IL-8 serum levels in patients with *Helicobacter pylori* infection and relation between serological markers

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In this study we aimed to investigate IL-8 levels in dyspeptic patients with *Helicobacter pylori (H. pylori)* infection and in control group. Our study group consists of 172 patients with dyspepsia who underwent upper gastrointestinal system endoscopy. On the day of taking gastric tissues, serum samples were taken. *H. pylori* were investigated from gastric tissues by invasive test methods as Gram staining, urease test and culture method. From serum samples anti-CagA IgA and IgG, anti-*H. pylori* IgM, IgA, IgG and IL-8 levels were studied by EIA. These 172 patients had positive invasive and/or serological test results. For the control group, 20 patients with negative *H. pylori* invasive and serological test results were included. Among 172 patients, 102 (59.3%) had normal IL-8 levels and 70 (40.7%) had high IL-8 levels. In 8 (40%) of 20 control patients IL-8 was high. The mean values of IL-8 were as 43.96 ± 13.65 pg/ml (min-max: 0 - 1477 pg/ml) and 42.03 ± 24.32 pg/ml (min-max: 0 - 483.6 pg/ml) in positive and negative groups, respectively. Our observations implicate that there was no significant difference in serum levels of IL-8 between *H. pylori* positive and negative patients.

Key words: *Helicobacter pylori*, interleukin (IL)-8, anti-*H. pylori* antibodies, anti-CagA antibodies.

INTRODUCTION

Presence of the *Helicobacter pylori* (*H. pylori*) in the stomach is usually associated with gastritis, peptic ulcers, duodenal and gastric carcinoma, and gastric lymphoma (Blaser, 2000; O’Mahony et al., 2004). Urease, catalase, oxidase enzymes, IL-8 secretion, vacuolating cytotoxin, protease and phospholipases are important virulence factors responsible for the diseases of *H. pylori* (Dunn et al., 1997). The local inflammation associated with *H. pylori* infection is characterized by an increased production of IL-1, IL-6, IL-8, and TNF-α (Gyulai et al., 2004). IL-8 is a potent T-cell and neutrophil chemoattractant (Sharma et al., 1998) and is the primary modulator cytokine in *H. pylori*-associated gastritis (Crabtree et al., 1994b).

*H. pylori* Pathogenicity Island encoding for CagA protein and other virulence factors increase the IL-8 expression and IL-8 triggers the neutrophil infiltration to the epithelium (Crabtree, 1996; Bayraktaroğlu et al., 2004). In this study, we aimed to investigate IL-8 serum levels in dyspeptic patients with *H. pylori* infection and in control group, and relation between serological markers such as anti-*H. pylori* IgM, anti-*H. pylori* IgA, anti-*H. pylori* IgG and anti-CagA IgA and IgG.

MATERIALS AND METHODS

Patients

Our study group consisted of 172 patients with dyspepsia who underwent endoscopy. On the day of taking gastric tissues, serum samples were also taken. These 172 patients had positive invasive and/or serological test results. For the control group, 20 patients with negative *H. pylori* invasive and serological test results were included. Patients who received antibacterial treatment or anti-ulcer treatment prior to endoscopes were excluded.
Table 1. Comparison of anti-CagA and anti-\(H.\text{pylori}\) antibodies with IL-8 levels.

<table>
<thead>
<tr>
<th>Anti-CagA IgA(-) IgG(-) n:78</th>
<th>IL-8 normal(^a) n (%)</th>
<th>IL-8 high(^b) n (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>n: 172</td>
<td>47 (60.3)</td>
<td>31 (39.7)</td>
<td></td>
</tr>
<tr>
<td>Anti-CagA IgA(-) IgG(+) n:47</td>
<td>29 (61.7)</td>
<td>18 (38.3)</td>
<td></td>
</tr>
<tr>
<td>Anti-CagA IgA(+) IgG(+) n:47</td>
<td>26 (55.3)</td>
<td>21 (44.7)</td>
<td></td>
</tr>
<tr>
<td>Anti-HP IgG(-) IgM(-) IgA(-) n:13</td>
<td>7 (53.8)</td>
<td>6 (46.2)</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>Anti-HP IgG(+) IgM(-) IgA(-) n:53</td>
<td>30 (56.6)</td>
<td>23 (43.4)</td>
<td></td>
</tr>
<tr>
<td>Anti-HP IgG(+) IgM and/or IgA(+) n:106</td>
<td>65 (61.3)</td>
<td>41 (38.7)</td>
<td></td>
</tr>
<tr>
<td>Control group n:20</td>
<td>12 (60.0)</td>
<td>8 (40.0)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)IL-8 concentration is below 8 pg/ml, and could not be detected by the assay.
\(^b\)IL-8 concentration is above 8 pg/ml.

Table 2. The median IL-8 values of patients in different groups with only high levels of IL-8.

<table>
<thead>
<tr>
<th>Anti-CagA IgA(-) IgG(-) n:31(^a)</th>
<th>Median IL-8 level (25 - 75 percentiles)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>n: 70(^a)</td>
<td>28.2 (20.6 - 56.65)</td>
<td></td>
</tr>
<tr>
<td>Anti-CagA IgA(-) IgG(+) n:18(^a)</td>
<td>32.45 (18.5 - 103.5)</td>
<td></td>
</tr>
<tr>
<td>Anti-CagA IgA(+) IgG(+) n:21(^a)</td>
<td>26 (16.5 - 47.3)</td>
<td></td>
</tr>
<tr>
<td>Anti-HP IgG(-) IgM(-) IgA(-) n:6(^a)</td>
<td>37.05 (26.1 - 86.7)</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>Anti-HP IgG(+) IgM(-) IgA(-) n:23(^a)</td>
<td>31.3 (19.75 - 66.35)</td>
<td></td>
</tr>
<tr>
<td>Anti-HP IgG(+) IgM and/or IgA(+) n:41(^a)</td>
<td>26.1 (16.5 - 58.0)</td>
<td></td>
</tr>
<tr>
<td>Control group n:8(^a)</td>
<td>45.55 (22.3 - 101.4)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) These numbers show patients with only high/positive levels of IL-8.

Assessment of invasive tests

\(H.\text{pylori}\) were investigated from tissues by conventional methods as Gram staining, urease test and culture method.

Assessment of serological tests

From serum samples anti-CagA IgA, anti-CagA IgG (Euroimmun, Hamburg, Germany), anti-\(H.\text{pylori}\) IgM, anti-\(H.\text{pylori}\) IgA, and anti-\(H.\text{pylori}\) IgG (IBL, Hamburg, Germany) were studied by Enzyme Immunoassay (EIA). All of the tests were studied according to the instructions of manufacturers.

IL-8 assay

IL-8 (Beckman-Coulter, Immunotech, France) was studied by EIA on sera of patients. At the beginning of the study, 20 ng/ml IL-8 solutions were prepared. From this solution, fresh dilution series were prepared and a series of 2000, 500, 125 and 31.2 pg/ml were achieved. The procedure of the assay was performed according to the instructions of the manufacturer. From the absorbance values of the standards, a standard curve was drawn. For the samples, corresponding IL-8 concentrations were read on the horizontal axis by locating the absorbance value of the sample on the vertical axis. The sensitivity of the assay was 8 pg/ml, and the values above 8 pg/ml were assessed as high levels, and the levels below 8 pg/ml were not detected by the assay and assessed as normal/negative IL-8 levels.

In statistical analysis of the data, two proportions z test, Kruskal-Wallis test, and variant analysis were used.

RESULTS

Among 172 patients, 102 (59.3%) had normal IL-8 levels and 70 (40.7%) had high IL-8 levels. In 8 (40%) of 20 control patients IL-8 was high. The mean values of IL-8 were as 43.96 ± 13.65 pg/ml (min-max: 0 - 1477 pg/ml) and 42.03 ± 24.32 pg/ml (min-max: 0 - 483.6 pg/ml) in positive and negative groups, respectively (p > 0.05). The comparison of IL-8 serum levels and serological markers of patient and control groups is given in the Table 1. Table 2 shows the median IL-8 values of patients in different groups with only high levels of IL-8.

DISCUSSION

In this study, we aimed to investigate the serum levels of IL-8 and compare these levels of patients with respect to the anti-\(H.\text{pylori}\) IgM, IgA, IgG and anti-CagA IgA and IgG antibody responses.

It is mentioned in the literature that, infection with \(H.\text{pylori}\) leads to increases in many mucosal proinflammatory and immunoregulatory cytokines such as IL-6, IL-8, TNF-\(\alpha\) (Bayraktaroğlu et al., 2004; Crabtree, 1998; Sharma et al., 1995). The primary modulator cytokine in
H. pylori-associated gastritis is IL-8 (Crabtree et al., 1994b). IL-8 is an important chemotactic and activating factor for neutrophils (Crabtree and Lindley, 1994). This cytokine plays a role in gastric mucosal injury caused by H. pylori (Xuan et al., 2005; Gionchetti et al., 1994). In the literature, mucosal IL-6 and IL-8 levels were found increased in H. pylori positive-dyspeptic patients (Gionchetti et al., 1994; Noach et al., 1994). It has been shown that IL-8 concentration in gastric biopsy specimens taken from H. pylori positive patients is increased (Reider et al., 2001). Also it is mentioned that, H. pylori infection up regulates the IL-8 gene expression and afterwards IL-8 synthesis and secretion from gastric cancer cell lines (Sharma et al., 1998; Crabtree et al., 1995b; Ogura et al., 1998).

As seen in the tables representing the comparison of IL-8 and antibody responses, there was no statistically significant difference in median of IL-8 levels of patients with high IL-8 values, and IL-8 positivity with respect to their anti-H. pylori IgG, IgM, and IgA antibodies (p > 0.05). Also when we compared the IL-8 levels of the control group with the patient group, we could not find any statistically significant difference (p > 0.05).

Fan et al. (1995) found that IL-8 concentrations in gastric juice and biopsy homogenate supernatant fluid were increased in patients with H. pylori infection compared with the patients without infection, but this difference was not observed for plasma IL-8 concentrations. Also Audibert et al. (2000) reported that there is an increased mucosal production of IL-8 that may be an important mediator of the inflammatory response. In studies performed on gastric cell lines, H. pylori is reported to induce IL-8 gene expression and then IL-8 synthesis and secretion (Crabtree et al., 1995b; Ogura et al., 1998; Orsini et al., 2000).

Xuan et al. (2005) investigated the IL-8 levels on gastric biopsy supernatants by ELISA and found that IL-8 levels in both the antrum and corpus were significantly higher in H. pylori positive patients than negative patients.

In a study of Crabtree et al. (1993), it is reported that local synthesis of IL-8 may be an important factor in regulating mucosal neutrophil infiltration and activation. In that study, it is also reported that, factors other than H. pylori, possibly bacterial products of oral flora and dietary components, may be responsible for the induction of IL-8 production in the patients without H. pylori infection. In our study, increased IL-8 serum levels may be due to factors other than H. pylori in the control group.

Bayraktaroğlu et al. (2004) could not detect statistically significant differences in the serum levels of IL-8, IL-6 and TNF-α with respect to the presence or absence of H. pylori determined by Giemsa staining of gastric tissues. CagA protein is encoded by the cagA gene which is one of the 31 genes of a pathogenicity island called the cag pathogenicity island (cag PAI) (Censini et al., 1996). The presence of this gene has been associated with more virulent strains (Censini et al., 1996) and diseases such as duodenal ulceration, gastric mucosal atrophy, and gastric cancer (Blaser et al., 1995). Several studies have reported that, cagA positive H. pylori isolates were able to induce IL-8 secretion, but cagA negative strains were not (Orsini et al., 2000; Crabtree et al., 1995a; Crabtree et al., 1994a). Audibert et al. (2000) confirmed the finding that cagA positive H. pylori strains induce significantly higher levels of IL-8 on Hep-2 cell cultures than cagA negative strains, but in that study some unexpected results were also obtained. For example, some cagA positive strains did not induce IL-8, whereas cagA negative strains induced IL-8 secretion. In our study, as seen in the tables, there was no statistically significant difference in IL-8 positivity of patients and median of IL-8 levels with respect to anti-CagA IgA and IgG antibodies (p > 0.05).

As we mentioned above, cagA is one of the genes present in the cag PAI. Several studies showed that most of the genes of cag PAI are associated with IL-8 induction (Censini et al., 1996; Akopyants et al., 1998), but the cagA gene is not required for IL-8 secretion (Sharma et al., 1995; Crabtree et al., 1995b). Audibert et al. (2000) reported that cagA negative strains inducing IL-8 secretion may have the cag PAI without the cagA gene. And the cagA positive but not IL-8 inducing strains may lack some of the curicial genes. In that study, the cagA status of the strains was determined by PCR and dot blot hybridization. But we only evaluated the serological results of anti-CagA IgA and IgG. That situation may be one of the limitations of our study.

In conclusion, our observations implicate that, serum IL-8 levels did not differ between H. pylori infected and uninfected persons, and between the subcategories of infected persons. This finding may indicate that, this infection remains local without inducing systemic inflammation. Investigating the levels of IL-8 and other cytokines in tissues, not on the sera, may be useful in determining the severity and/or clinical outcomes of the infectious diseases. Determining the presence of cagA gene of strains and, anti-CagA antibodies on sera of patients may only be the indicator of cag PAI. But we suggest that, cagA may not be the certain indicator of IL-8 induction. In further studies, researches investigating different gene regions associated with IL-8 induction should be planned.

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REFERENCES


