Evaluation of serum levels of Proinflammatory Cytokines IL-8, IL-17, and IL-22 in Helicobacter pylori infection and their association with the degree of gastritis histopathology in a sample of Iraqi patients

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Abstract

Gastritis can be defined as histological inflammation of the gastric mucosa. It can be classified according to the time course of the disease as acute or chronic, histological findings, anatomic location, and pathological mechanisms. The objective of this study was to evaluation of serum levels of the proinflammatory cytokines IL-8, IL-17 and IL-22 in Helicobacter pylori infection and their association with the degree of gastritis histopathology in a sample of Iraqi patients. The case-control prospective study consists of 60 patients who attended the Gastrointestinal Tract Center at Al-Kindy Teaching Hospital during the period from December 2019 to April 2020. In addition, the control group included 60 apparently healthy individuals. Biopsies from the gastric antrum and/or body mucosa were used to assess the severity of chronic inflammation, neutrophil infiltration, atrophy, intestinal metaplasia. Serum samples were obtained to determine H. pylori infection, circulating interleukin (IL)-8, IL-17, and IL-22. Results showed that the Patients’ ages with gastritis ranged from 18-75 years. The body mass index revealed that 33.33% of the patients were obese and 35% of them were overweight. Most of the patients with active chronic gastritis and superficial chronic gastritis had positive titers for anti-H. pylori IgG antibody (167.89 ± 3.18 IU/ml) and (150.74± 1.45 IU/ml) respectively, which was significantly different from the control group (4.36 ± 0.29 IU/ml) (P=0.0001). Histopathological analysis showed that all subjects experienced chronic inflammation, while neutrophil infiltration was found 36.66% and there was significant association between serum levels of IL-8, IL-17, and IL-22 with a degree of chronic inflammation and neutrophils infiltration. In conclusion, the most common cause of gastritis was H. pylori with histopathological lesions, showing neutrophils infiltration and chronic gastric mucosal inflammation associated with increased levels of IL-8, IL-17, and IL-22 in serum.

Keywords: Biopsies, Cytokines, Gastritis, Histopathology, H. pylori.

Introduction

Gastritis can be defined as histological inflammation of the gastric mucosa. It can be classified according to the time course of the disease whether acute or chronic, histological findings, anatomic location, and pathological mechanisms. The most common cause of gastritis is Helicobacter pylori (H. pylori) infection, tobacco smoking, alcohol, use of non-steroidal anti-inflammatory drugs (NSAIDs) or steroids, autoimmune gastritis, collagenous gastritis, sarcoidosis, eosinophilic gastritis, and lymphocytic
gastritis. Patients with chronic gastritis may end up with Gastric atrophy (GA), gastric ulcer, and metaplasia in the intestine of the gastric mucosa (GIM) and may develop gastric adenocarcinoma (GC), which is the fifth most common cancer all over the world and causes mortality as a result of delayed diagnosis. *H. pylori* may introduce alterations in its genetics, produce many virulence factors, toxins, and proteins, and stimulates miscellaneous adaptation processes throughout its adhesion and colonization. In addition to that, it changes its shape from spiral to coccoid to persist in the gastrointestinal tract (GIT).

Therefore, the pathogenicity and virulence of *H. pylori* are sophisticated interactions among virulence factors, host, and environmental factors. The host’s immune responses play an important role in controlling the infection. Interleukine-8 (IL-8) is an important mediator in *H. pylori*-associated gastritis. In infected individuals, The T-helper (Th)-CD4+ lymphocytes in the gastric lamina propria become polarized and hyporesponsive by Th1/Th17 cells that are under the control of Treg (CD25+) cells. Th17 in the gastric mucosa plays an important role in gastric pathogenesis via secretion of IL-17 that affects the activity of antimicrobials and the immune response inside the gut environment. On the other hand, IL-22 is a multifunctional cytokine that helps to maintain tissue homeostasis and regulate immune responses. Its role in *H. pylori* infection is not well understood, but it is known to play a critical role in tissue repair and regeneration, especially in barrier tissues like the gut. This study was designed to an evaluation of serum levels for the proinflammatory cytokines IL-8, IL-17, and IL-22 in *Helicobacter pylori* infection and their association with the degree of gastritis histopathology.

**Patients and methods:**

This is a case-control prospective study including 60 patients, 30 males and 30 females who attended the Gastrointestinal Tract Center at Al-Kindy Teaching Hospital during the period from December 2019 to April 2020 with the cooperation of the medical and nursing staff of the Endoscopy Unit. The inclusion criteria were patients who were complaining of epigastric pain, dyspepsia, nausea, vomiting, and upper-gastrointestinal bleeding presenting as hematemesis and/ or Malena. The exclusion criteria were patients with carcinoma of the stomach and esophagus. The patients who underwent endoscopic examination were kept fasting for at least 8 hours and the examination was performed under local pharyngeal anesthesia by using fiber optic endoscope: GIF-H260; Olympus, Tokyo, Japan display screen; Olympus OEV-261H liquid crystal display monitor; Olympus, Tokyo, Japan for diagnosis confirmation with biopsies for histopathological studies. The second group was the control group that included 60 apparently healthy individuals that matched the gastritis patients in sex and age, but without any gastric disorders or complaints.

Ethical clearance was obtained from Al-Kindy Teaching Hospital's Scientific and Ethical Committee as well as Al-Kindy College of Medicine. Informed consent was obtained from each participant prior to the commencement of the study.

**Gastritis histological analysis**

Sixty biopsies of the mucosa of the gastric antrum and stomach body were used to determine the degree of histological gastritis. Patients’ biopsy samples were first fixed in 10% formalin, and then they were embedded in paraffin. After that, they were stained using Hematoxylin- Eosin and examined by the pathologists of Al-Kindy Teaching Hospital in a blinded technique. The histopathological study included: the degree of neutrophils infiltration, chronic gastric mucosal inflammation, mucosal atrophy, and intestinal metaplasia according to the most recent Sydney System, they were graded as (0) normal, (1) mild, (2) moderate, and (3) severe.

**Serological examination**

One hundred twenty sera samples were collected from both gastritis patients and apparently healthy individuals for serological examination. Enzyme-linked immunosorbent assay kit (ImmunoLab, Germany) used for the determination of the quantity of anti- *H. pylori* IgG antibodies in the sera of the study groups. Serum levels of IL-8 was measured according to manufacturer instructions, using ELISA kit from Beckman Coulter Marseille, France. While, ELISA kits of IL-17 and IL-22 were obtained from Al-shkairate Company, Jordon.

**Statistical Analysis:**

Statistical Analysis System (SAS) program, 2018 was used in this study. The Least Significant Difference (LSD) test (Analysis of Variation, ANOVA) was performed to compare between means. The Chi-square test was used to determine an association between categorical variables in a significant way (0.05 and 0.01 probability). In this study, the odd ratio and confidence interval were estimated.
Results

This study included 60 patients with gastritis confirmed by gastroscopy and histopathology. Their ages ranged from 18-75 years old. Most of them were between 30-50 years old (56.67%) which was not significantly different from the control group (53.33%) as shown in fig.1. Male to female ratio was 1:1 (50% males and 50% females) is nearly frequency-matched with the control group as demonstrated in fig.2. Regarding body mass index, Fig.3 illustrated that the percentage of individuals that have a normal weight (18.5-25) and obese (BMI more than 30) tended to be lower in the control group when compared with the patients' group (25% versus 31.67% and 31.67% versus 33.33%, respectively). In contrast, the control group tended to be more overweight (25.1-30) in comparison with the patients' group (43.33% versus 35%, respectively) but the differences did not reach the significance level. The main cause of gastritis in those patients was infection with \textit{H. pylori}. Most of the patients with active chronic gastritis and superficial chronic gastritis had positive titers for anti-\textit{H. pylori} IgG antibody (167.89 ± 3.18 IU/ml) and (150.74 ± 1.45 IU/ml) respectively, which was significantly different from the control group (4.36 ± 0.29 IU/ml) (P=0.0001) as shown in table 1.

Gastric biopsies were taken from the patients and sent for histopathologic study. The most common findings were moderate chronic gastric mucosal inflammation seen in 29 (48.33%) of the cases followed by mild chronic gastric mucosal inflammation 21 (35%) and severe gastric mucosal inflammation 10 (16.66) as shown in Table 2. Furthermore, histological analysis confirmed that only 22 (36.66%) of cases were infected with \textit{H. pylori} as presented in table 2.

Concerning cytokines levels of IL-8, IL-22, and IL-17 in patients with gastritis, there was a significant increase (P= 0.0001, 0.0001, and 0.0062) in the serum (336.95± 19.41, 38.77 ± 1.01, 18.84 ± 0.16 pg/ml), respectively, as compared with the control group (122.48± 12.07, 17.69 ± 1.34, and 14.09 ± 0.8 pg/ml) as shown in Table 3. In addition, analysis association between cytokines serum levels with the degree of gastritis histopathology revealed that, the percentage of patients that showed increased in the cytokines levels of IL-8, IL-17, and IL-22 were significantly associated with moderate and severe chronic inflammation [OR ( 95% CI): 1.26 (0.82-2.15); 1.33;0.86-2.09) and 1.07:(0.72-1.87) respectively, P≤0.05] and neutrophil infiltration of gastric mucosa [OR ( 95% CI) were 1.74 (0.92-3.05); 2.19:(1.15-4.22) and 1.56: (1.02-3.97) respectively, P≤0.01] as demonstrated in Table 4.

Figure 1. Distribution of study samples based on age groups.
Figure 2. Distribution of study samples based on gender

Figure 3. Distribution of study samples based on Body Mass Index (BMI).

Table 1. The mean level of anti- *H. pylori* IgG antibody concentration in study groups.

<table>
<thead>
<tr>
<th>Study groups</th>
<th>Number</th>
<th>Level of anti- <em>H. pylori</em> IgG antibody (IU/ml) (Mean ± SD)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active chronic gastritis</td>
<td>22</td>
<td>167.89 ± 3.18 a</td>
<td>0.0001 **</td>
</tr>
<tr>
<td>Superficial chronic gastritis</td>
<td>38</td>
<td>150.74± 1.45 a</td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>60</td>
<td>4.36 ± 0.29 b</td>
<td></td>
</tr>
</tbody>
</table>

Means with different letters in the same column were significantly different.

** (P≤0.0001).
Table 2. Histopathologic grading of gastritis according to Sydney System.

<table>
<thead>
<tr>
<th>Histological results</th>
<th>Normal (0) No. (%)</th>
<th>Mild (1) No. (%)</th>
<th>Moderate (2) No. (%)</th>
<th>Severe (3) No. (%)</th>
<th>Total No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophil infiltration</td>
<td>38 (63.33)</td>
<td>12 (20)</td>
<td>6 (10)</td>
<td>4 (6.67)</td>
<td>22 (36.66)</td>
</tr>
<tr>
<td>Chronic gastric mucosal inflammation</td>
<td>0</td>
<td>0</td>
<td>21 (35)</td>
<td>29 (48.33)</td>
<td>60 (100%)</td>
</tr>
<tr>
<td>Mucosal atrophy</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Intestinal metaplasia</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>H. pylori positive by histopathology</td>
<td>38 (63.33)</td>
<td>10 (16.66)</td>
<td>7 (11.66)</td>
<td>5 (8.33)</td>
<td>22 (36.66)</td>
</tr>
</tbody>
</table>

Table 3. Cytokines levels in patients and control groups.

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Serum level (pg/ml)</th>
<th>P-value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8</td>
<td>336.95± 19.41</td>
<td>0.0001 **</td>
<td></td>
</tr>
<tr>
<td>IL-17</td>
<td>38.77 ± 1.01</td>
<td>0.0001 **</td>
<td></td>
</tr>
<tr>
<td>IL-22</td>
<td>18.84 ± 0.16</td>
<td>0.0062 **</td>
<td></td>
</tr>
</tbody>
</table>

** (P<0.01).

Table 4. Association among cytokines levels of IL-8, IL-17, IL-22 and degree of chronic inflammation and neutrophil infiltration.

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Chronic inflammation</th>
<th>Neutrophil infiltration</th>
<th>P-value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8</td>
<td>High</td>
<td>22 (36.66%)</td>
<td>0.037</td>
<td>1.26 (0.82-2.15)</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>17 (28.33%)</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>IL-17</td>
<td>High</td>
<td>7 (11.66%)</td>
<td>0.039</td>
<td>1.33 (0.86-2.09)</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>20 (33.33%)</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>IL-22</td>
<td>High</td>
<td>14 (23.33%)</td>
<td>0.041</td>
<td>1.07 (0.72-1.87)</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>16 (26.66%)</td>
<td>*</td>
<td></td>
</tr>
</tbody>
</table>

* (P<0.05), ** (P<0.01).

Discussion

In this study, the highest incidence of gastritis was observed to occur between the third and fifth decades. Age can be a crucial factor for having gastritis due to thinning stomach lining with aging because of aging is related to a decreased rate of gastric epithelial cell turnover and a decreased ability to repair the gastric mucosa due to decreased levels of prostaglandin in the gastric mucosa. Additionally, lifestyle factors such as stress, smoking, and diet may contribute to the development of gastritis. This result agrees with other studies that have shown that aging is associated with inflammatory changes in the gastric body, which can also contribute to the development of gastritis. In the present study,
serological tests for *H. pylori* IgG showed the highest positive frequency when compared with the histological examination that showed only 22 (36.66%) from cases were infected with *H. pylori* and IgG antibodies titer was higher in active chronic gastritis than superficial chronic gastritis. Previous studies reported that the prevalence of *H. pylori* infection increased in the Middle East region, and the rate was more in subjects with gastritis and this prevalence was varied between studies performed in different countries and within the same country. The serological index of IgG antibodies cannot predicate whether the individuals have a previous or current infection. IgG antibodies can be found even after the bacteria clearance and IgG level could be decreased to 40-50% by 6-month post-treatment, and only 25% of successfully treated patients show complete disappearances of IgG10-13.

One of the risk factors for gastritis is high body mass index as seen in this research (BMI of more than 30 kg/m² was associated with gastritis 33.33%). This study is in accordance with previous studies illustrated that obese patients had higher *H. pylori* infection and gastritis than lean patients with BMI less than 25 kg/m². High BMI leads to lipid peroxidation, oxidative stress, decreased antioxidant activity all of which led to oxidative DNA damage that increased the risk of gastric cancer14-16. The histopathological tests showed that all subjects experienced chronic inflammation, while neutrophil infiltration was found 36.66% and atrophy and intestinal metaplasia were not found in the present study. The degree of gastritis histopathology results was varied among studies17, 18. This difference may be due to patient selection, duration of the disease, underlining causes, race of the patients included in the study, sample size, and the timing of biopsies during the course of the disease. The mucosa of stomach corpus either undergo superficial injury that heals through histopathologic changes of surface cells like foveolar hyperplasia or it may suffer a deep injury like chronic inflammation caused by *H. pylori* leading to pyloric pseudopyloric metaplasia, loss of parietal cells that secrete acids i.e atrophic gastritis, foveolar cells expansion, and turning of chief cells which secretes enzymes in the deep antral gland that is similar to mucous cells. Chief cells can be reprogrammed into paligenosis and mucus secreting spasmolytic polypeptide which expresses metaplasia cells stimulated by IL-13 secreted from innate lymphoid cells (ILC2s)18. This study demonstrated the significant elevation in the levels of IL-8, IL-17 and IL-22 as compared with control samples. Compatible with a previous study, the present study demonstrated that *H. pylori* stimulate Th CD4+ cells and Th17 to secret proinflammatory cytokines IL-17A, IL-17F, IL-21 and IL-22, which have antimicrobial response and control bacterial colonization. IL-22 and IL-17 act synergistically in the stimulation of chemokines and antimicrobials like lipocalin (LCN), IL-8 and some β-defensins within gastric epithelial cells that inhibit growth of *H. pylori*19. Infection with *H. pylori* induces IL-17 secretion in the mucosa of the stomach that induces IL-8 secretion via ERK 1/2 MAP kinase pathway activation. IL-8 works by attracting neutrophil to induce inflammation, while T regulatory cells (Tregs) works on suppressing the reaction of mucosal inflammation initiated by IL-17. Gastric inflammation is stimulated by host factors like IL-6, IL-1β, TGF-β1, tumor necrosis factor TNF-α, IL-22, IL-21, IL-18 and IL-17, and the *H. pylori* factors like vacuolating cytotoxin A (vacA) and proteins cytotoxin associated gene A (cagA)20. So, inhibition of these cytokines may be used as a potential therapeutic opportunity in the treatment21.

Conclusion

The most common cause of gastritis was *H. pylori* with histopathological lesions showing neutrophil infiltration and chronic gastric mucosal inflammation associated with increased levels of IL-22, IL-8, and IL-17 in the serum.

Acknowledgment

Many thanks to the staff of the Gastrointestinal Tract Center at Al-Kindy Teaching Hospital.
Author’s Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any figures and images that are not ours have been included with the necessary permission for republication, which is attached to the manuscript.
- Authors sign on ethical consideration’s approval.
- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.

Author’s Contribution Statement

M.J.Sh. devised the project, samples collection and responsible on carried out ELISA technique. B.M.M.

interpretation of histological reports and writing the manuscript. R.H. and Dh. S.N. data analytic.

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تقييم مستويات السيتوكينات الموالية للالتهاب Helicobacter pylori

1. define the histological section, cytokines, stomach inflammation, histopathological changes

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الخلاصة

يعرف التهاب المعدة بأنه التهاب نسيجي للطبقة المخاطية المبطنة للمعدة ويصنف إجمالاً على مدة المرض إلى التهاب حاد أو مزمن. وذلك اعتماداً على النتائج السريرية والوقت التشريحي والإدمان. هدفت الدراسة الحالية إلى تقدير مستويات السيتوكينات الموالية للالتهاب Helicobacter pylori في المرضى المصابين بالتهاب المعدة. قام المرضى بداء أي احتقان مزمن من المعدة. تضمنت الدراسة 60 مريضاً مصاباً، والتي حددت أن 33.33% من المرضى كاناً يعانون من السمنة الشديدة و35% كاناً يعانون من الوزن الزائد، معظم المرضى كاناً يعانون من تهاب المعدة النشط وتهاب المعدة المزمن. وكان P=0.0001 عند التحليل التسلسلي أن جميع المرضى كاناً يعانون من تهاب المعدة المزمن، بينما 36.6% كاناً يعانون من ارتفاع خلايا الدم البيضاء المعتدلة وكان هناك ارتفاع معدل من مستويات السيتوكينات 8 و22 ودرجة التهاب الغشاء المخاطي المزمن وارتفاع خلايا الدم البيضاء الفراغ. هذه النتائج تشير إلى أن التهاب المعدة المزمن هو إصابة بيكتريا Helicobacter pylori النسيجية المرضية والتي تنتج ارتفاع خلايا الدم البيضاء المعتدلة، مستويات السيتوكينات الموالية لالتهاب Helicobacter pylori.

الكلمات المفتاحية: الخزع النسيجي، السيتوكينات، التهاب المعدة، السيتوكينات الموالية، المثلث المجهرية.