

Introduction: *Helicobacter pylori* (*H. pylori*) is a non-invasive microorganism causing intense gastric mucosal inflammatory and immune reaction. *H. pylori*-induced gastric mucosal cytokine overproduction has been clearly documented previously. The stomach has a large surface area and continuous spill-over of locally produced cytokines into the blood stream is a possibility. There are few and conflicting data on circulatory proinflammatory cytokine levels in patients with *H. pylori* infection.

Materials and methods: Forty-two dyspeptic patients were enrolled into the study. The presence of *H. pylori* infection was diagnosed with antral histopathologic examination. After overnight fasting; serum samples were obtained from each patient to determine circulating interleukin (IL)-6, IL-8 and tumor necrosis factor- α (TNF- α) levels.

Results: *H. pylori* was shown in 30 cases using Giemsa stain in antral histopathologic evaluation. Twelve cases were negative for *H. pylori* staining. Both the age and sex distribution had an insignificant difference in both *H. pylori*-positive and *H. pylori*-negative groups. The mean circulatory levels of IL-6, IL-8 and TNF- α in both groups were not different. The situation was same in respect to the serum levels of these cytokines and the degree of inflammation, *H. pylori* density and activation scores according to Sydney classification.

Conclusion: We could not show elevated circulatory levels of IL-6, IL-8 and TNF- α in *H. pylori*-infected cases. We believe that *H. pylori*-related cytokine activation become concentrated on gastric mucosa and this pathogen-induced local inflammatory cascade does not cause changes in circulatory levels of these cytokines. Moreover, there is no correlation between the levels of serum cytokines and Sydney parameters.

Key words: *Helicobacter pylori* infection, Interleukin-6, Interleukin-8, Tumor necrosis factor- α , Sydney parameters

Serum levels of tumor necrosis factor- α , interleukin-6 and interleukin-8 are not increased in dyspeptic patients with *Helicobacter pylori*-associated gastritis

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Introduction

In many gastrointestinal infections, the inflammatory response induced by the pathogen can be an important contributor to mucosal damage and epithelial dysfunction. Possibly the best example of this is the infection with *Helicobacter pylori* microorganisms. *H. pylori* leads to mucosal increases in many proinflammatory and immunoregulatory cytokines, and also increases in members of the chemokine group of peptides.¹ As a most probable target in *H. pylori* infection, and a major interface between the host and pathogen, the epithelial cell initiates acute mucosal inflammation and interacts with the other mucosal cell populations via a cytokine network.² These two responses may be regulated differentially

following induction of cytokines involved in the inflammatory cascade, including tumor necrosis factor- α (TNF- α), interleukin (IL)-1, IL-6, and IL-8.^{3–6}

The stomach has a large surface area. A suggestion that the chronic gastric mucosal inflammation induced by *H. pylori* potentially may have systemic effects based on increases in serum proinflammatory cytokines seems not to be bizarre. Although cytokine-based gastric mucosal immune response to *H. pylori* infection has been documented very well, very minute data on circulating levels of particular proinflammatory cytokines are available. Moreover, the present few data are contradictory.^{7–10} In our study, we investigated circulating levels of IL-8, IL-6 and TNF- α in patients with *H. pylori* infection.

Research design and methods

Patients

Forty-two patients with dyspeptic symptoms enrolled into the study. Patients who received anti-bacterial treatment for *H. pylori* and anti-ulcer drugs 1 month prior to the study or who had undergone vagotomy or stomach resection were excluded.

Assessment of the state of *H. pylori* infection

The state of *H. pylori* infection was assessed histologically. The biopsy specimens were fixed in 10% formalin and embedded in paraffin. *H. pylori* was determined by Giemsa staining. The density of *H. pylori* was graded as 'none', 'mild', 'moderate', and 'severe' according to the Sydney system,¹¹ and scored on a 0–3 scale. When at least one of the two biopsies of each patient yielded a positive result, the patient was considered as positive for *H. pylori*.

Histological assessment of gastritis

Sections were also stained with hematoxylin and eosin. The severity of chronic and acute inflammation, atrophy, and intestinal metaplasia was graded as 'none', 'mild', 'moderate', and 'severe' according to the Sydney system and scored on a 0–3 scale.¹¹

IL-8, IL-6 and TNF- α assays

Blood samples were taken after overnight fast and after 30 min in the supine position to determine serum IL-8, IL-6 and TNF- α . Samples were stored at -20°C . Serum cytokine concentrations were determined in triplicate using a commercial enzyme-linked immunosorbent assay (Immulite IL-8, Immulite IL-6, Immulite TNF- α ; DPC, Los Angeles, CA, USA). The sensitivities of these assays are approximately 2 pg/ml, 1 pg/ml and 1.7 pg/ml for IL-8, IL-6 and TNF- α , respectively.

Statistical analysis

Results are expressed as the mean \pm standard deviation. In the comparison between groups, statistically significant differences were assessed by the chi-square test and the Mann–Whitney U-test. $p < 0.05$ was considered statistically significant.

Results

Our study group had a mean age of 44.5 ± 11.9 years (20–65 years). The male to female ratio was 20/22. The presence of *H. pylori* was seen in 32 patients. Twelve cases were negative for the presence of

H. pylori. Both the age and sex distribution had an insignificant difference in both groups. The Sydney classification scores in our cases are presented in Table 1. None of our cases had severe inflammation and atrophy. The situation was not different in respect to serum levels of these cytokines and the degree of inflammation, *H. pylori* density and activation scores according to the Sydney system ($p > 0.05$) (Table 2).

We could not detect statistically significant differences in the serum levels of these cytokines with respect to the presence or absence of *H. pylori* ($p > 0.05$).

Discussion

In the present study, we scrutinized the levels of circulatory cytokines, which may theoretically increase as a consequence of intense gastric mucosal cytokine activation. We know that infection with *H. pylori* leads to increases in many mucosal proinflammatory and immunoregulatory cytokines such as IL-6, IL-8, IL-1 β , TNF- α , interferon- γ , and platelet-activating factor.¹ However, the primary modulator cytokine in *H. pylori*-associated gastritis is IL-8.¹² *H. pylori* pathogenicity island encoding for Cag A protein and other virulence factors increases expression of IL-8, which in turn triggers the neutrophilic infiltration and induces cytokine release adherence to the epithelium.¹³ If we consider the total gastric surface area, which is nearly 700 cm², it is not unwise to think that *H. pylori*-associated gastric local mucosal response may have systemic effects.¹⁴ As an example; one of these effects may be through hypergastrinemia. TNF- α increases serum gastrin levels by decreasing the somatostatin content in D cells in animal models.¹⁵ IL 8 increases the basal gastrin release from canine G cells.^{16,17} As is generally known, gastrin has many extra-gastric effects, especially on intestinal and colonic epithelial growth and enteric immune response.^{18,19}

In the present study we aimed to investigate whether we can show increased serum proinflammatory cytokines such as TNF- α , IL-8 and IL-6 in *H. pylori*-infected patients without systemic diseases. We could not demonstrate any increase in circulatory cytokines in *H. pylori*-positive patients. Although

Table 1. Sydney scores of our patients

	Score 0	Score 1	Score 2	Score 3
<i>H. pylori</i>	12 (28.6)	25 (59.5)	5 (11.9)	–
Inflammation	–	15 (35.7)	27 (64.3)	–
Activation	22 (52.4)	20 (47.6)	–	–
Atrophy	–	–	–	–
Metaplasia	40 (95.2)	2 (4.8)	–	–

Data presented as n (%).

Table 2. The mean serum cytokine levels in respect to *H. pylori* positivity, activation and severity of inflammation

	<i>H. pylori</i>			Activation			Inflammation		
	(Score 0, n = 12)	(Score 1+2+3, n = 30)	p value	Absent (Score 0, n = 22)	Present (Score 1+2+3, n = 20)	p value	Mild (Score 1, n = 15)	Moderate (Score 2, n = 27)	p value
Age (years ± SD)	40.8 ± 12.7	46.0 ± 11.5	0.253	43.6 ± 12.3	45.5 ± 11.8	0.597	42.2 ± 12.7	45.7 ± 11.6	0.454
TNF-α (pg/ml ± SD)	11.8 ± 4.5	12.7 ± 5.0	0.587	12.2 ± 4.8	12.7 ± 4.9	0.623	11.5 ± 4.1	12.9 ± 5.2	0.372
IL-6 (pg/ml ± SD)	4.4 ± 3.6	4.4 ± 2.8	0.301	4.2 ± 3.2	4.58 ± 2.9	0.215	4.0 ± 3.4	4.6 ± 2.9	0.111
IL-8 (pg/ml ± SD)	8.3 ± 5.6	10.8 ± 8.6	0.079	9.4 ± 8.2	10.93 ± 7.7	0.391	9.0 ± 5.2	10.8 ± 9.1	0.423

SD, Standard deviation.

mucosal cytokine production was found to correlate well with the intensity of gastric mucosal inflammation, serum cytokine levels did not show correlation with Sydney parameters, including the gastric epithelial inflammatory scores, *H. pylori* density and activation, in the present study.³ In the literature, some authors reported increased circulatory TNF-α levels while serum IL-8 levels remained normal in *H. pylori*-infected persons.⁷ Some others reported elevated serum TNF-α levels in patients with CagA-positive *H. pylori* infection.⁸ IL-1, IL-6 and IL-8 were all also elevated in Cag A-positive cases.^{20,21} We did not determine the Cag A status of our patients. Thus, we do not know whether most or all of our patients were infected with a Cag A-negative strain. However, we know that our patients neither had endoscopic sign of gastroduodenal ulceration nor had severe gastritis on histopathology. Therefore we possibly had patients infected with less pathogenic strains of this bacterium such as with Cag A-negative *H. pylori*. The other possibility may be the inhibition of further inflammation by a gastric protective cytokine IL-10.²² Although we do not measure its levels on gastric mucosal extracts and circulation, we know that IL-10 secreted by gastric mucosa has inhibitory effects on gastric mucosal immune response associated with local proinflammatory cytokine activation.

As a result of contradictory reports, including ours, we do not know whether the continuous spill-over of cytokines into the blood stream could be possible as a consequence of gastric mucosal cytokine activation and increased synthesis. If true, this may be a potential threat in patients with *H. pylori* infection for some 'extra-gastric' pathologies, such as coronary heart disease, primary Raynaud phenomenon, sideropenic refractory anemia and delayed growth in children.²³⁻²⁸ We think that further studies are needed to make firm conclusions on *H. pylori*-induced changes in the circulatory cytokine levels.

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Received 10 November 2003

Accepted 8 December 2003