

IL-10, TNF- α and IFN- γ Levels in Serum and Stomach Mucosa of *Helicobacter Pylori*-Infected Patients

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ABSTRACT

H. pylori is a human pathogen that colonizes the epithelium of the stomach. The host immune response may influence the disease process, where cytokines play important roles in the development of disease. In this study, the concentrations of selected cytokines in the gastric antrum and stomach body mucosa and also in the serum were evaluated.

Eighty patients according to their rapid urease test were divided into two groups: *H. pylori* positive (n=39) and *H. pylori*-negative (n=41). The concentrations of cytokines in biopsies and serum were determined by ELISA method. The mean TNF- α and IFN- γ levels in the infected group were significantly higher than that of uninfected patients. In contrast, IL-10 level in most patients was undetectable. The mean antral of stomach TNF- α and IFN- γ levels were significantly higher than that of the stomach body.

IFN- γ serum level showed positive correlation with antrum and stomach body levels, whereas no correlation was found in TNF- α in different samples.

Higher levels of TNF- α and IFN- γ in antral indicate that the colonization of bacteria in the antrum may be higher than stomach body (culture results from two sites support this statement). Increased serum level of IFN- γ indicates the activation of circulating-T cells against infection. Induced *H. pylori*-related TNF- α is concentrated in gastric mucosa and this pathogen does not cause any significant change in the serum level of this cytokine. Therefore *H. pylori* by inducing certain inflammatory cytokines but not IL-10 may contribute to the process of disease development.

Keywords: *Helicobacter pylori* infection; IFN- γ ; IL-10; TNF- α

INTRODUCTION

Infection with *Helicobacter pylori* (*H. pylori*) is one

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of the most common bacterial infections. This organism causes chronic gastritis, gastric and duodenal ulcers and could be the cause of some neoplasm (gastric cancer and MALT lymphoma).^{1,2} In addition to virulence factors of *H. pylori*, the role of host responses in this process is persistence of bacteria in the mucosa that cause unclear.

Inflammatory changes are related to the neutrophilic infiltrations in the acute conditions and macrophagic infiltrations in the chronic conditions of the inflammation.³ Infection with *H. pylori* results in innate and acquired immune responses that cause release of immune mediators by epithelial and immune cells. The primary mechanism of T-cell immune response versus infection is secretion of cytokines by host. The main functions of these cytokines are regulatory, but increased levels of them may be attributed to mucosal damage and epithelium dysfunction.⁴

Previously IL-10 had been designated as a cytokine synthesis inhibitory factor (CSIF). This interleukin is produced by the CD4+ Th2 cells and inhibits the cytokine production of Th1 cells.⁵ Tumor necrosis factor alpha (TNF- α) is produced mainly by activated macrophages as well as T cells, and has many important biological functions.⁶ Th1 cells and natural killer (NK) cells produce IFN- γ and cause promotion of cell-mediated immune responses.⁷ Although cytokine based mucosal response to *H. pylori* has been documented, but only a few studies compared mucosa and serum levels of cytokines. Therefore in this study, we investigated mucosal of stomach (body and antrum) and circulating levels of IL-10, TNF- α and IFN- γ in patients suspected of *H. pylori* infection.

PATIENTS AND METHODS

In this case/control study, we evaluated patients referring to Endoscopy unit of Afzalipour hospital in the city of Kenman. Selection and differentiation of the two groups (Case/Control) were based on clinical examinations in endoscopy, rapid urease test and anti-*H. pylori* IgG. Biopsies from stomach and blood samples of patients for this research work were acquired with consent as well as fulfilling the condition of non-antibiotic or H2 blocker drug consumption during two weeks before sampling.

The Patients number in case group was 39 (49%) and control group was 41 (51%). Mean age \pm SD in case group was 58 \pm 16 and in control group was 38 \pm 13. Male/Female ratio also in case group and control group was 20/19 and 19/22 respectively.

Gastric Biopsy Specimens

Four biopsies samples were collected from each patient's stomach (2 from body and 2 from antral of stomach). Transport media included 750 μ l normal saline (for cytokine assay in mucosal samples) and Stuart media (for culturing samples).

Blood Specimens

From each patient 10 ml blood was collected for cytokine assay in their serum.

Isolation and Identification of *H. pylori*

Biopsy samples were homogenized using a tissue homogenizer and then cultured on *Brucella* agar plates (containing 10% sheep blood and antibiotics of polymyxin B, trimethoprim and vancomycin) and also urea broth under microaerophilic condition (5% CO₂) for 5 to 7 days. *H. pylori* identification was based on morphology and biochemical tests.⁸

Separation of case (infected) and control (uninfected) groups was based on rapid urease test on gastric biopsies as well as presence of IgG antibodies (anti-*H. pylori*) in the sera of patients using Enzyme-Linked Immunosorbent Assay (ELISA) method (Monobind Inc. USA). Case group had positive urease test and serum IgG antibody level was >20U/ml (according to the manufacturer guidelines).

Cytokine Assay

Biopsy samples (antrum and body of stomach) were weighted and 10mg of each sample was homogenized separately in 750 μ l of normal saline. Supernatants were obtained by centrifugation at 12000 \times g for 10 min at 4°C. Serum was also separated from venous blood by centrifugation (2000 \times g for 15 min at 4°C). All samples were frozen at -20°C in sterile tubes until used for cytokine measurements by ELISA method, using commercial kits (Bender Med Systems, Austria). Total cytokine concentrations in samples was expressed as pg/g (for biopsies) and pg/ml (for serum).

Statistical Analysis

Results were expressed as the mean \pm SD. Comparisons between groups were analyzed by chi-square and *t* student tests. The Pearson correlations were used and *P*<0.05 was considered statistically significant.

RESULTS

The mean IFN- γ concentration showed that there was significant difference between case and control groups. Also mucosal concentrations of TNF- α (but not serum concentration) in case group was statistically higher than the control group, while IL-10 level between groups was not statistically different (Table 1).

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Table 1. Concentrations of cytokines in case and control groups

Cytokines	Groups	Case		Control		P-value
		Mean±SD	Mean±SD	Mean±SD	Mean±SD	
IFN- γ	Stomach	Antral (pg/g)	398±296	30.1±44.8		<0.0001
		Body (pg/g)	120±84.9	9.6±17.3		<0.0001
	Serum(pg/ml)		1.53±1.82	0.045±0.352		<0.0001
TNF- α	Stomach	Antral (pg/g)	1880±20	347±267		<0.0001
		Body (pg/g)	732±524	153±146		<0.0001
	Serum(pg/ml)		0.0026±0.0160	0.00244±0.0156		0.972
IL-10	Stomach	Antral (pg/g)	0.26±1.14	0.081±5.21		0.170
		Body (pg/g)	2.6±16	52±220		0.162
	Serum(pg/ml)		0.028±0.0161	0.11±0.705		0.336

There was not significant correlation between serum levels and mucosal levels of IL-10 and TNF- α , but the correlation was significant for IFN- γ . Significant correlation was found between antrum and body of stomach for IFN- γ and TNF- α , though their levels in antrum were significantly higher than the body of stomach. The analysis of cytokines concentration (TNF- α , IFN- γ and IL-10) in the gastric mucosa and serum showed no correlation with culture positivity, sex and age. Pearson correlations of TNF- α and IFN- γ with serum anti-*H. pylori* IgG levels were significant (Table 2). The results of biopsy culture showed that 23% of antrum samples were positive, whereas stomach body samples did not reveal any positive culture.

DISCUSSION

The balance between Th1 and Th2 cell responses is important in disease outcome. A Th1 cells response is characterized by the production of IFN- γ and Th2 response is characterized by the cytokines of IL-4 and IL-10 that may function to downregulate IFN- γ .⁹ Previous reports have shown that increase in proinflammatory cytokines may affect in the pathological process of the disease. TNF- α can be found in chronic inflammatory conditions such as Crohn's disease and osteoclast formation, where it has

some effects in the inflammatory triad.^{10,11} In our study, concentrations of TNF- α in the mucous of stomach (antrum and body) of *Helicobacter pylori*-infected group were higher than uninfected group. It seems that *H. pylori* with its virulence factors causes induction of cytokine response in the mucosa of gaster. It demonstrated that *H. pylori* secreted a 19 kDa Tumor necrosis factor-alpha (TNF- α) inducing protein (Tip α) that by DNA-binding activity can enhance expression of TNF- α gene in the stomach. Tip α also can cause gastric cancer progression.¹²

Guiraldes et al. reported that in gastric mucosa of *H. pylori*-infected patients, IL-1 β , IL-8 and TNF- α concentrations were increased.¹³ Some other workers also have demonstrated that serum TNF- α level elevated in patients with *H. pylori* infection and over expression of this cytokine due to this pathogen also involved in induction of tumor and promotion of stomach cancer.^{14,15}

Serum level of TNF- α in most patients of our study was undetectable. It seems that cells producing this cytokine (e.g. macrophages) are mainly infiltrated in gastric mucosal tissue and also soluble TNF receptors (sTNF-Rs) in serum, depending on its concentration, may in some cases inhibit the effects of TNF which is in agreement with several other studies.^{16,17}

Table 2. Pearson correlation between cytokines concentrations and serum anti-*H. pylori* IgG levels

Topics	IFN- γ			TNF- α			IL-10		
	Stomach		Serum	Stomach		Serum	Stomach		Serum
	Antrum	Body		Antrum	Body		Antrum	Body	
Pearson correlation	0.801	0.722	0.304	0.828	0.653	-0.092	0.230	-0.146	-0.119
P-value	<0.0001	<0.0001	0.006	<0.0001	<0.0001	0.416	0.06	0.195	0.293

Bayraktaroglu et al. showed that serum levels of IL-6, IL-8 and TNF- α were not increased during infection with *H. pylori*.¹⁸ Fan *et al.* also reported that there were not significant differences between *H. pylori* infected and uninfected patients with regard to plasma TNF- α and IL-8 concentrations.¹⁹

Comparison of TNF- α concentration in antrum and body of stomach in this study indicates that antrum level was higher than that of stomach body. It seems that any part of the stomach may be colonized, but mucus-secreting epithelium of the antrum is the favored site, as our results from two samples (antrum and body) cultures supports this statement.⁸ In the literature, some authors reported that IL-8, IL-6 and TNF- α levels in antrum of patients infected with *H. pylori* were higher than that of uninfected.²⁰

However there are other reports which have shown mucosal levels of proinflammatory cytokines such as IFN- γ in patients with *H. pylori* associated gastritis were increased.^{21,22} In the present study, mucus IFN- γ level in antrum of patients was higher than that of control group. Infection with *H. pylori* increases secretion of cytokines such as IL-12 and IFN- γ in gastric mucus, because chronic infections cause immune system to shift toward Th1 response.²³ Results of Perpetto also indicate that Th1 related cytokines (IFN- γ) may contribute to the pathogenicity of *H. pylori*.²⁴ Sawai et al in a mouse model suggested that IFN- γ although may be involved in protection against *H. pylori* infection but in a long-term infection IFN- γ may play an important role in gastric inflammation.²⁵

In our study, the IFN- γ serum level in case group was higher than the control group. This may be due to the activation of circulating T-cell response. A similar study has already been reported by Quiding-Jarbrink *et al.* who found that the blood stream T cells in *H. pylori*-infected individuals produce IFN- γ to bacterial antigens in cell culture media.²⁶

Other cytokines such as IL-10 has been reported as a key modulating agent in various diseases. During host response to infection, IL-10 is like a natural suppressor in immune reactions.²⁷ Our data suggest that, there was not significant difference in IL-10 levels in mucus (antrum and body) and in serum. In addition, this cytokine in most of patients was undetectable. This may probably be due to the low effect of Th2 cell response to *H. pylori*. Some other workers clearly showed that the poor ability of *H. pylori* to stimulate IL-10 may be due to the low endotoxin activity of its

LPS.²⁸ It has been shown that the LPS of *E. coli* has a much higher endotoxic activity (approximately 500-fold). Therefore the ability of *H. pylori* to stimulate host immune system is relatively weak. Szkaradkiewicz *et al.* showed that the IL-10 levels in sera of patients were not dependent on infection with *H. pylori*, while increased concentration of this cytokine was accompanied in advanced cancer of stomach.^{29,30}

Generally, the present study demonstrates that chronic infection due to *H. pylori*, induce inflammatory agents (such as IFN- γ and TNF- α), but its ability to induce IL-10 is poor. IFN- γ serum concentration showed positive correlation with mucosal level. This may mean that for detection of this cytokine, biopsy samples could be exempted, but not for TNF- α . Further studies will be necessary to confirm this conclusion.

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