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Genetic Aspects of Helicobacter-Associated Diseases in the Gastroduodenal Tract of Children

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Abstract

The genetic structure of Helicobacter in children with gastroduodenal pathology and the regional distribution of the pathogenic genes of Helicobacter pylori (*CagA* and *VacA*) were established. Almost all the Helicobacter pylori (HP) strains showed the presence of gene toxicity (*tox*+) of 71.7%. The spectrum of toxigenic HP genes shows the following distribution: *CagA*+ defined in 70 persons (81.3%), *VacA* + determined in 62 individuals (72.1%). In this study, the specific distribution patterns of the causative agent of the pathogenic genotypes are established in the various diseases of the gastroduodenal region in children, and the comparative topological characterization of the bacterial inoculation of the gastrointestinal tract with Helicobacter pylori of different genotypes is presented.

Key words: *children, Helicobacter pylori (CagA and VacA), genotyping.*

Introduction

Recently, an uncontrolled increase in the frequency of gastroduodenal pathology among children has been observed [1]. According to the Center for Ukrainian Health Statistics, the incidence of diseases of the digestive system in children has increased in 2011 compared with 2000, by 56.8%, while the prevalence of diseases of this class, during the same period, showed an increase from 88.9 to 149.9‰. Most researchers have linked this high prevalence of infection with Helicobacter pylori (HP) [2,3]. In the study of the phenotypic and genotypic characteristics of HP, an exceptionally high genetic diversity of strains was revealed and almost every patient was found to possess a unique strain, leading to some form of pathology of the gastroduodenal region. It is well known that all the HP strains are not equally pathogenic [4]. The most virulent strains are *VacA* and *CagA*-positive types, which stimulate the synthesis of the epithelial cells of the inflammatory mediators of the gastric mucosa – viz, the cytokines, followed by the subsequent infiltration of the mucosa by these inflammatory cells and ultimately the release of the reactive oxygen metabolites.

The purpose of this study is to discover the prevalence of the toxic genotypes of helicobacter infection in children and to determine the regional specificities of their distribution.

Material and Methods

Research was conducted employing the randomization method, taking into account the main provisions of the ICH-GCP and Helsinki Declaration on Biomedical Research and their subsequent views (Seoul, 2008), the Council of Europe Convention on Human Rights and Biomedicine (2007) and the recommendations of the Bioethics Committee at the Presidium of the Academy of Medical Sciences of Ukraine (2002) and by the endorsement of the Bioethics Committee of the Bukovinian State Medical University. Clinical examinations were done in 120 children with chronic gastroduodenal pathology (CGDP (HP+)), age between 7 and 18 years (main group) and in 120 children with CGDP (HP-) of the same age (group of

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comparison). Patients of the main group were further divided into two subgroups: Group A with 86 children having CGDP (HP+) with the selected method of polymerase chain reaction (PCR) toxigenic strain (*CagA, VacA* and *CagA + VacA*) and Group B including 34 patients with CGDP (HP+) with the selected PCR nontoxic strain. All the children were subjected to general clinical tests as mandatory requirement, including ultrasound ("Aloka SSD-1000", Japan), and fiberoptic gastroduodenoscopy ("Pentax FG - 24V", Japan) to verify the diagnosis, according to the "Sydney system"(1990) and biopsy sampling from the gastric antrum and duodenal bulbs ("Sydney-Houston system", 1996). For the diagnosis and the study of the gene structure and polymorphism of HP, the polymerase chain reaction (the standard test system to determine the DNA of the HP (Insta Gene Matrix, Bio Rad, USA thermocycler Eppendorf and sequencer CEQ 8000, Beckman Coulter, Germany), *CagA*-positive and *VacA*-positive strains of HP)) was employed.

Results were statistically processed using the computer software package "STATISTICA V.6.0». Quantitative and serial parameters are presented as the mean (M) ± standard deviation (SD), quality - as the absolute number of observations and proportion (in %) of the total number of patients for the sample as a whole or relevant group.

Results

Based on the pathology of the children, both the groups were divided into four subgroups, as follows: the 1st group included 35 (14.6%) children with chronic gastritis (CG), the 2nd with 76 (31.6%) children having chronic gastroduodenitis (CGD), the 3rd third had 52 (21.7%) children with erosive gastroduodenitis (EGD), and the 4th contained 77 (32.1%) children with stomach and duodenal ulcers (SDU). The frequency of HP invasion in our study was 85.7%, which concurs with the literature data [4]. The results are presented in Table 1.

From this research it can be concluded that the HP genotypes possessing both the determinants of pathogenicity (precisely *CagA+ VacA+)* are found more often (71.7 ± 9.6%; p <0.05) and are more inoculated. Simultaneously, *CagA- VacA+* the pathogenic strains of HP inoculate larger areas than the strains *CagA + VacA-* (78.8 ± 8.7% *vs* 48.5 ± 5.3%; p <0.05). Therefore, this is indicative that VacA + plays a role in invasiveness along with HP. The degree of inoculation is also observed to vary depending upon the availability of the HP pathogenicity factors. Thus, we determined that the HP strains containing *CagA* and *VacA* cause only the high and medium degrees of mucosal inoculation. HP strains negative for the pathogenic strains cause the low and medium degrees of inoculation. A thorough analysis was performed regarding the distribution of HP strains with pathogenicity factors depending on the clinical variant (Table 3 and 4).

The highest number of HP strains detected on biopsy of the mucosal membrane, as well as the number of toxigenic HP strains detected was characteristically found in children suffering with SDU as a whole in 57 children (74%) and 45 children (78.9%), respectively. This is why SDU is found to occur 2.8 times more often during the clinical course as the background infection of HP. It should be noted that the distribution of the pathogenic genes in such patients is dominated by the *VacA+* strains (27 persons, 60.0%) and *CagA+* strains (18 persons, 40.0%). This survey data proves that patients with SDU HP+ have a greater inoculation area and a significantly higher incidence of the highest degree of inoculation with Helicobacter.

The patients with EGD present a similar picture. Helicobacter-associated EGD was recorded 2.5 times higher than EGD infection without HP. Among those patients with EGD HP+ the toxic strains were determined in 71.4% cases: 21 individuals (84%) with *VacA+* and 19 (76%) with *CagA+* strains. Another characteristic noted was a high degree of mucosal inoculation of HP, especially of the gastric antrum.

Table 1
Results of genotyping of helicobacter infection

Helicobacter strains									
HP (tox+), n=86								HP (tox -), n=34	
CagA + VacA+		CagA + VacA-		CagA- VacA+		Total		CagA - VacA-	
Abs.	%	Abs.	%	Abs.	%	Abs.	%	Abs.	%
46	53.4	24	27.9	16	18.6	86	71.7	34	28.3

The density of all the strains showing the presence of the toxigenic gene (*tox+*) is 71.7% for all the HP strains. The spectrum of the toxin genes of HP are distributed as follows: *CagA+* defined in 70 children (81.3%) and *VacA+* determined in 62 individuals (72.1%). Thus, among strains of the HP is defined heterogeneity of the natural increase in the biochemical activity of the bacteria with presence of the *CagA* gene in them. Therefore, HP is more often found in the antral stomach (71.7 ± 9.6%) and less frequently in the duodenal bulb (28.3 ± 4.9%), p <0.05. As our studies revealed the heterogeneity of HP in the composition of the toxigenic genes in the microorganisms, an interesting fact that arose was the dependence of the inoculation frequency of HP in various portions of the gastroduodenal region based on the presence of an antigenic spectrum (Table 2).

Slightly lower frequencies of lesions with HP were seen in patients with CGD. Of the 76 cases, 36.8% were found infected with the HP-associated variant, indicating that it occurs 1.8 times less than in patients with EGD, and almost three times lower when compared with patients with SDU. In 60.0% of the cases of CGD diagnosed, those with toxigenic strains of HP included 9 patients with75.0% *VacA+* and 3 patients with 25.0% *CagA+* strains.

Very few samples with HP were found on biopsy of CO in children with CG (8 patients out of 35, 22.9%) with an even distribution of the toxigenic and non-toxigenic HP strains. This draws our attention to the predominance of low and moderate mucosal inoculation with HP.

Table 2
The frequency of inoculation of different parts of the gastroduodenal area with strains of helicobacter infection

Helicobacter strains	Topical localization					
	Antral part of stomach		Body of stomach		Duodenum	
	Abs.	%	Abs.	%	Abs.	%
CagA+ VacA+ (n=46)	44	95.6*	26	56.5	8/11	72.7
CagA+ VacA– (n=24)	10	41.6*	7	29.2	2/11	18.2
CagA– VacA+ (n=16)	12	75.7	10	62.5	1/11	9.1
CagA– VacA– (n=34)	25	73.5*	15	44.1	-	-
CagA+ (n=70)	54	59.3	33	56.8	10/11	90.9
CagA– (n=50)	37	40.6	25	43.1	-	-
VacA+ (n=42)	56	61.5	36	62.1	1/11	9.1
VacA- (n=58)	35	38.5	22	37.9	2/11	18.2
Tox+ (n=86)	66	76.7*	43	50.0	9/11	81.8
Tox- (n=34)	25	73.5*	15	44.1	2/11	18.2

Note*: difference is precise between measuring variations of frequency of helicobacter detection in antral and body parts of stomach (P <0.05).*

Table 3
Frequency of Helicobacter infection in children against nosological form of illness

Nosological form	Helicobacter positive		Helicobacter negative	
	Abs.	%	Abs.	%
Chronic gastritis n=35	8	22.9	27	77.1*
Chronic gastroduodenitis n=76	20	26.3	56	73.7*
Chronic erosive gastroduodenitis n=52	35	67.3**	17	32.7
Peptic ulcer n=77	57	74.0**	20	26.0

Note: *difference is precise * - about helicobacter positive variations in nosologies, ** - with chronic erosive gastroduodenitis and peptic ulcer with presence of helicobacter (P <0.05).*

Table 4
Division of helicobacter infection in accordance to presence of genes of pathogenicity and nosological form of pathology

Nosology	HP (tox+)		HP (tox-)	
	Abs.	%	Abs.	%
Chronic gastritis n=8	4	50.0	4	50.0
Chronic gastroduodenitis n=20	12	60.0	8	40.0
Chronic erosive gastroduodenitis n=35	25	71.4*	10	28.6
Peptic ulcer n=57	45	78.9*	12	21.1
Total, n=120	86	71.7	34	28.3

Note: **Pφ- difference is precise between measuring variations of frequencies of toxigenic strains in patients with chronic erosive gastroduodenitis and peptic ulcer.*

Conclusion

The more often the pronounced, destructive, inflammatory process occurs in the mucosa of the gastroduodenal region of the gastrointestinal tract in children, the more often are the HP bacteria detected and the more pronounced are their toxigenic effects. These research results provide an opportunity to identify precisely which *VacA+* strains of HP are the major determinants of the virulence of the microorganism that plays an important role in the invasiveness and degree of colonization of the gastroduodenal mucosa.

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