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Full length research paper

Relation between Helicobacter pylori cagA Status and Risk of complications Peptic Ulcer in children

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Pathogenicity genes of Helicobacter pylori studied in children with complicated peptic ulcer disease. Found that patients with complicated ulcer disease prevails serotype I (CagA+ VacA+) Helicobacter pylori. The risk of complicated of Helicobacter-associated peptic ulcer disease increases to 8,18 times (95% CI [2,03-32,9], χ2 = 10,22, p<0,01) for the presence of Helicobacter pylori CagA gene and to 5,37 times (95% CI [5,24-18,9], χ2 = 7,44. p <0,01) in the presence of serotype I (CagA+ VacA+). Thus, CagA gene Helicobacter pylori is a factor that determines the nature of the disease in children.

Keywords: children, peptic ulcer, strains of Helicobacter pylori (CagA, VacA).

Introduction

Analysis of the causes and risk factors for a complicated course is a crucial direction in the study of peptic ulcer [Carvalho A.S. (2010); Sorokman T. et al., (2012)] Complications of peptic ulcer disease include gastrointestinal bleeding, obstruction, penetration and perforation ulcer. In the light of modern concepts of complications of greatest interest is the analysis of the role of aggressive Helicobacter pylori (H. pylori) - one of the most common chronic infections in man. H. pylori is commonly present in the human stomach, and investigations over the past years have focused on its relation to ulcer disease [Арутин Л.И. et al., (1998)]. H. pylori colonizes persistently in the gastric mucosa, leads to chronic mucosal inflammation and increases the risk of gastric and duodenal ulceration. However, there are distinct differences in the extent of inflammation among H. pylori -infected patients [Сорокман Т.В. et al. (2008)].Question that determines the development of complicated forms of the disease is the most sophisticated and up to date has not been resolved [Lin H.J. (2009)]. Most authors have suggested that the nature of the disease depends on intraspecific diversity of strains of H. pylori, the degree of infection H. pylori gastric mucosa, as well as the genetic characteristics of the microorganism, the state of host immune system and other factors [Garcна-Iglesias P. et al.,(2010); Сокольник С.В. (2012)].H. pylori strains are highly diverse, yet a fundamental distinction among strains is the CagA pathogenicity island, a region of about 40 kb that is present or absent in the H. pylori chromosome [Исаев Г.Б. (2004)]. One gene, CagA, was the first discovered gene on the island and is a marker for its presence. The H. pylori strains CagA+ and CagA– differ substantially in their biology, in that the former are much more interactive with the host, injecting the CagA protein into epithelial cells and inducing a more profound tissue response [Говорун В.М. et al., (2002); Blecker U. (2010)]. H. pylori strains may occupy different microniches in the stomach according to CagA status, which may affect the microecology of the stomach with consequent differences in clinical outcome [Carvalho A.S. (2010)].The present study was conducted to assess the role of virulent strains of H. pylori in the formation of complicated peptic ulcer in children.

Materials and methods

The study involved 57 children aged 7 to 18 years, patients with Helicobacter-associated peptic ulcer that were hospitalized in the Regional Pediatric Clinical

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Table. The frequency distribution of serotypes *Helicobacter pylori* in children with peptic ulcer disease in children depending on the presence of complications

Serotype *H. pylori*	Children with uncomplicated ulcer (n=38)	Children with complicated ulcer (n=19)
CagA+VacA+ (n=16)	6 / 15,8 %	10 / 52,6 %*
CagA+VacA- (n=15)	9 / 23,7 %	6 / 31,6%
CagA-VacA+ (n=13)	12 / 31,6 %*	1 / 5,3 %
CagA-VacA- (n=13)	11 / 28,9 %	2 / 10,5 %

Note. * - p<0,05

Hospital and Municipal Children Clinical Hospital (Chernivtsi), including 19 individuals with complicated disease course. Patients who had not received H. pylori eradication therapy and had not been treated with proton pump inhibitors, not with gastric pH neutralizers during the three months prior to the endoscopy were included in the study. The mean age of children was 12,9±2,9 years. Participants' eating habits, socio-demographic factors, family history of gastritis or ulcers and other were recorded via surveys.For each patient, endoscopy was carried out using gastroscope (Pentax FG – 24P, Japan). From each patient, we took two biopsies from the antrum, corpus, or ulcer edge; one specimen was immediately fixed in formalin for histological testing and the other was placed in buffer solution H. pylori diagnosis. The histological sections were stained with hematoxylin-eosin and evaluated by a pathologist using the updated Sydney System criteria [Dixon M. *et al.* 1996]. Endoscopic observation and histopathological confirmation were used to determine patient pathology.H. pylori infection was screened by histological examination, the rapid urease test, or antibodies against H. pylori. Patients were diagnosed as H. pylori positive if one or more of these diagnostic methods was positive.Genotyping of virulence genes CagA, VacA performed by polymerase chain reaction (PCR) using specific primers (Insta Gene Matrix, Bio Rad (USA) termotsykler Eppendorf sekvenator and CEQ 8000, Beckman Coulter (Germany)). Section amplification products was carried out in 2% agarose gels. The results documented using video systems and processed using the software package "Molecular Analyst Software".Statistical analysis of the data was performed using computer software package «Statistica 6.0». Significance of differences was assessed by Pearson ($\chi 2$), with differences considered authentic $p<0,05$. The role of risk factors in the development of peptic ulcer complications were evaluated in terms of odds ratio (OR) with a 95% confidence interval. OR>1 indicates an increased risk of complications when exposed to the factor.

Results and Discussion

The majority of patients with complicated peptic ulcer noted gastrointestinal bleeding (11 children (57,9 %)); in 7 (36,8 %) patients diagnosed ulcer perforation and and only 1 (5,3%) child was with obstruction. Analysis of complications depending on the location of the ulcer showed some differences: significantly more gastrointestinal bleeding in children diagnosed with an ulcer in the duodenum than gastric ulcer (72,7 % and 27,3 %, $p<0,05$). However, perforated ulcers frequently noted in children with gastric ulcer (71,4% and 28,6 %, $p<0,05$) localized mainly in prepyloric area. Thorough examination of children with/without complications of peptic ulcer did not reveal their any co-morbidity. Complications in peptic ulcer infection of H. pylori histological method identified in 94,7% of cases, uncomplicated was in 84,2%. In the structure of infection in complicated ulcers significantly dominated cases with a high degree of infection H pylori (77,8%), whereas in patients without complication was mild cases the degree of infection H. pylori (71,9%).Using PCR DNA positive for H. pylori from gastric biopsy specimens in patients with peptic ulcer children in both groups was obtained in 100%. Spectrum of toxicity genes revealed that their share of children with complicated peptic ulcer disease was significantly greater than in children with uncomplicated ulcer disease (89,5% and 71,1%, $p\varphi <0,05$).

In general, children with peptic ulcer disease CagA gene frequency was 54,4% and was slightly leaching than gene VacA (50,9%), $p\varphi>0,05$. The same trend was observed, but with significant difference in children with ulcer complications: CagA gene revealed in 16 (84,2%) children, the gene VacA was in 11 (57,9%) patients ($p\varphi<0,05$). However, in children with uncomplicated disease significantly more frequently diagnosed gene VacA (18 (47,4%)) than gene CagA (15 (39,5%)), $p\varphi <0,05$.The frequency distribution of serotypes of H. pylori in children with peptic ulcer disease showed no significant differences (Table). But it should be noted that children with ulcer complications unlike patients with uncomplicated course of the disease, significantly predominant serotype I (CagA+ VacA+) was 52,6% and 15,8% respectively ($p\varphi<0,05$), whereas as the latter were significantly more determined serotype Ib (CagA-VacA+) was 31,6% and 5,3% ($p\varphi<0,01$).Analysis of epidemiological indicators found that the risk of ulcer

complications in children will grow up to 8,18 times during infection with strains of H. pylori CagA gene (OR = 8,18, 95% CI [2,03-32,9], $\chi 2$ = 10.22, $p<0.01$) and to 5,37 times in the presence of serotype I (CagA + VacA +) - OR = 5,37, 95% CI [5,24-18,9], $\chi 2$ = 7.44, $p <0,01$).It is known that 90% of patients with peptic ulcer disease often exhibit strains that have phenotypes CagA and VacA. Cytotoxin CagA - phenotype is a marker of bacterial virulence strain [Blecker U. (2010); Thomas G. *et al.*, (2013)]. Infection with this strain determines the characteristics of the colonization of the mucosa and leads to a more severe course of the disease with severe clinical manifestations [Garcia-Iglesias P. *et al.*, (2011)]. It is proved that the development of H. pylori-dependent inflammatory process is only possible under certain conditions high virulence of the bacteria on the one hand, which is caused by the presence of pathogenicity genes (including CagA), and a decrease in host defenses on the other [Lin H.J. (2009); Сорокман Т.В. *et al* ., (2008)].

In our study just found a relationship between the presence of H. pylori gene pathogenicity CagA and the development of complications of peptic ulcer disease in children.

Conclusions

The results of these studies confirm and extend our understanding of the role that carriage of CagA+ strains play in complicated peptic ulcer disease. That CagA+ strains is associated with enhanced risk of complications ulcer disease. Thus, all H. pylori strains are not equal in their relation to disease, and physicians should consider H. pylori strain characteristics, such as CagA status, in attempting to optimize care for their patients.

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