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Circulating Interleukin 37 and the Chemokine CXCL9 Studies on Gastroduodenal Disorders with *H. pylori* Infection

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Abstract: The ability of the bacterium *Helicobacter pylori* to survive in a gastric environment causes gastroduodenal disorders (GD) due to its virulence factors. The aim of the study host cell recognizes bacterial shape and pathogen-associated molecular patterns (PAMPs) by toll-like receptors (TLRs), which trigger the immune system. The study involved 165 patients diagnosed with gastroduodenal disorders and 32 healthy individuals, the patients attended the Gastroenterology Teaching Hospital and a private clinic during the period from April 2021 to January 2023. The physicians diagnosed them with gastritis, peptic ulcers, and gastric cancer by OGD. The IL37 and CXCL9 level in sera were measured by ELISA. This study aimed to find the effect of age, gender and blood group on the susceptibility of GD related to *H. pylori* and its effect on interleukin 37 and chemokine CXCL9. The current findings confirm that GD affects the elderly and males more than young people and females. The susceptibility increases with the ABO phenotype and the O+ blood group exhibited the highest incidence of GD. A significant increase in IL37 and CXCL9 levels in patients' serum compared with the healthy group was reported. The IL37 concentration in gastritis, peptic ulcer, and gastric cancer, and the healthy group was as follows: 637.3 ± 125.3, 598.7 ± 158.2, 177.06 ± 67.49, and 127.08 ± 13.41, respectively. While the concentration of CXCL9 level in serum in patients suffering from gastritis, peptic ulcer, gastric cancer, and healthy individuals was as follows: 586.06 ± 701.47, 589.94 ± 778.86, 364.16 ± 215.08, and 111.85 ± 54.28, respectively. The peptic ulcer group was the highest among the others, followed by the gastritis group and gastric cancer. It was concluded that significant relationship between the biomarkers (IL-37 and CXCL9) and gastroduodenal disorder patients in patients with *H. pylori*-infected especially gastritis and peptic ulcers.

Keywords: IL37, CXCL9, *H. pylori*, Gastritis, Peptic ulcer, Gastric cancer.

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Introduction

The bacterium *Helicobacter pylori* (*H. pylori*) was described by Marshall and Warren in 1984 as a spiral-shaped, gram-negative bacterial pathogen that is positive for urease, catalase, and oxidase (1). This bacterium is the main cause of peptic ulcers, gastritis, and stomach cancer and was identified and classified as a class I carcinogen in 1994. In 2005, it was considered the most widespread etiologic agent of

infection-related malignancies, which account for 5.5% of the worldwide cancer burden (2). The bacterium causes 90% of duodenal ulcers and 70% to 90% of stomach ulcers (3). This bacterium may be investigated with either invasive or non-invasive techniques. Endoscopy and biopsy are required for invasive techniques such as histological investigation, culture, and a rapid urease test to diagnose the infection. Non-invasive methods are classified into two types: active tests and passive tests (4). Active tests include the urea breath test and the detection of *H. pylori* antigen in blood or stool, while passive tests include serology, urine, and near-patient tests, which are markers of *H. pylori* exposure but do not indicate whether an active infection is ongoing (5). Cytotoxin-associated (cagA) and vacuolating cytotoxin (vacA) virulence factors have been identified as influencing colonization and disease severity (6). Gonciarz and his colleagues (7) reported that the mechanism of developing *H. pylori* infection by injecting cagA and vacA into host cells results in changes in cell morphology and characteristics that can lead to malignancy. The bacterium decreases intracellular actin transport, induces inflammatory reactions, and destroys tight cellular junctions by cagA. The host cell recognizes microbial-associated molecular patterns (MAMPs) and pathogen-associated molecular patterns (PAMPs) by toll-like receptors (TLRs) (8), to activate NF-κB which prevents cell death by regulating the transcription of many genes responsible for cell survival, proliferation, and inflammation. The immune cells, especially macrophages, dendritic cells (DCs), neutrophils, mast cells, and T- and B-lymphocytes, recognize LPS and the shape of the bacterium, then increase in the stomach to release pro- and anti-inflammatory cytokines (9). Cytokines are small proteins released from specific cells to communicate with immune and non-immune cells in an immune response. There are two types of cytokines: interleukins and chemokines (10). The cytokine interleukin-37 (IL37) is an anti-inflammatory cytokine that restrains innate immunity and raises inflammation and autoimmune diseases(11) while Khalaf *et al*. reported IL37 not increased with β-Thalassemia (12). It is related to *H. pylori* infection, peptic ulcers, and carcinogenesis (13). The chemokine CXCL9 inhibits pathogens from penetrating intestinal crypts through its antibacterial activity, therefore protecting the host against immunopathology. Additionally, it is overexpressed in gastric cancer tissue relative to normal gastric tissue (14) and also offers contradictory activity in the development of cancer in *H. pylori*-infected children and adults (15).

Materials and methods

A total of 197 individuals have been involved in this study, including 165 gastroduodenal disorder patients and 32 healthy individuals for comparison. The studies included age, gender, blood group, and *H. pylori* infection for all of them, and 88 individuals were involved in the immunological assay, including 66 gastroduodenal disorder patients and 22 healthy individuals for comparison. Studies excluded patients who were not infected with *H. pylori* and had chronic conditions such as hypertension, diabetes, or celiac disease. The patients' groups attended to the Gastroenterology Teaching Hospital and the doctor's private clinic and

suffered from chronic epigastric pain, weight loss, and anaemia. The physicians diagnosed them with gastritis, peptic ulcers, and gastric cancer by oesophagogastric duodenoscopy (OGD).

Detection of *H. pylori*

The bacterium was detected using the rapid test for the *H. pylori* antibody kit (Biozek, Netherlands). The antibody of the bacterium in whole blood was captured by anti-human IgG immobilized in the test line region by adding one drop of blood and diluent then waiting until the lines were observed. This test has two lines: the first one is C (control), and the second is T (test). When the C line appears alone, it's considered negative but regarded positively when both C and T.

Measurement of circulating IL37 and CXCL9 levels in sera

The measurement of IL37 and CXCL9 in sera was performed by using Boster Picokine™ (USA) kits for human cytokines IL37 and CXCL9 concentration. The measurement was performed by a pre-coated enzyme-linked immunosorbent assay (ELISA) in both healthy and gastroduodenal patients with a 96-well strip plate that was pre-coated with an antibody specific for IL37 and CXCL9 according to the kit manufacturing company guidelines. The sensitivity and detection range of the kits were as follows: <10 pg/ml and 15.6 - 1,000 pg/ml for IL37, <2 pg/ml and 31.2 - 2,000 pg/ml for CXCL9. The optical density (OD) was read at 450 nm by using a microplate reader within 15 minutes.

Statistical analysis

The data were analyzed using the software: IBM SPSS V26 and Microsoft Excel was used for graphics. The results reported in this study were expressed as mean ± SD. A one-way ANOVA was used to test continuous variables (CXCL9 and IL37). Duncan's Multiple Range Test was used to test for significant differences between groups' means. The chi-square test of independence was used to describe the demographic characteristics of the study groups. Probability values less than 0.05 were considered significantly different (16, 17).

Results and discussion

Age and gender distribution of the samples

The demographic characteristics of subjects, including age and gender, are shown in Tables (1 and 2). The total number of patients was 165 (66 females and 99 males; mean age 42.45 ± 16.02 years; ranging from 15–71 years in three groups: gastritis, peptic ulcers, and gastric cancer. On the other hand, results showed the prevalence of GD increased significantly ($p = 0.001$) with age; it was also noticed a higher susceptibility to infection in the age group 30-39 years, which recorded 20.6% of the total percentage of patients, followed by the group 50-59, which recorded 19.4%, the group ≥ 60, which recorded 18.8%, the group ranging between 40-49 years, and the 20-29 group, which recorded 16.4 and 15.1%, respectively. The lower incidence of the disease occurred in the age group of less than 20 years, which recorded 9.7%. Similar findings were recorded by Karam (2019) and Shehab *et al.* (2021) (18,19), and a study done in Karbala reported middle-aged patients (16–45 years) had a larger prevalence (30%) than younger patients (1–15 years) (8%) and elderly patients (61–75) years representing 12% (20).

It is concluded from the present study that gastroduodenal disorder is more common in elderly individuals

than others, which may be due to decreasing the ability of protection and reparative capacity for the gastric mucosa layer, which has two different repair mechanisms. The first mechanism is mucosal restitution by migration of viable epithelial cells from gastric pits and glands, and the second mechanism is cell division replacement of lost cells. These mechanisms are affected by gastrointestinal hormones and growth factors that regulate the reparation of mucosal membranes, like EGF (epidermal growth factor) and TGF-α (transforming growth factor alpha), which activate the intrinsic tyrosine kinase (Tyr-k) of their epidermal growth factor receptor (EGF-R), and its activation decreases with age. In addition, polyamines and prostaglandins are also thought to be involved in gastric mucosal reparative processes that decrease with advancement with age (21). In this study, the incidence of gastroduodenal disorders was higher in men than in women. The female has a stronger immune response and antibody production due to the additional protection provided by the extra copy of chromosome X. Although this chromosome is exposed to X chromosome inactivation mechanisms, some genes escape from silencing and skewed inactivation, which may contribute to an immunological advantage (22). Several X-linked genes have roles in both the innate and adaptive immune systems, like TLR7 and TLR8, and estrogen stimulates the immune system, whereas testosterone suppresses it.

These results agree with the findings of Kalaf *et al*. (23) and Zhu *et al.* (24) who reported that the infection rate in men is significantly higher than that in women while disagreeing with those of Majeed and Khoshnaw (25) and Lafta and Al-Faisal (26) who reported a high prevalence of *H. pylori* in women.

Table (1): Age distribution in patients suffering from gastroduodenal disorders and control according to endoscopic exanimation.

Age group	Group number (%)			
	Gastritis	Peptic ulcer	Gastric cancer	Total patients
< 20	10 (11.6)	4 (8.2)	2 (6.7)	16
20-29	22 (25.6)	2 (4.1)	1 (3.30)	25
30-39	19 (22.1)	12 (24.5)	3 (10.0)	34
40-49	15 (17.4)	9 (18.4)	3 (10.0)	27
50-59	11(12.8)	9 (18.4)	12 (40.0	32
≥ 60	9 (10.5)	13 (26.5)	9 (30.0)	31
Total	86 (100)	49 (100.0)	30 (100)	165
Chi-Square Tests		**P-value**	**0.001****	

**** (P≤0.01) is significant.**

There was no significant difference between males and females in the gastritis group, which recorded 50% in both, as shown in Table 4-2. These findings agree with KSA's, which reported no statistically significant variation in infection rates between females and males from different age groups (27). Our findings matched up with research carried out in Korea, which showed the frequency of the incidence of peptic ulcers was higher in males than females (28). There was a significantly higher incidence of gastric cancer in males than in females; these results are reported by Lou *et al.* (29).

Table (2): Gender distribution in gastroduodenal disorder patients.

Gender	Group number (%)			Total
	Gastritis	Peptic ulcer	Gastric cancer	
Male	43(50.0)	37 (75.5)	19 (63.3)	110 (55.5)
Female	43(50.0)	12 (24.5)	11 (36.7)	87 (44.2)
Total	86 (100.0)	49 (100.0)	30 (100.0)	197 (100.0)
Chi-Square Tests		P-value	0.001**	

**** (P≤0.01) is significant.**

Detection of *H. pylori* by rapid test cassette

The collected blood from gastroduodenal disorder patients and healthy individuals was used to detect *H. pylori* IgG antibodies by the immunochromatographic method, as shown in Figure 1. All the patients included in the current study were positive for *H. pylori* infection (the negative ones were excluded from the study), and the healthy were negative for the infection.

Figure (1): Results of immunochromatographic test the NC is the negative result (left), and 1-5 is the positive result.

The level of IL37 concentrations in serum

Out of 197 subjects, 88 were subjected to measuring the concentration levels of IL37 and CXCL9 in sera by ELISA. The subjects were divided into four groups: gastritis, peptic ulcer, gastric cancer, and healthy. The IL37 level in serum was increased in gastroduodenal group patients compared with the healthy group; the means of concentrations ± SD were illustrated in Table (3) in gastritis, peptic ulcer, gastric cancer, and healthy were as follows: 637.3 ± 125.3, 598.7 ± 158.2, 177.06 ± 67.49, and 127.08 ± 13.41, respectively.

Table (3): Serum level of IL37 in gastroduodenal disorder patients and healthy group.

Group	Mean concentrations of IL37 ± SD	SE	P-value
gastritis	637.30 ± 125.29 a©	25.57	0.0005*
Peptic ulcer	598.77 ± 158.82 a	33.86	0.0005*
Gastric cancer	177.059 ± 67.49 b	14.73	0.336
Healthy	127.08 ± 13.41 b	3.076	-----
Dunnett t-tests	Healthy compared with patients' groups P-value ≤ 0.001**		

¥: One way ANOVA was used to test between groups ©Means that do not share a letter are significantly different. * (P≤0.05) is significant.

As shown in Table 3, the comparison of patient groups with healthy indicated exhibited highly significant differences ($p \leq 0005$) between gastritis and healthy (637.30 ± 125.29 *versus* 127.08 ± 13.41, respectively) and peptic ulcer with healthy (598.77 ± 158.82 *versus* 127.08 ± 13.41, respectively) and non-significant differences between gastric cancer and healthy (127.08 ± 13.41 versus 127.08 ± 13.41, respectively). IL-37 is an anti-inflammatory cytokine that plays a regulatory role in the immune system. As reviewed by Yan and his colleagues (2019), the cytokine IL37 is an anti-inflammatory cytokine that plays a regulatory role in the immune system (11).

It suppresses pro-inflammatory cytokines and has a positive impact on *H. pylori* infection (30). In the present study, the increase of IL37 in patients with gastritis and peptic ulcers was significantly high due to the stimulation of TLRs expression that triggers pro-inflammatory cytokines and the accumulation of different immune cells in stomach tissue like lymphocytes (B and T), macrophages, mast cells, neutrophils, and dendritic cells that highly expressed the *IL37* gene in the *H. pylori* infection. Attah *et al*. (31) reviewed *H. pylori*-induced ulcer formation that can harm the stomach epithelial barrier and its protective function. A weakening of this function can aid in the translocation of *H. pylori* virulence factors and inflammatory mediators into the circulation, resulting in a systemic inflammatory response These results matched with several previous studies (11, 13, 30).

Our findings compared the gastric cancer patients' group with the healthy one, which indicated a slight increase in IL37 in patients and a decrease in the gastric cancer group compared with gastritis and peptic ulcer groups (177.06 ± 67.49 *versus* 637.30 ± 125.28 and 598.77± 158.82); although the cancer patients were *H. pylori* positive, there was a down regulation in its level compared to gastritis and peptic ulcer groups. While there is limited information available on IL-37 and gastric cancer, some studies have shown that IL-37 expression may be reduced or dysregulated in colorectal cancer tissues compared to normal tissues (32). IL-37 is believed to have tumour-suppressive properties in various types of cancers. However, the specific role of IL-37 in gastric cancer is still being investigated, and research on this topic is ongoing. This suggests that IL-37 levels may not increase in gastric cancer but rather exhibit altered expression patterns. The reasons behind this dysregulation are not yet fully understood. It is possible that other factors, such as genetic alterations or epigenetic modifications, contribute to the downregulation of IL-37 in gastric cancer. The findings of the current study disagree with those reported by Zhang *et al.* (2019), who found that IL37 was significantly higher in gastric cancer patients than in healthy controls(33).

The level of CXCL9 concentration in serum

The circulating CXCL9 level in serum was increased in gastroduodenal group patients compared with the healthy group; the means of concentrations ± SD were illustrated in Table (4) in gastritis, peptic ulcer, gastric cancer, and healthy were as follows: 586.06 ± 701.47, 589.94 ± 778.86, 364.16 ± 215.08, and 111.85 ± 54.28, respectively. The peptic ulcer

group was the highest among the others, followed by the gastritis group and gastric cancer.

As shown in Table (4), the comparison of patient groups with healthy groups indicated significant differences (P≤ 0.020) between these disorders and the healthy group. In these disorders, various factors like infection with *H. pylori*, tissue damage, or an imbalance in the immune response can trigger the release of pro-inflammatory cytokines, including interferon-gamma. These results agree with George *et al*. (34) and Zhang *et al*.(35), who reported that CXCL9 is elevated with *H. pylori* infection, which is primarily induced by interferon-gamma and plays a role in recruiting immune cells, particularly T cells, to the site of inflammation. This recruitment of immune cells can contribute to the inflammatory response observed in gastroduodenal disorders (35). Weakening of the epithelial barrier and its function can aid in the translocation of *H. pylori* virulence factors and inflammatory mediators into the circulation, resulting in a systemic inflammatory response (31) in gastritis and peptic ulcer conditions.

Table (4): Serum level of CXCL9 in gastroduodenal disorder patients and healthy group.

Group	Mean concentrations of CXCL9 ± SD	SE	P-value
gastritis	586.06 ± 701.47 a©	137.57	0.016
Peptic ulcer	589.94 ± 778.86 a	166.05	0.020
Gastric cancer	364.16 ± 215.08 ab	46.93	0.339
Healthy	111.85 ± 54.28 b	12.45	-----
Dunnett t-tests	Healthy compared with patients' groups (P< 0.020*)		

¥: One way ANOVA was used to test between groups ©Means that do not share a letter are significantly different.

The CXCL9, -10, and -11/CXCR3 axis controls immune cell migration, differentiation, and activation, which results in tumour suppression (paracrine axis). However, several studies have found that this axis is involved in tumour development and metastasis (autocrine axis) (36). These results agree with the findings of Raja *et al.* (37), Tokunaga *et al.* (36), and Zhang *et al*. (35), who reported CXCL9 upregulation in gastric cancer patients.

Conclusions

It is concluded that a significant relationship between the biomarkers (IL-37 and CXCL9) and gastroduodenal disorder patients infected with *H. pylori* especially gastritis and peptic ulcer. These biomarkers can be used as laboratory parameters predicting the severity of the disorders and the ability to cancer progress.

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