes of ulcer 1-2 mm (12,61 ± 0,31 mmol/l in plasma and 45,37 ± 1,1 mmol/l in saliva) and 3-5 mm (11,19 ± 0,2 mmol/l and 38,90 ± 1,0 mmol/l in saliva) as compared with the NO level by ulcer size > 6 mm (10,13 ± 0,6 mmol/l in plasma and 36,21 ± 0,9 mmol/l in saliva), p <0,05.

Significantly higher concentration of NO in plasma (12,16 ± 0,3 mmol/l) and saliva (43,35 ± 1,1 mmol/l) was observed in the presence of duodenogastral reflux in comparison with patients with gastroesophageal reflux (10,03 ± 0,2 mmol/l in plasma and 38,21 ± 1,0 mmol/l in saliva), p<0,05. Significantly higher NO level in plasma (13,95 ± 0,9 mmol/l) was in patients with increased acid productive function of the stomach as compared with patients with normal stomach acid production (10,25 ± 0,7 mmol/l) with a similar trend in saliva, p <0,05.

Molecular genetic studies of children with UD (identification of G954S polymorphism of iNOS gene promotor in terms of single nucleotide substitutions) was conducted considering the importance and relevance of the NO value in the pathology of gastrointestinal tract (tab., fig.) [4, 7].

"Wild" G allele and GG genotype met with almost equal frequency in children of both groups (p>0,05). This indexes of UD were less than one, indicating a possible protective effect of this allele and genotype.

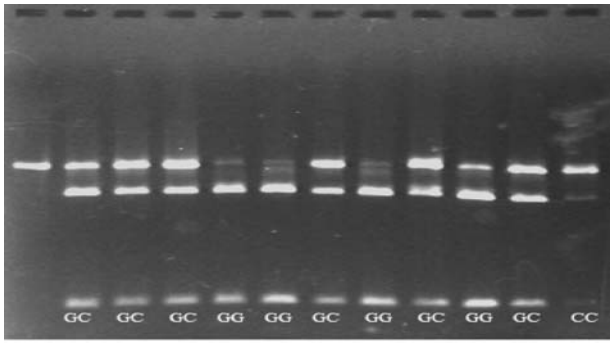

Fig. Gene i-NOS (G954C) polymorphism in children with ulcer disease

There was the following distribution of iNOS (G954C) genotype in patients with UD: homozygotes for G-allele – 47,5%, heterozygotes – 38,3%, homozygotes for C-allele – 14,2%. The total prevalence of mutant C-allele of iNOS amounts 52,5%. Analysis of epidemiological indicator of UD showed that risk of the disease development increases in 1,3 times by the presence of GC genotype and in 3,9 times by the presence of genotype CC. It should be noted, that CC genotype was diagnosed in children with complicated course of UD.

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

Probable higher level of NO in biological fluids was observed in patients of main group and homozygotes for G-allele of comparison group, and probably lower – in homozygotes for C-allele (p<0,05). In heterozygotes NO concentration was significantly lower, and in children with CC genotype was higher than in patients with GG genotype (p<0,05). We found a strong direct correlation between the NO level in children with UD and the presence of GG genotype in this category of children (r=0,97, p<0,05) and a strong probable inverse correlation with CC genotype (r=-0,96, p <0,05).

The presence of abnormal C allele worsens the course of disease and contributes to larger size of ulcer defect. In homozygotes with GG genotype size of ulcer defect was significantly lower as to heterozygotes and homozygotus persons by C allele (p<0,05) in the tendency to its increase in patients with CC genotype as compared with heterozygotes (p<0,05).

Conclusions

1. Equivalent correspondence of the nitrogen monoxide content in saliva (41,06 ± 1,9) mmol/l and plasma (11,62 ± 0,9) mmol/l of children with peptic ulcer disease, and probably its lower its rates in children of comparison group (in saliva (56,08 ± 1,8) mmol/l and plasma (15,84 ± 0,8) mmol/l) has been established. NO level in the blood is significantly lower in H. pylori-associated ulcer disease, duration

of disease more than 3 years, remission, severe clinical course, ulcers sizes more than 6 mm ($p<0,05$), significantly higher – by increased of acid productive function of the stomach, presence of duodenolgastric reflux ($p<0,05$) and is independent upon age, sex, and localization of ulcer defects.

2. In patients with peptic ulcer disease distribution of iNOS genotype (G954C) was the next: homozygotes for G-allele – 47,5%, heterozygotes – 38,3%, homozygotes for C-allele – 14,2%. The total prevalence of mutant C-allele iNOS was 52,5%. CC genotype of iNOS gene (G954C) increases the risk of ulcer disease in 3,9 times (CI [0,21-73,83], $x^2 =9,6$, $p=0,0007$).

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ЗМІНИ КОНЦЕНТРАЦІЇ МОНООКСИДУ НІТРОГЕНУ В ДІТЕЙ, ХВОРИХ НА ВИРАЗКОВУ ХВОРОБУ

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Резюме. У дітей, хворих на виразкову хворобу, рівень монооксиду нітрогену достовірно підвищений в крові та слині при гелікобактерній інфекції, знижується при тривалості захворювання більше трьох років, та при тяжкому перебігу хвороби, не залежить від віку дитини, локалізації виразкового дефекту. У хворих на виразкову хворобу розподіл генотипу iNOS (G954C) був наступним: гомозиготні особи по G-алелю – 47,5 %, гетерозиготні особи – 38,3 %, гомозиготні особи по С-алелю – 14,2 %. Загальна поширеність мутантного С-алеля iNOS становить 52,5 %.

Ключові слова: виразкова хвороба, монооксид нітрогену, діти, індукована синтаза монооксиду нітрогену.

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ИЗМЕНЕНИЯ КОНЦЕНТРАЦИИ МОНООКСИДА АЗОТА У ДЕТЕЙ, БОЛЬНЫХ ЯЗВЕННОЙ БОЛЕЗНЬЮ

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Резюме. У детей с язвенной болезнью уровень монооксида азота достоверно повышен в крови и слюне при хеликобактерной язвенной болезни, снижается при длительности заболевания более трех лет и при тяжелом течении болезни, не зависит от возраста ребенка, локализации язвенного дефекта. У больных язвенной болезнью распределение генотипа iNOS (G954C) было следующим: гомозиготные особи по G-алели – 47,5 %, гетерозиготные особи – 38,3 %, гомозиготные особи по С-алели – 14,2 %. Общая распространенность мутантной С-алели iNOS составляет 52,5 %.

Ключевые слова: язвенная болезнь, монооксид азота, дети, индуцированная синтаза монооксида азота.

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