The System Endotoxinemia and Antiendotoxin Immune Response in Children with Peptic Ulcer Disease, Associated with Helicobacter Pylori Virulent Strains

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Abstract

The purpose of this study was to determine the systemic endotoxemia and antiendotoxin immune response markers in children with peptic ulcer disease, associated with cagA and vacA positive H. pylori strains.

Methods. 60 children, aged 12 to 17 years, all with H. pylori-associated peptic ulcer disease. 32 patients with peptic ulcer disease with persistence cagA and vacA H. pylori strains were in group I, and 28 patients with peptic ulcer disease, associated with cagA and vacA negative H. pylori strains, were in group II. The control group consisted of 20 healthy children. The diagnosis of H. pylori
was carried out by two methods: the rapid urease test with biopsy material and the urea breath test. Genotyping of H. pylori in biopsy specimens of gastric mucosa with the determination of cagA and vacA genes was performed by PCR. Concentrations of lipopolysaccharide of Gram-negative bacteria, level of lipopolysaccharide-blinding protein and antibodies (classes IgG, IgM, IgA separately) to the Core region of endotoxin were conducted in blood serum of patients.

Results. The persistence of H. pylori infection in children with peptic ulcer disease leads to higher levels of endotoxemia. The persistence of H. pylori with vacA and cagA genotype leads to a more significant elevation of systemic endotoxemia markers. It was revealed that infection with virulent strains of H. pylori leads to a significant increase in the concentration of lipopolysaccharide-blinding protein in serum. It was established that infection with virulent H. pylori strains is associated with greater oppression of antiendotoxin immunity links. Persistence of virulent H. pylori strains was also combined with inhibition of anti-LPS-IgG, anti-LPS-IgM and anti-LPS-IgA levels.

In conclusion, microbial disbalance in patients with PUD is accompanied by increased serum level of lipopolysaccharide and systemic endotoxemia. Prolonged exposure to pathological endotoxemia can be considered as a factor in the maintenance of duodenal inflammatory and destructive process on the background of persistent H. pylori infection and inhibition of antiendotoxin immune response. We have established that persistence of virulent strains of H. pylori leads to significant increased endotoxin level and depression humoral antiendotoxin protection.

Keywords: lipopolysaccharide of Gram-negative bacteria, children, virulent strains, H. pylori, peptic ulcer desease, antiendotoxin immune response

Introduction

H. pylori infection is present in more than the half of the world’s population. Most individuals are infected during early childhood; in developing countries, 50 % of children are infected by the age of 5 years [2,3]. However, not all infected people exhibit diseases associated with this bacterium. Recent studies suggest the main etiological role of H. pylori infection in development of chronic gastroduodenal pathology and the formation of its most severe forms in patients of different age groups. However, the pathogenetic aspects of the development of peptic ulcer disease (PUD) in children on the background of persistence of H. pylori remain poorly studied.

Several virulence genes have been studied and established in the literature as determining factors in pathogenesis of gastroduodenal pathology, such as cagA (cytotoxin-associated gene A) and vacA (vacuolating cytotoxin A) genes. H. pylori infection with a strain, containing cagA and vacA genes, was associated
with the development of ulcer disease, showing the importance of these genes to gastric diseases outcomes [1,5].

Nowadays, the depletion of regulatory mechanisms of the immune response and the biochemical protection due to increased immune load on the body is considered as one of the pathogenic mechanisms of the development of the mucosal destruction and the duodenal ulcer [1,6]. In case of chronic persistence of H. pylori the microecological balance of the digestive tract is damaged. The concentration of intraluminal endotoxins is significantly increased in case of excessive growth of pathogenic or opportunistic pathogens, especially Gram-negative bacteria. Endotoxins of Gram-negative bacteria (lipopolysaccharide (LPS)) are the most active intestinal toxins [4]. They maintain chronic inflammatory processes in the organism after admission to the systemic circulation in case of pathology. Lipopolysaccharide-binding protein (LBP) is constitutively present in blood of a healthy person; it is synthesized chiefly by hepatocytes. The key function of LBP is to detect, bind and sequentially present bacterial endotoxin to genetically encoded receptors which are present on leukocytes and other cells, and that provide increased sensitivity of receptors to pathogen and amplification of a signal of infection risk. LBP is able to detect molecular structures of gram-negative microorganisms. An important component of antiendotoxin immunity response is humoral antibacterial protection. It is known that the human body almost always synthesizes antibodies to LPS intestinal microbiota. Their appearance is connected with the penetration of microflora and its toxins through the intestinal wall.

**Aim**

The purpose of this study was to determine the systemic endotoxemia and antiendotoxin immune response markers in children with PUD, associated with cagA and vacA positive H. pylori strains.

**Methods**

60 children, aged 12 to 17 years, all with H. pylori-associated PUD. 32 patients with PUD with persistence cagA and vacA H. pylori strains were in group I, and 28 patients with PUD, associated with cagA and vacA-negative H. pylori strains were in group II. The control group consisted of 20 healthy children. The diagnosis of H. pylori was carried out by two methods: the rapid urease test with biopsy using the test system "Helpil" ("AMA", Russia) and the urea breath test using the test system "Helic" with detector tubes ("AMA", Russia). Genotyping of HP in biopsy specimens of gastric mucosa with the determination of cagA and vacA genes was performed by PCR using sets of "Helicopol" ("Liteh", Russia).

Concentrations of LPS of Gram-negative bacteria was determined by using adapted to clinic LAL (Lumulus Amebocyte Lysate) the test «E-toxate» («Sigma Chem. Co.», USA) in EU/ml. Quantitative determination of LBP in serum of patients was detected by enzyme immunoassay ELISA («HyCult biotechnology», Netherlands) in mcg/ml. Determining the level of antibodies
(classes IgG, IgM, IgA separately) to the Core region of endotoxin was conducted in blood serum by using a test EndoCab (Endotoxin Core Antibody ELISA, «HySult biotechnology», Holland) by enzyme immunoassay ELISA (in MU/ml). LPS obtained from four Gram-negative bacteria (Escherichia coli, Salmonella typhimurium, Klebsiella aerogenes, Pseudomonas aeruginosa) were used as the antigens. Each of LPS contained completely inside core-part, but there were no external parts or an O-specific polysaccharide chain.

Statistical analysis of the obtained results was carried out in MedCalc Version 13.0.2.0. The data were presented as mean (X̄) and error of mean (±m). To compare the main parameters of patients’ groups, ANOVA or the rank Kruskal-Wallis test were used. To compare categorical variables between groups, the Chi-square test (χ²) and the Fisher F exact test were used. A calculated difference of P<0.05 was considered significant.

Results

Table 1 shows levels studied serum markers of system endotoxinemia and immune antiendotoxin response in children with PUD, associated with different strains of H. pylori.

Table 1.
Average values of systemic endotoxemia and antiendotoxin immunity markers in studied patients, depending on the virulence of H. pylori genotype

<table>
<thead>
<tr>
<th>Serum markers</th>
<th>Group I (n=32)</th>
<th>Group II (n=28)</th>
<th>Control group (n=20)</th>
<th>Level of differences, P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS, EU/ml</td>
<td>2,3±0,1*²</td>
<td>1,6±0,1¹</td>
<td>0,52±0,04¹²</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td>LBP, ng/ml</td>
<td>29,8±1,7*²</td>
<td>20,6±1,1*¹</td>
<td>6,7±0,7¹²</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td>IgA EndoCab MU/ml</td>
<td>69,3±4,4*²</td>
<td>90,0±5,4¹</td>
<td>104,8±6,7¹</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td>IgG EndoCab MU/ml</td>
<td>73,1±4,2*</td>
<td>90,1±4,6</td>
<td>107,2±5,3¹</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td>IgM EndoCab MU/ml</td>
<td>74,6±3,5*²</td>
<td>91,0±3,2*¹</td>
<td>110,2±4,3¹²</td>
<td>&lt;0,001</td>
</tr>
</tbody>
</table>

Note: * – statistically significant difference (P<0.05) from control group; 1 – statistically significant difference (P<0.05) from group I; 2 – statistically significant difference (P<0.05) from group II
The effect of virulent strains of H. pylori persistence to the condition of the system entoxinemia was studied. It was found that patients who were infected with virulent strains of H. pylori revealed a higher level of LPS in the serum (Table 1). The level of significance of differences between groups – p<0.001.

The average level of LPS in children of group I was 2.3±0,1 EU/ml, which was statistically significantly (p<0.05) higher compared to patients of group II (1.6±0,1 EU/ml) and patients of the control group.

The level of LBP in patients of group I was 29.8±1.7 ng/ml, that was statistically significant (p<0.05) higher compared to this marker in children of group II (20.6±1.1 ng/ml). It was found that the average levels of LBP in both groups of children with PDU, associated with H. pylori were significantly (p<0.05) higher compared to the control group. The level of significance of differences between groups was p<0.001.

The average level of anti-LPS-IgA in patients of group I was 69.3±4.4 EU/ml, which was statistically significantly (p<0.05) lower compared to the control group and group II. The mean value of anti-LPS-IgA in children of group II was 90.0±5.4 EU/ml.

The average value of anti-LPS-IgG in the blood serum of patients infected with virulent strains of H. pylori was 73.1±4.2 EU/ml, which was statistically significantly (p<0.05) lower compared to control group. In patients of group II the mean value of this marker was 90.1±4.6 EU/ml.

Levels of anti-LPS-IgM in patients of II group I and group II were also statistically significantly (p<0.05) lower compared to the control group. The average value of this marker in patients with persistence of H. pylori virulent strains was 74.6±3.5 EU/ml, that was statistically significantly lower (p<0.05) compared to children of group II (91.0±3.2 EU/ml).

Discussion

The genome of H. pylori has several genes that cause cytotoxic effect of this microorganism. VacA and cagA genes play important role in colonization and are responsible for the development of gastric diseases. The persistence of H. pylori infection in children with PUD leads to higher levels of endotoxemia. The presence of H. pylori with vacA and cagA genotype leads to a more significant elevation of systemic endotoxemia markers. All children of the control group had endotoxinemia within the physiological range which did not exceed 1.0 EU/ml. It was revealed that infection with virulent strains of H. pylori leads to a significant increase in the concentration of LBP in serum. It was established that infection with virulent H. pylori strains is associated with greater oppression of antiendotoxin immunity links. Persistence of virulent H. pylori strains was also combined with inhibition of the level of anti-LPS-IgG, anti-LPS-IgM, and anti-LPS-IgA.
Conclusion

Thus, microbial disbalance in patients with PUD, associated with H. pylori is accompanied by increased serum level of LPS and systemic endotoxemia. Prolonged exposure to pathological endotoxemia can be considered as a factor in the maintenance of duodenal inflammatory and destructive process on the background of persistent H. pylori infection and inhibition of antiendotoxin immune response. We have established, that persistence of virulent strains of H. pylori leads to significantly increased endotoxin level and depression of humoral antiendotoxin protection.

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Ethical declaration: All patients have given their written informed consent for participation in the study.

Conflict of interests: none declared.

References


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