Evaluation of Inflammatory Biomarker IL-8 in *Helicobacter Pylori*-Associated Gastritis

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ABSTRACT

Helicobacter pylori gastritis has been recognized as a significant risk factor for the development of gastric cancer. This study aimed to evaluate the role of interleukin-8 (IL-8) in *Helicobacter pylori*-Associated Gastritis disease progression. A total of 65 participants in the present study 35(58%)with symptoms of gastritis, were diagnosed and included in the study, along with 25 healthy controls. Gastric biopsy samples (mucus layer) were collected, and IL-8 concentrations were measured using an ELISA assay (Solarbio Human IL-8 ELISA Kit). Absorbance was recorded using a Fisher Scientific ELISA plate reader. Patients with gastritis who tested positive for *Helicobacter pylori* by PCR analysis—using glmM-specific primers—showed significantly elevated levels of IL-8 compared to healthy controls (17.78 pg/mL vs. 9.05 pg/mL, $p < 0.001$). These findings demonstrate a marked increase in IL-8 levels in patients with *H. pylori*-induced gastritis, underscoring its critical role in mediating inflammatory responses and contributing to disease pathogenesis.

Key words: IL-8, glmM, PCR,Helicobacter. pylori, *ureC*

INTRODUCTION

Helicobacter pylori is a Gram-negative, spiral-shaped bacterium that colonises the gastric mucosa and is a major cause of chronic gastritis**,** peptic ulcer disease, and gastric cancer. The pathogenesis of *H. pylori*-induced gastritis involves a complex interaction between bacterial virulence factors and the host immune response[1].One of the key mediators in this process is Interleukin-**8 (IL-8)**, a potent pro-inflammatory chemokine produced primarily by gastric epithelial cells. Upon infection with *H. pylori*, bacterial components, especially virulence factors such as CagA and lipopolysaccharide (LPS)—activate intracellular signalling pathways, most notably the NF-κB pathway[2]**.** This activation leads to increased transcription and secretion of IL-8. Elevated IL-8 levels play a crucial role in the recruitment and activation of **neutrophils**, which release reactive oxygen species (ROS) and proteolytic enzymes, resulting in epithelial cell damage and sustained inflammation characteristic of chronic active gastritis[3**]**.For the accurate detection of *H. pylori*, molecular techniques such as the polymerase chain reaction (PCR**)** are widely used due to their high sensitivity and specificity compared to conventional diagnostic methods. PCR allows for the amplification of specific bacterial DNA sequences directly from gastric biopsy specimens[4,5].

A commonly targeted gene in PCR assays is the **glmM gene** (also referred to as *ureC*)[6,7]. This gene encodes phosphoglucosamine mutase, an essential enzyme involved in bacterial cell wall biosynthesis[8,9]. The glmM gene is highly conserved in *H. pylori*, making it a reliable molecular marker for its identification. Detection of the glmM gene via PCR confirms the presence of *H. pylori* infection, even in cases with low bacterial load[10,11].Several studies have demonstrated a strong correlation between PCR-confirmed ***H. pylori*** infection (glmM-positive samples) and elevated levels of IL-8 in gastric tissues. This association highlights the role of IL-8 as an indicator of infection-induced inflammation. Increased IL-8 expression reflects the intensity of the host immune response and is often linked to the severity of gastritis[12].*H. pylori* infection triggers IL-8 production through activation of inflammatory pathways, leading to neutrophil-mediated mucosal damage[13]. The use of PCR targeting the glmM gene provides a precise method for detecting the bacterium, and the combined assessment of glmM positivity and IL-8 levels offers valuable insight into the diagnosis and

pathogenesis of **gastritis[14]**. The study investigated whether inhibiting IL-8 could reduce disease severity due to its role in causing tissue damage.

MATERIALS AND METHODS

Subjects and sample collection

In this case, the control enrolled 2 groups: a case group of patients who have gastritis disease and a control group that does not have gastritis disease. Both groups were selected from individuals referred to a gastroenterology hospital in Al- Marjan city in January 2026. Gastric biopsies were obtained from 60 persons candidate undergoing upper gastrointestinal endoscopy. Before the biopsy collection, all participants took part in an interview using a standardised questionnaire to gather demographic information. . Specimens were obtained from patients aged 18 to 65 years. The patient group consisted of 60 individuals participating, 35(58%) diagnosed with *Helicobacter. pylori* (15 males and 20 females), while the control group included 25 individuals without *Helicobacter. pylori* (12 males and 13 females).

biopsy specimens were prepared from the patient's gastritis. The study received approval NO.3 in 13\3\2026 from the Research Ethics Committee of the Training and Human center, Iraq. In addition, informed consent was obtained from all participants before their inclusion in the study.

Cytokine assay:-

Centrifuge mucus layer of the biopsy at 3000 rpm for 1 minute for homogenization. Perform the assay immediately or aliquot and store samples at or below -20°C. Avoid repeated freeze-thaw cycles.The Enzyme-Linked Immunosorbent Assay (ELISA) method was used to measure cytokines by using commercial kits for human IL-8 ELISA immunoassay kit (Solarlab, China). The manufacturer's instructions were carefully followed to and each plate was tested before use to confirm the calibration curve measured IL-8standards (17.78 pg/ml vs. 9.05 pg/ml) within the stated limits of the assay. An ELISA reader (The BioTek 800 TS absorbance reader/Germany) measured the kits made use of concentrated Biotin- conjugated with anti- IL8 antibody.The substrate color reaction at 450 nm. The optical density values obtained with the known samples were used to calculate the quantity of IL-8 .

DNA extraction:-

Gastric biopsy specimens were preserved in normal saline at -20°C. DNA extraction from the gastric biopsies was carried out following the manufacturer's instructions using the Geneaid Biotech DNA extraction kit (Presto, UK). The extracted DNA specimens were stored at -20°C until use. The quality and amount of DNA extracts were evaluated using a NanoDrop spectrophotometer (DeNovix, USA).

Molecular Detection H. pylori by PCR

The molecular identification of H. pylori was done by the detection of gene glmM-specific primers (Table 1), which were used to produce 140bp product. PCR amplification of DNA was performed by thermal cycler in final mixture volume of 25 µl (GoTaq® G2 Green Master Mix, Promega, USA). The conditions achieved optimum for amplification glmM gene were consisting of an initial denaturation of target DNA at 95 °C for 3 min (stage 1), followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at (57) °C for 1 min and extension at 72 °C for 30 s (stage 2). The final stage included only one cycle for extension 8 min at 72 °C. Eight microliters from PCR products were subjected to electrophoresis on 2% (wt/vol) agarose gel with 80 voltages for 90 min using horizontal electrophoresis apparatus and 1X TAE as a running buffer. The gel was stained with RedSafe DNA staining dye (INTRON© – Korea) and PCR bands were visualized gel documentation instrument under ultraviolet light. Statistical analysis Statistical analyses were carried out using IBM SPSS Statistics 23 (IBM Corp., Armonk, NY, USA). Categorical variables were presented as frequencies (percentages). Group differences were evaluated using the Student's t-test or the Mann–Whitney U test for normally and non-normally distributed continuous variables, respectively. Differences in categorical variables were assessed using Chi-square or Fisher's exact tests. Statistical significance was set at $P < 0.05$.

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Table 1: Specific primer and their sequences used for the diagnosis of *H. pylori* infection.

Gene	Seq (3-5`)	PCR product (bp)	Annealing Temp.	Reference
glmM	GGTCTTGCTGTCACTTATAGATGG	140	(57) °C	[15]
	GGTCTTGCTGTCACTTATAGATGG			

Ethical approval statement

The research protocol was reviewed and approved by the Ethical Review Committee of the Training and Human center, Iraq (certificate NO.3 in 13\3\2026). Informed consent was obtained from all participants prior to sample collection. Participants were provided with comprehensive information about the study's objectives, procedures, potential risks, and benefits, ensuring voluntary participation. Confidentiality and anonymity of all patient data were strictly maintained throughout the study. All collected samples were used solely for the purpose of this research and were handled with utmost care to adhere to ethical and scientific standards.

RESULTS AND DISCUSSION

Demographic information of the subjects:

Out of the 60 participants included in this study,35 individuals diagnosed with gastritis disease, with a mean age of 34.63 ± 14.234 years (15 males and 20 females), comprised the case group. The control group consisted of 25 individuals without H. pylori infection, with a mean age of 31.24 ± 11.875 years (12 males and 13 females) as in Table 2.

Table 2:- Demographic comparison of age and gender distribution between patients and the control group.

Demographic	Control (n = 25)	Patients (n = 35)	P-value
Age (years) Mean ± SD	31.24 ± 11.875	34.63 ± 14.234	0.284
Gender (Female/Male) N (%)	12/13(48.0/52)	35/30(48.8/51.3)	0.887

Diagnosis of *H. pylori* infection:-

A PCR assay for the detection of Helicobacter pylori in gastric biopsy specimens with specific primers for the glmM gene amplification was used. Bacteria were found in 35 (58%) out of 60 participants, by glmM PCR, a PCR-based assay to detect the H. pylori was also developed. To assess the likelihood of detection of H. pylori genes. In Figure (2), showed 35 (58%)specimens were positive for H. pylori infection while another specimens consider control group as negative for H. pylori (41.66%).

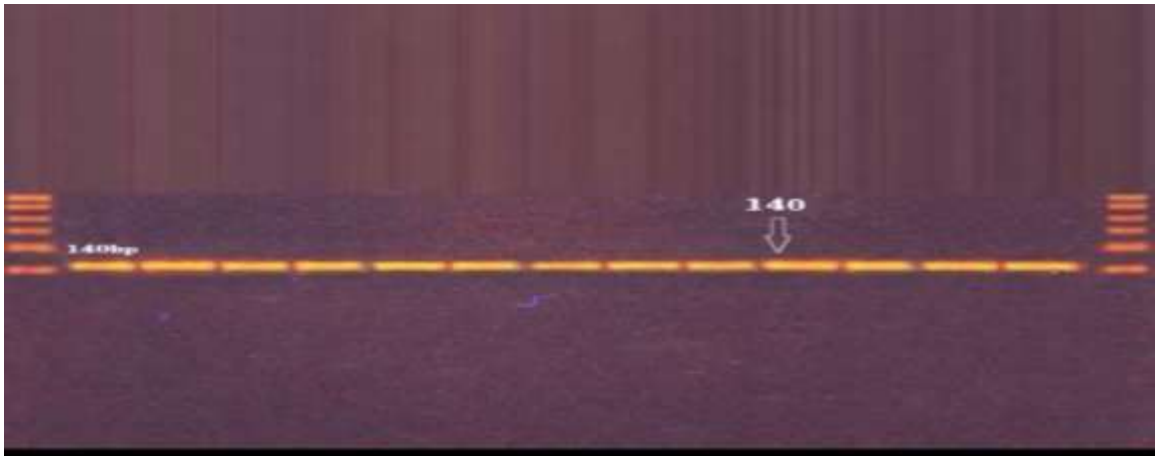


Figure a:

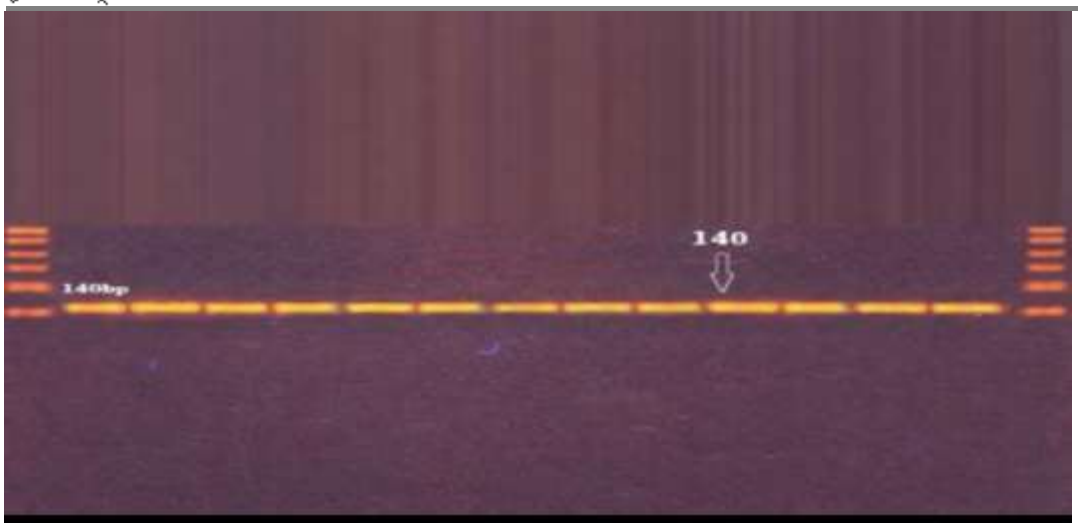


Figure b:

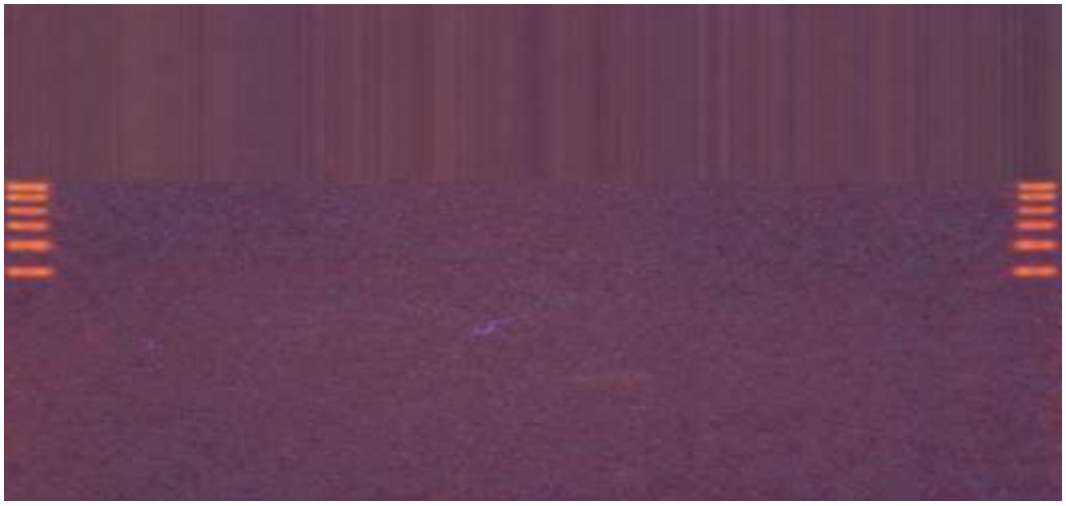


Figure C:

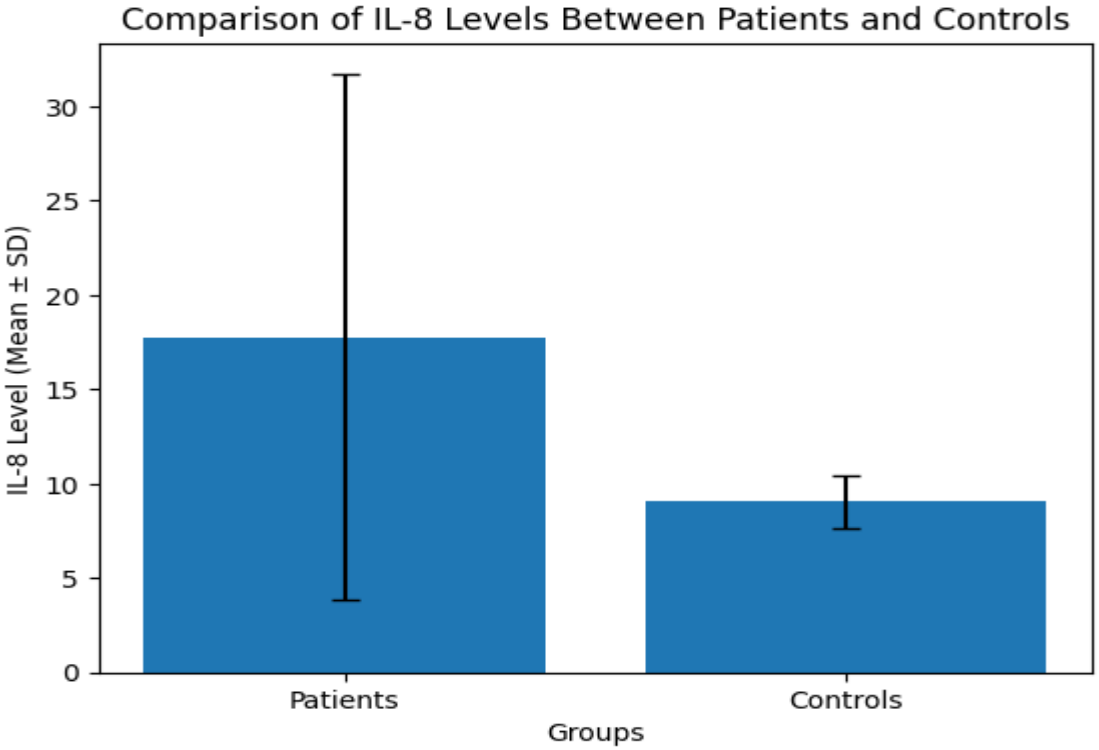
Figure (a,b,c) :- PCR products for glmM gene detection in *P. aeruginosa*. A single, specific 140 bp band was detected in lane 35 confirming the presence of the glmM gene in that isolate. and lanes L show molecular weight markers ranging from 400–1500 bp.and L(1-35)positive,36-60 negative results.

Estimate of IL8 concentrations between patients and control groups:-

Table 3, presents the comparison of IL-8 levels between patients and healthy controls. The mean serum IL-8 concentration in patients (n = 35) was significantly higher (17.78 ± 13.961) compared to the control group (n = 25), which exhibited a mean value of 9.05 ± 1.404. The patient group showed a markedly higher standard deviation, indicating substantial variability in IL-8 levels among patients, whereas the control group demonstrated relatively consistent values. Statistical analysis revealed a highly significant difference between the two groups ($p < 0.001$), confirming that the elevation of IL-8 in patients is statistically significant, as in figure2. These findings suggest that IL-8 levels are elevated in patients and may reflect increased inflammatory activity associated with the disease condition, supporting its potential role as a biomarker of inflammation.

Table 3: Comparison of IL-8 levels between patients and controls.

Groups	Number	IL-8(mean ± Std. deviation)	p-value
Patients	35	17.78±13.961	<0.001**
Controls	25	9.05±1.404	



Figure(2): Comparison of IL-8 Levels between patients and the control groups.

DISCUSSION

The present study investigated the relationship between *Helicobacter pylori* infection and serum IL-8 levels among patients with gastritis compared to healthy controls. The demographic data showed no statistically significant differences between patients and controls in terms of age ($p = 0.284$) or gender distribution ($p = 0.887$), indicating that both groups were comparable and that the observed differences in IL-8 levels are unlikely to be influenced by these variables. Molecular detection using PCR targeting the *glmM* gene revealed that 35 (58%) of the participants were positive for *H. pylori*, confirming the presence of infection in the patient group. PCR-based detection is considered a sensitive and specific method, supporting the reliability of infection status classification in this study. A key finding of this study is the significant elevation of serum IL-8 levels in patients compared to controls (17.78 ± 13.961 vs. 9.05 ± 1.404, $p < 0.001$). This result indicates enhanced inflammatory activity in patients with *H. pylori*-associated gastritis. IL-8 is a well-known pro-inflammatory chemokine that plays a crucial role in neutrophil recruitment and activation, contributing to mucosal inflammation and tissue damage in gastric diseases. The high standard deviation observed in the patient group suggests considerable variability in IL-8 levels, which may be attributed to differences in disease severity, bacterial virulence factors (such as *cagA* and *vacA*), or host immune response. These findings are consistent with several recent studies. For instance, a study by [16], and **[17],** reported significantly elevated IL-8 levels in patients infected with *H. pylori*, highlighting its role in gastric inflammation. Similarly, **[18],** demonstrated that *H. pylori* infection induces IL-8 production in gastric epithelial cells through activation of NF-κB signaling pathways. Furthermore, **[19],** found that IL-8 levels were significantly higher in patients with peptic ulcer disease compared to controls, supporting its potential as a biomarker for disease progression. The agreement between the current findings and previous studies reinforces the concept that IL-8 is a key mediator in the pathogenesis of *H. pylori*-associated gastric disorders. The elevated levels observed in this study further support its potential use as a non-invasive biomarker for inflammation and possibly for monitoring disease severity.

CONCLUSION

In conclusion, the present study demonstrates a significant increase in IL-8 levels in patients with *H. pylori*-associated gastritis compared to healthy controls. These findings highlight the important role of IL-8 in gastric inflammation and support its potential utility as a biomarker in clinical assessment.

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Authors' contributions

All authors contributed to the conception and design of the study, performed the experiments, and analysed the data. They also participated in the study design and coordination, and contributed to drafting the manuscript. All authors have read and approved the final version of the manuscript.

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