Serum Levels of Interleukin-2 and Interleukin-6 among *Helicobacter Pylori* Positive Patients in Relation to Prognosis of Gastro-duodenal Disease

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Abstract

Background: *Helicobacter pylori* is the most widespread pathogenic associated with gastro-duodenal Disease. Interleukin 2 and interleukin 6 are the main cytokines involved in host immune response to *H.pylori* infection. The aim of the present study are to assess the serum level of IL-6 and IL–2 in *H.pylori* infected patients and to study their association with gastric endoscopy findings. **Methods:** One hundred and seven suspected patients and 19 healthy were recruited. The confirmed positivity of *H.pylori* detection was based on a rapid urease test (R.U.T) and stool antigen test. Serum concentrations of IL-2 and IL-6 were measuring by ELISA. **Results:** Seventy-five (70%) patients were positive for *H.pylori.* Both interleukins were found at higher levels in patients than in healthy (p- value <0.05). Interestingly, the level of IL-2 was lower in patients infected with *H.pylori* (43.40 pg/ml) than those who were not infected (85.2 pg/ml), while the level of IL-6 was higher in patients infected with *H.pylori* (117 pg/ml) than those not infected (40 pg/ml), p-value <0.05. Furthermore, the increasing levels of both interleukins were correlated with disease progress.

Keywords: *Gastritis; Gastric cancer; Helicobacter pylori; IL-6, IL-2; Peptic ulcer.*

Introduction

H.pylori infection induces strong immune responses but the host is still unable to clear the organism from the mucosa. The first-line of defense against *H.pylori* infection are Gastric epithelial cells, these cell express a Toll-Like Receptor[1], and the Nucleotide-binding oligomerization domain NOD-like receptor family members[2]. Recognition of *H.pylori* by these molecular on gastric cells leads to the activation of intracellular signaling pathways that culminate in the induction of various genes involved in host defense including the production of interleukins (IL-8), (IL-6), (IL-1β, chemokine, and antigen presenting molecules[3]. Many of these molecules act as a chemotactic factor for neutrophils and lymphocytes, and a proliferative response with a dense infiltrate of these cells in the gastric mucosa, resulting in a chronic active gastritis. *H.pylori* also induces gastric mucosal infiltration by dendritic cells and T and B cells, and stimulates secretion tumor necrosis factor (TNF)-α, IL-12, IL-10, transforming growth factor (TGF)-β, and interferon (IFN)-γ [4]. Gastro duodenal diseases caused by *H.pylori* lead to different pathological

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outcomes in relation to host's immune response. One of these outcomes is changing cytokine serum levels [5]. T- helper cells (Th1) secrete (IL-2), and (IFN-γ) that increase proinflammatory cytokines, and promote both neutrophil recruitment and macrophage activation[6]. Activated Th1 produce high levels of IL-2 when properly stimulated through both the TCR and the CD28 costimulatory molecule. *H.pylori* also stimulate the production of IL-2 in the gastric immune response [7]. *H.pylori* cytotoxin Vacuolating (VacA) inhibits Th1 proliferation by inducing arrest of the G1 / S cell cycle through interference with the cell receptor/IL-2 signaling pathway. The (VacA) prevents the activation of CD4 + cells. IL-6 is a multifunctional cytokine produced by monocytes, [Th2], macrophages and intestinal epithelial cells. It synthesized upon the stimulation of TLRs by *H.pylori* lipopolysaccharide or upon stimulation of cells by (TNF)[8]. IL-6 act as a messenger between innate and adaptive systems in the mechanisms of host defense by stimulating (Th2) to produce IFN-γ and activates B cells to secret immunoglobulin [9,10]. The aim of the present study was to assess the serum level of IL-6 and IL-2 in *H.pylori* infected patients and to study their association with gastric endoscopy findings.

Materials and Methods

Study population

The study was conducted in the Endoscopy Unit of a specialist center for the digestive system and liver at Al Sadr Teaching Hospital, Basrah – Iraq on 107 patients with Gastro-duodenal Diseases (suspected *H.pylori* infection) and 19 healthy individuals. The eligible patients had been confirmed to have *H.pylori* with chronic gastritis or peptic ulcer through clinical and laboratory examinations, and histopathologically confirmed to have gastric cancer by endoscopic biopsy. Informed consent was obtained from all participants, and a questionnaire regarding age, job, marital status, residential address, number of endoscopic, smoking, symptoms, and family history of Gastro-duodenal Diseases. Exclusion criteria were as follows: taking antibiotics in past 4 weeks, proton pump inhibitors in past 2 weeks or H2-blocker agents in past one week, taking immunosuppressive agents, active gastrointestinal bleeding, pregnancy, breast-feeding and history of gastrostomy. All individuals were tested for *H.pylori* fecal antigen and all patients tested positive *H.pylori* stool antigen were confirmed by R.U.T.

Assessment of serum IL-2 and IL-6

Serum concentrations of IL-2 and IL-6 were measured by commercial enzyme linked immunosorbent assay (ELISA) kit (KOMABIOTECH, KORA, IL-2 Lot No:42223, IL-6 Lot No:46223). The tests were performed according to the manufacturer's instructions. Briefly, standards and samples were pipetted into the 96 wells plate, coated with antibody specific for Human IL-2 and IL-6. IL-2 and IL-6 present in samples were bound to the wells by the immobilized antibody. Then, the wells were washed, and biotinylated anti-Human IL-2 and IL-6 antibody was added. After washing away unbound antibodies, HRP conjugated streptavidin was pipetted into the wells. The wells were washed and a TMB substrate solution was added to the wells. The intensity of the color was measured at 450nm. The results were calculated according to standard curve by reducing the data using ELISA reader's computer software capable of generating standard curve-fit.

Statistical Analysis

Statistical Package for Social Sciences (SPSS) version 25 was used. P-value of <0.05 was considered a clue for the presence of significance.

Results

One hundred and seven patients with Gastro-duodenal Diseases (suspected *H.pylori* infection) and

19 healthy were recruited. The confirmed positivity of *H.pylori* detection was based on a R.U.T and stool antigen test; 75 (70.1%) patients were positive for *H.pylori.* Age group ≤ 30 years (39.3%), females (65.1%), and O blood group (58.7%) showed higher rate *H.pylori* infection (table 1).

Both interleukins (IL-2 & IL-6) were found at higher levels in patients than in healthy with significant differences (p-value <0.05) (table2&3). Interestingly, the level of IL-2 was lower in patients infected with *H.pylori* (43.40 pg/ml) than those not infected (85.2 pg/ml), whilst the level of IL-6 was higher in patients infected with *H.pylori* (117 pg/ml) than those not infected (40 pg/ml), p-value <0.05 (table2). Furthermore, the increasing level of both interleukins was correlated with disease progress (Table2&3).

Table 1. Distribution of *H.pylori* status according to blood group

Blood group	H.pylori status			p-value
	HP +	HP-	Total	
A	19 (25.3%)	7 (21.9%)	26	0.015*
B	10 (13.3%)	13 (40.6%)	23	
O	44 (58.7%)	11 (34.4%)	55	
AB	2 (2.7%)	1 (3.1%)	3	
Total	75 (100%)	32 (100%)	107	

Table 2. Serum level of IL-2 in relation *H.pylori* infection and gastric endoscopy findings.

Category	IL-2 Level (median) pg/ml	Kruskal-Wallis Test	P-value
Study populations			
Patients	43.6	134.5	0.0001
Healthy people	31.1		
H.pylori status			
H.pylori Pos+	43.40	34.96	0.0001
H.pylori Neg-	85.2		
Gastric endoscopy findings			
Gastritis	40	22.54	0.001
Peptic ulcer	52		
Gastric cancer	103.9		

Table 3. Serum level of IL-6 in relation *H.pylori* infection and gastric endoscopy findings.

Category	IL-6 Level (median) pg/ml	Kruskal-Wallis Test	P-value
Study populations			
Patients	107.5	3.500	0.001
Healthy people	10.0		
H.pylori status			
H.pylori Positive	117	56.5	0.0001
H.pylori Negative	40		
Gastric endoscopy findings			
Gastritis	104	30.56	0.001
Peptic ulcer	167		
Gastric cancer	495		

Discussion

In the present study, we found that the prevalence of infection with *H pylori* was 70.1% of patients with gasto-doudenal disorders. This finding was higher than that found in in previous study Iraq (55.8%) [11], and in neighboring countries, e.g., 46.5% Saudi Arabia[12], 83.5% Iranian population[13], 49.7% in Kuwait[14], but lower than those found in Jordan (88.6%) [15] and in Turkey (66.3%) [16]. In Egypt was (70%) [17]. The prevalence of *H.pylori* infection in china 62%, Korea 66.9%, Pakistan 74.4% [18]. These variations in the prevalence rates of *H.pylori* across the world might be attributed to different factors such as living standards, socioeconomic status, geographical location and ethnicity. In addition to the variability in the (*H.pylori*) detection methods, size of the study and exclusion of prior used of antibiotic [19]. Although there was no statistically significant difference observed among the studied age groups of patients, the occurrence of the disease was higher in people aged ≤ 30 (39%). These findings agreed with other studies which reported that young people are the most effected age group who suffer from gastroduodenal disorders[20]. However, many studies showed high prevalence in young as well as old age [21]. No statistically significant difference associated between sex and gasto-doudenal a disorder was observed in our study, though the occurrence of gastrointestinal disorders was higher in females (56.1%) compared to males (43.9%). These results corresponded with numerous previous research and studies[21]. In contrast, other studies reported that the occurrence of *H.pylori* infections is higher in males than in females [23,24]. The present results revealed a relationship between ABO blood type and *H.pylori*, we found that the patients with blood group O (58.7%) were more prone to *H.pylori* infection than others groups (P=0.012). Similar studies demonstrated that the distribution

of ABO blood groups in *H.pylori* positive patients were A=31.4%, B=15.4%, AB=25.0% and O=53.7%, with a statistically significant link for blood group O (p=0.05) [25]. The association of *H.pylori* with blood group antigens may be related to the Lewis blood group system (Lewis b antigen) which acts as a receptor for *H.pylori*. This antigen is most frequently found on blood group O , that people with blood group O have more *H.pylori* adhesions and have a higher density of colonized *H.pylori* [26]. In our study we found that the IL-2 level was significantly down regulated in the serum of the *H.pylori* pos+ patients when compared with the *H.pylori* neg- patients, which is similar with those obtained by Dlugovitzky et al.[5]. Interleukin-2 secreted mainly by T helper cells (Th1) and other immune cells [6]. The *H.pylori* vacuolating cytotoxin A (VacA) protein can interact with lymphocytes, resulting in blockage of IL-2-mediated T cell proliferation[27]. By its ability to induce vacuolization of epithelial cells, has also been revealed as an inhibitor of T cells signaling and proliferation by inducing a G1/S cell cycle arrest through the interference with the T cell receptor/IL2 signaling pathway[28]. On other hand, IL-6 levels in patients infected with *H.pylori* were higher than in uninfected patients. The association between serum IL-6 levels and *H.pylori* found in the current study could play an important role epidemiologically and clinically. The association observed in this study is consistent with the result obtained by previous study [29], which showed that *H.pylori*-positive Japanese had a higher level of IL-6. Our findings suggest that a strong immune response to *H.pylori* enhanced the systemic inflammation, which was reflected in an increased level of serum IL-6. In addition, the increasing levels of IL-6 in peripheral blood of the patients is likely to be associated with ulceration inflammation, macrophage stimulation and active secretion by the neutrophils and vascular endothelial cells. Once attached to the gastric epithelial cells, *H.pylori* incites an immune response characterized by activated inflammatory and immunologically competent cells such as neutrophils, lymphocytes and monocytes release IL-6, IL-8 and IFN-gamma. As a result, the serum levels of IL-6 increase[30]. In this study we describe the level of IL-2 and IL-6 in the serum with relation to the gastric endoscopic findings in gasto-doudenal disease associated with *H.pylori*. We found IL-2; IL-6 levels significantly rise with the progress of gastric endoscopy findings. The results obtained in the present study regarding the level of IL-2 is comparable with those obtained by Sugimoto et al.[9], who found that the serum levels of IL-2 were higher in Gastric cancer patients[9]. Also, in our findings of IL-6 level were similar with a study carried by Wu et al.[30], who found that the IL-6 levels in the serum of gastric cancer patients were significantly elevated[31]. Sugimoto et al., also found that IL-6 controls the development of chronic inflammatory diseases and the Serum levels of IL-6 are higher in patients with gastric cancer than gastritis [9]. Other studies also showed that the overproduction of IL-6 was responsible for the pathogenesis of various inflammatory diseases[30]. IL-2 and IL-6 play an active role in the pathogenesis of gastro-duodenal disease. Several studies observed the correlation of IL-2 and IL-6 levels in the patient serum with the severity of gastro-duodenal disease[10].

Conclusion

Serum IL-2 and IL-6 were markedly higher in *H.pylori* infected patients and the levels of the both interleukins were significantly higher in patients with peptic ulcer(s) and gastric cancer. However, IL-2 level was lower in *H.pylori* positive patients than *H.pylori* negative patients.

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