Investigating the Role of IFN-γ, IL-1β, IL-10, and GM-CSF in the Pathophysiology of Chronic Gastritis: Clinical Correlations and Immunological Assessments

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Abstract

Background: Chronic gastritis is a prevalent gastrointestinal condition often associated with *Helicobacter pylori* (*H. pylori*) infection and influenced by lifestyle factors such as diet, obesity, alcohol consumption, stress, pain relievers abuse and smoking. Here, we investigated the role of cytokines in pathophysiology of the disease, and evaluated the diagnostic efficacy of urea breath test (UBT) and *H. pylori* stool antigen (HP S-Ag) tests.

Methods: A cohort of 125 patients with chronic gastritis and a control group (27 participants) were analysed. Demographic data and clinical symptoms were collected. H. pylori test using UBT and H.P S-Ag was conducted. Serum cytokine levels (IFN-γ, IL-1β, IL-10, and GM-CSF) were measured. The diagnostic performance of UBT and H.P S-Ag was evaluated and Spearman's correlation analysis was conducted to determine association between the cytokines measured and UBT delta over baseline (DOB) values as well as H.P S-Ag levels.

Results: The mean age of patients was 57.08 years, with a significantly higher body mass index (BMI) of 30.56 kg/m² compared to 28.14 kg/m² in controls ($p = 0.0033$). Smoking was more prevalent among patients (30.4%) than controls (12.0%; $p = 0.0030$). Significant elevations in H. pylori positivity were observed in chronic gastritis patients, with UBT values at 38.84 ppm and H.P S-Ag levels at 0.20 µg/mL (both $p < 0.0001$). Serum cytokines, including IFN-γ, IL-1β, IL-10, and GM-CSF, were significantly elevated in patients compared to controls. Correlation analyses revealed strong positive associations between UBT DOB and BMI ($r_s = 0.2077$), as well as with cytokines (IFN-γ: $r_s = 0.5745$; IL-1β: $r_s = 0.3422$; IL-10: $r_s = 0.5102$). The diagnostic performance of UBT showed high sensitivity (99.20%) while H.P S-Ag demonstrated superior specificity (93.33%). Both tests had similar overall accuracy rates (UBT: 89%; H.P S-Ag: 90%).

Conclusion: The study highlights the significant associations of chronic gastritis with obesity and smoking, while elevated cytokine levels indicate a complex immune response linked to H. pylori infection. Both UBT and H.P S-Ag tests are effective diagnostic tools, with a combined approach enhancing diagnostic accuracy. Further research is needed to explore the underlying mechanisms of these associations and their implications for patient management.

Keywords: Chronic gastritis, Helicobacter pylori, urea breath test (UBT), H. pylori stool antigen (HP S-Ag), IFN-γ, IL-1β, IL-10, GM-CSF.

Introduction

Chronic gastritis is a relatively common disorder characterized by persistent gastric mucosal inflammation which over time, results to significant morbidity. This condition can arise from different causes including infectious agents, autoimmune triggers and chemical irritants such as non-steroidal anti-inflammatory drugs (NSAIDs). *Helicobacter pylori* (*H. pylori*) infection is a primary cause of chronic gastritis, implicated in various gastric diseases especially peptic ulcers and gastric cancers [1-3]. To ensure effective management of gastritis, timely and accurate diagnosis is essential, and this usually involves clinical evaluation, endoscopy, and histological examination of gastric biopsy specimens [4]. Recent advancements in non-invasive methods of diagnosis such as the urea breath test (UBT) and stool antigen testing for *H. pylori*, have enhanced our ability to detect this pathogen, however the underlying immunological mechanism involved in the pathophysiology of gastritis remains to be fully deciphered.

The pathophysiology of chronic gastritis is complex and involves a dynamic interplay of microbial factors and host immune response. Cytokines, which are critical mediators of the immune response play significant roles in the inflammatory processes associated with chronic gastritis. Due to their diverse functions in inflammation and immunomodulation, interferon-gamma (IFN-γ), interleukin-1 beta (IL-1β), interleukin-10 (IL-10), and granulocyte-macrophage colony-stimulating factor (GM-CSF) have attracted particular interest [2, 5, 6].

IFN-γ is a crucial cytokine produced predominantly by T helper 1 cells and natural killer cells. It promotes the activation of macrophages as well as expression of major histocompatibility complex (MHC) molecules, thereby facilitating the immune response against infections, including *H. pylori* [7]. Elevated levels of IFN-γ have been associated with greater inflammatory responses and increased mucosal damage in gastric tissues [8]. On the other hand, IL-1β, which is a pro-inflammatory cytokine that is produced by activated macrophages and epithelial, is involved in orchestrating the inflammatory response in chronic gastritis. It contributes to the recruitment of inflammatory cells to the site of infection and plays a role in the release of other pro-inflammatory cytokines [9]. In contrast however, is IL-10, which is an anti-inflammatory cytokine that is known for its role in the inhibition of cytokine synthesis and promotion of tissue repair. It functions by limiting excessive inflammation which is otherwise characteristic of chronic diseases, thereby preventing exposure to prolong inflammation that can cause tissue damage [5]. Finally, GM-CSF plays a role in the differentiation and survival of eosinophils and macrophages, which are essential for maintaining mucosal immunity and promoting a balance immune response in the gastrointestinal tract [10].

Given the crucial roles played by these cytokines, monitoring their serum levels in patients with chronic gastritis and evaluating with how they relate with to conventional diagnostic test is crucial. While previous studies have primarily focused on the presence of *H. pylori* and its pathogenic mechanisms, few have comprehensively examined the cytokine profiles in conjunction with clinical diagnosis and non-invasive tests for *H. pylori* detection. This study aimed to bridge this gap by assessing serum levels of in patients diagnosed with chronic gastritis through clinical, endoscopic and histological methods, and correlating these finding with non-invasive diagnostic test such as the urea breath test and stool antigen assays.

The comparative analysis of traditional diagnostic methods with cytokine profiles could pave the way for more sensitive and specific diagnostic approaches in identifying and managing chronic gastritis.

Patients and Methods

Study design and subjects

This study is a cross-sectional case control study involving 125 adult patients (aged 18 years and above) clinically diagnosed with chronic gastritis based on histological examinations and endoscopy at the Imam Al-Hussain Medical City, from June to November, 2024. The patients were age-matched with 75 healthy participants with no medical evidence of chronic gastritis and were not on any medication, and this served as the control group. All participants underwent a comprehensive medical history review and physical examination by a gastroenterologist. The exclusion criteria for patient recruitment included individuals with history of gastrointestinal malignancies or significant gastric surgery, those with autoimmune diseases or severe inflammatory conditions, current or recent (within the last month) use of medications affecting gastric acid secretion (e.g., proton pump inhibitors, H2 blockers, NSAIDs) as well as individuals with systemic infections. Ethical approval for the study was sought and obtained for the Research and Ethics Committee of the Imam Al-Hussain Medical City, and all the participant provided written informed consent prior to commencement of the research. Medical records and questionnaire-based interviews were used to gather clinical and sociodemographic information from the patients and the control group, including age, body mass index (BMI), common symptoms, marital status, smoking status, and family history.

Urea breath test for *H. pylori*

The ^{13}C-UBT BreathID HP Lab – IDKit HP Two (Meridian Bioscience, United States) was used. First, the baseline sample of exhaled air was collected before administering urea and the patient was asked to take a deep breath and exhale completely into a collection bag. Subsequently the subject was given a 200 mL solution containing 75 mg of ^{13}C-labelled urea mixed with citric acid to ingest, and was waited for 30 minutes without eating or drinking. After the waiting period, the procedure was repeated and the patient's exhaled air was collected into a second collection bag. The exhaled air samples were analysed within 24 hours using an infrared spectrometer S4 T-STAR (Bruker Nano GmbH, Berlin, Germany) which quantifies the ratio of ^{13}C to total CO_2 (which is ^{12}C) in expired air within three minutes. The difference between the baseline readings and those taken after urea ingestion is referred to as the delta over baseline (DOB). A DOB greater than 4.0 ppm after thirty minutes, which is typically considered indicative of *H. pylori* infection [11], was adopted.

H. pylori Ag in stool test

To measure the levels of *H. pylori* antigen in stool (H.P S-Ag), the subjects were provided with instructions on how to collect fresh stool sample, avoiding collecting urine or tissue paper and ensuring that the sample is free from contamination. The sample were placed in a pre-labelled, sterile container within few hours to testing, to ensure antigen stability. Approximately 30 mg of the sample was collected by 3 random stabs into the sample container using a collection stick to avoid scooping, and was transferred into a test tube containing the extraction buffer. The mixture was the thoroughly vortexed to homogenize the sample and ensure that the antigens were adequately extracted into the solution. The sample was the centrifuged at 2000 x g for 10 minutes, after which the supernatant was carefully collected for testing. The Atlas *H. pylori* Antigen ELISA Test Kit (Atlas Medical, Berlin, Germany) was used to quantitatively measure levels of *H. pylori* antigen in the sample as per manufacturer's instructions. Antigen concentration < 0.045 µg/mL was considered Negative, while > 0.055 µg /mL was Positive. Samples with concentration 0.045 – 0.055 µg/mL were considered equivocal, the test was

repeated and the samples were re-categorized as Negative or Positive, otherwise, they were eliminated from the study and replaced.

Measurement of serum cytokine levels

Blood samples were obtained by venepuncture following disinfection of the antecubital fossa with 70% ethanol from all the study subjects. Five millilitres of blood were drawn and dispensed into a gel tube and was allowed to rest for 15 to 30 minutes at room temperature prior to serum preparation. Serum was prepared by centrifugation at 10,000 g for 15 minutes and the supernatants were dispensed into Eppendorf tubes and stored at -20 °C until use. Serum levels of IFN-γ, IL-1β, IL-10, GM-CSF were measured using commercial ELISA kits: The Human Interferon gamma ELISA Kit (IFN-γ) (Sigma-Aldrich, Massachusetts, United States), Human Interleukin 1β, IL-1β ELISA Kit (Cusabio, Houston, United States), the human IL-10 ELISA kit (Cell Sciences Inc., Canton, Massachusetts, United States), and ELISA MAX™ Deluxe Set Human GM-CSF kit (BioLegend Inc, San Diego, CA, United States) adhering to the manufacturers' protocols.

Statistical analysis

Descriptive statistics was used to summarize demographic and clinical data, (mean ± standard deviation for continuous variables; frequencies and percentages for categorical variables).

Chi-square tests was used to compare the prevalence of *H. pylori* infection between patients and controls, the differences in UBT DOB, *H. pylori* Ag and cytokine levels between patients with chronic gastritis and healthy controls was assessed using the Mann-Whitney U test for non-normally distributed data, while Spearman's correlation coefficient was used to evaluate the relationship between cytokine levels and UBT as well as stool *H. Pylori* Ag outcomes. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of both UBT and H.P S-Ag were assessed using the patients' clinical diagnosis as gold standard. The IBM SPSS Statistics v.25.0 (IBM Corporation, Armonk, New York) was used and A p-value of < 0.05 was considered statistically significant.

Results

Demography and clinical characteristics of the study subjects

As summarized in Table 1, the mean age of patients with chronic gastritis was 57.08±11.85 years, while the control group had a mean age of 53.68±9.92 years, with a p-value of 0.0561, indicating no significant difference in age between the two groups. Regarding sex distribution, 37.6% of chronic gastritis patients were male compared to 45.4% in the control group. The BMI was significantly higher in the chronic gastritis group, with a mean of 30.56±7.67 kg/m² compared to 28.14±5.00 kg/m² in controls ($p = 0.0033$). Marital status did not show a significant difference between the groups; 83.2% of patients with chronic gastritis were married compared to 69.33% in the control group ($p = 0.2807$). Smoking status revealed a significant disparity, with 30.4% of chronic gastritis patients reporting current smoking compared to only 12.0% in the control group ($p = 0.0030$). Family history of chronic gastritis was present in 24.8% of patients versus 17.3% in the control group, which did not reach statistical significance ($p = 0.2172$). Symptoms associated with chronic gastritis were also assessed, with abdominal pain reported by 55.2% of patients. Other common symptoms included fatigue (50.4%), weight loss (43.2%), and belching (30.4%). Additionally, symptoms such as vomiting (19.2%), light-headedness (23.2%), loss of appetite (8.8%), blood in stool (4.8%), blood in vomit (13.6%), and indigestion (28.0%) were reported.

Table 1: General characteristics of the study subjects

	Chronic Gastritis *(n = 125)*	**Control** *(n = 75)*	**p-value**
Age, *years (Mean±SD)*	57.08±11.85	53.68±9.92	0.0561
Sex, n (%)			
Male	47 *(37.6)*	34 *(45.4)*	
Female	78 *(62.4)*	41 *(54.6)*	
BMI, *Kg/m²*	30.56±7.67	28.14±5.00	0.0033*
Marital status			
Yes	104 *(83.2)*	52 *(69.33)*	0.2807
No	21 (16.8)	23 *(30.67)*	
Smoking status			
Yes	38 *(30.4)*	9 *(12.0)*	0.0030*
No	87 *(69.6)*	66 *(88.0)*	
Family history, n *(%)*			
Yes	31 *(24.8)*	13 *(17.3)*	0.2172
No	94 *(74.2)*	62 *(82.7)*	
Symptoms, *n (%)*			
Abdominal pain	69 *(55.2)*		
Vomiting	24 *(19.2)*		
Fatigue	63 *(50.4)*		
Weight loss	54 *(43.2)*		
Light-headedness	29 *(23.2)*		
Belching	38 *(30.4)*		
Loss of appetite	11 *(8.8)*		
Blood in stool	6 *(4.8)*		
Blood in vomit	17 *(13.6)*		
Indigestion	35 *(28.0)*		

BMI; body mass index, SD; standard deviation, *Statistical significance at p < 0.05

***H. pylori* test findings and serum cytokine levels of the patients vs. control**

The results of the *H. pylori* test findings and serum cytokine levels for patients with chronic gastritis in comparison to the control group are presented in Table 2. Among the chronic gastritis patients, a significant proportion tested positive for *H. pylori*, as measured by the UBT which showed a marked elevation in UBT DOB values compared to the control group (i.e., 38.84±14.05 ppm, chronic gastritis vs. 2.85±1.99 ppm control ($p < 0.0001$)). Similarly, the levels of *H. pylori* antigen in stool (H.P S-Ag) were significantly elevated in patients, with a mean of 0.20±0.13 µg/mL compared to 0.031±0.015 µg/mL in controls ($p < 0.0001$). Regarding serum cytokines, IFN-γ levels were significantly higher in chronic gastritis patients (259.17±105.51 pg/mL) compared to controls (97.09±31.98 pg/mL; $p < 0.0001$). Elevated IL-1β levels were also observed in the patient group, with a mean of 124.46±71.45 pg/mL, compared to 66.15±22.74 pg/mL in the control group ($p < 0.0001$). For IL-10, serum levels were significantly higher in chronic gastritis patients (155.04±84.71 pg/mL) compared to controls (54.67±34.25 pg/mL; $p < 0.0001$). The GM-CSF levels also showed a significant

difference, with chronic gastritis patients having a mean of 93.54±45.68 pg/mL compared to controls with 75.46±30.70 pg/mL (p = 0.0012).

Table 2: *H. pylori* test findings and serum cytokine levels of the patients vs. control

	Chronic Gastritis *(Mean±SD)*	**Control** *(Mean±SD)*	**p-value**
UBT DOB, (ppm)	38.84±14.05	2.85±1.99	< 0.0001*
H.P S-Ag *(µg/mL)*	0.20±0.13	0.031±0.015	< 0.0001*
IFN-γ, *(pg/mL)*	259.17±105.51	97.09±31.98	< 0.0001*
IL-1β, *(pg/mL)*	124.46±71.45	66.15±22.74	< 0.0001*
IL-10, *(pg/mL)*	155.04±84.71	54.67±34.25	< 0.0001*
GM-CSF, *(pg/mL)*	93.54±45.68	75.46±30.70	0.0012*

UBT DOB; urea breath test delta over baseline, H.P S-Ag; *Helicobacter pylori* antigen in stool, IFN-γ; interferon-gamma, IL-1β; interleukin 1 beta, GM-CSF; granulocyte macrophage colony stimulating factor, SD; standard deviation, *Statistical significance at p < 0.05

The serum cytokine levels of the patients in comparison with the control group is graphically presented in Figure 1.

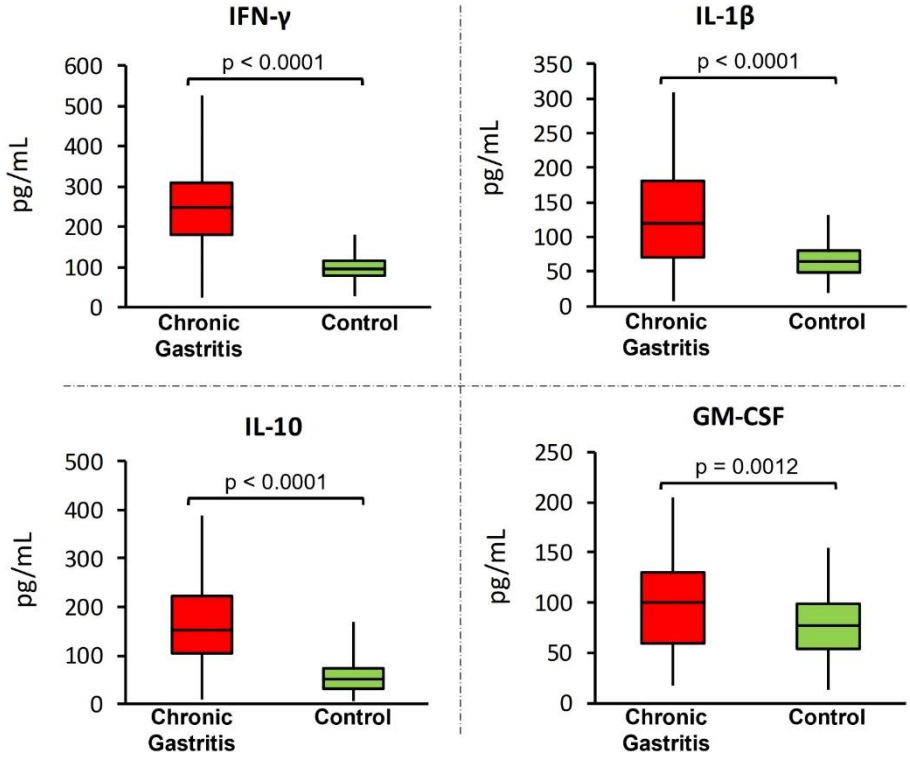

Figure 1: Comparison of serum cytokine levels between patients with chronic gastritis and control subjects.

Box plots showing the concentrations of IFN-γ, IL-1β, IL-10, and GM-CSF measured in pg/mL. Data are presented as median with interquartile ranges for both groups. The red boxes represent serum cytokine levels in chronic gastritis patients, while the green boxes represent levels in control subjects. Significant differences are indicated by p-values: IFN-γ ($p < 0.0001$), L-1β ($p < 0.0001$), IL-10 ($p < 0.0001$), and GM-CSF ($p = 0.0012$), demonstrating elevated cytokine levels in patients with chronic gastritis compared to controls.

Serum cytokine levels in the subjects with positive UBT vs. positive H.P S-Ag results

We compared serum cytokine levels between subjects who tested positive for the UBT and those who tested positive for H.P S-Ag (Table 3). A total of 145 subjects were categorized as UBT^+ while 115 were classified as H.P $S\text{-}Ag^+$. The analysis revealed no significant differences in cytokine levels between these groups. As summarized in Table 3, the mean IFN-γ levels were 225.59±109.66 pg/mL in the UBT^+ group and 246.34±108.28 pg/mL in the H.P $S\text{-}Ag^+$ group ($p = 0.2225$). Similarly, IL-1β levels showed no significant variation, with a mean of 115.96±70.99 pg/mL for UBT^+ subjects and 122.68±73.65 pg/mL for H.P $S\text{-}Ag^+$ subjects ($p = 0.4965$). IL-10 levels also did not differ significantly, with UBT^+ subjects exhibiting a mean of 143.41±91.30 pg/mL compared to 155.45±94.36 pg/mL in the H.P $S\text{-}Ag^+$ group ($p = 0.2846$). Finally, GM-CSF levels were similar across both groups, with means of 91.38 ± 44.44 pg/mL for the UBT^+ group and 94.36 ± 45.93 pg/mL for the H.P $S\text{-}Ag^+$ group ($p = 0.7489$). These findings are graphically presented in Figure 2.

Table 3: Serum cytokine levels in the study subjects with positive UBT vs. positive H.P S-Ag results

	UBT^+ *n = 145 (Mean±SD)*	**H.P $S\text{-}Ag^+$** *n = 115 (Mean±SD)*	**p-value**
IFN-γ, *(pg/mL)*	225.59±109.66	246.34±108.28	0.2225
IL-1β, *(pg/mL)*	115.96±70.99	122.68±73.65	0.4965
IL-10, *(pg/mL)*	143.41±91.30	155.45±94.36	0.2846
GM-CSF, *(pg/mL)*	91.38±44.44	94.36±45.93	0.7489

UBT^+; urea breath test positive, H.P $S\text{-}Ag^+$; *Helicobacter pylori* antigen in stool positive, IFN-γ; interferon-gamma, IL-1β; interleukin 1 beta, GM-CSF; granulocyte macrophage colony stimulating factor, SD; standard deviation

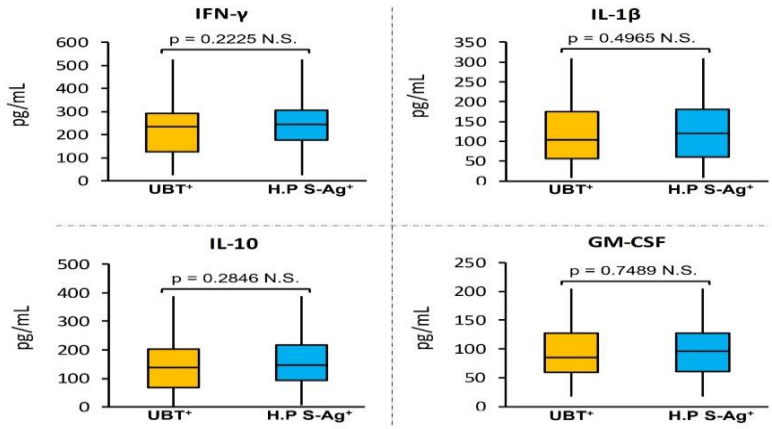

Figure 2: Comparison of serum cytokine levels between subject's positive for the UBT and H.P S-Ag

Box plots show the concentrations of IFN-γ, IL-1β, IL-10, and GM-CSF measured in pg/mL for each group. No significant differences were observed between the two groups for any cytokine, as indicated by non-significant p-values: IFN-γ (p = 0.2225 N.S.), IL-1β (p = 0.4965), IL-10 (p = 0.2846), and GM-CSF (p = 0.7489). Data are presented as median with interquartile ranges, highlighting comparable cytokine levels between UBT+ and H.P S-Ag+ subjects. N.S. (not significant).

Performance of UBT and H.P S-Ag tests in the diagnosis of chronic gastritis

The performance of the UBT and H.P S-Ag was evaluated to assess their diagnostic efficacy for chronic gastritis using their clinical diagnosis (combination of histological observation and endoscopy). The results, detailed in Table 4, highlight key metrics including sensitivity, specificity, PPV, NPV, and overall accuracy for each testing method. The UBT exhibited a high sensitivity of 99.20%, while the H.P S-Ag test had a lower sensitivity of 88.00%. In terms of specificity, the H.P S-Ag test outperformed the UBT, achieving a specificity of 93.33% compared to 72.00% for the UBT. The PPV was higher for the H.P S-Ag test at 95.65%, compared to 85.52% for the UBT. Conversely, the NPV for the UBT was notably higher at 98.18%, while the H.P S-Ag test had an NPV of 82.35%. Overall, the accuracy rates of both tests were similar, with the UBT at 89% and the H.P S-Ag test at 90%.

Table 4: Performance of UBT and H.P S-Ag test in the diagnosis of chronic gastritis

	Method of Chronic Gastritis Diagnosis	
	UBT	**H.P S-Ag**
Sensitivity %	99.20	88.00
Specificity %	72.00	93.33
PPV %	85.52	95.65
NPV %	98.18	82.35
Accuracy %	89	90

UBT; urea breath test, H.P S-Ag; *Helicobacter pylori* antigen in stool, PPV; positive predictive value, NPV; negative predictive value

Association between age, BMI, cytokine levels, and UBT DOB as well as H.P S-Ag levels

The results of Spearman's correlation analysis are presented in Table 5. For UBT DOB, the analysis showed a significant positive correlation with BMI (r_s = 0.2077, p = 0.0032). There were also strong positive correlations with cytokine levels: IFN-γ (r_s = 0.5745, p < 0.0001), IL-1β (r_s = 0.3422, p < 0.0001), and IL-10 (r_s = 0.5102, p < 0.0001). Conversely, the correlation between age and UBT DOB was not significant (r_s = 0.0886, p = 0.2124). The GM-CSF also showed a weaker correlation (r_s = 0.1814, p = 0.0101) compared to the other cytokines. Regarding H.P S-Ag levels, a significant positive correlation was observed with BMI (r_s = 0.1623, p = 0.0216) and the cytokines IFN-γ (r_s = 0.5408, p < 0.0001), IL-1β (r_s = 0.2467, p = 0.0004), and IL-10 (r_s = 0.4575, p < 0.0001). However, age (r_s = 0.1018, p = 0.1514) and GM-CSF (r_s = 0.0797, p = 0.2619) did not demonstrate significant correlations with H.P S-Ag levels.

Table 5: Spearman's correlation (rho) analysis showing the association between age, BMI, cytokine levels, and UBT DOB as well as H.P S-Ag levels

	UBT DOB		H.P S-Ag	
Parameter	**r_s**	**p (2 tailed)**	**r_s**	**p (2 tailed)**
Age	0.0886	0.2124	0.1018	0.1514

BMI	0.2077	0.0032*	0.1623	0.0216*
IFN-γ	0.5745	< 0.0001*	0.5408	< 0.0001*
IL-1β	0.3422	< 0.0001*	0.2467	0.0004*
IL-10	0.5102	< 0.0001*	0.4575	< 0.0001*
GM-CSF	0.1814	0.0101*	0.0797	0.2619

UBT DOB; urea breath test delta over baseline, H.P S-Ag; *Helicobacter pylori* antigen in stool, r_s; Spearman's rank correlation coefficient, BMI; body mass index, IFN-γ; interferon-gamma, IL-1β; interleukin 1 beta, GM-CSF; granulocyte macrophage colony stimulating factor, SD; standard deviation, *Statistical significance at $p < 0.05$

Discussion

The present study first explored the demographic and clinical characteristics of patients with chronic gastritis in comparison with the control group. Since the patients and the control were age-matched, the statistical similarity observed between the study groups was expected. However, our findings revealed noteworthy distinctions between the study groups, particularly concerning BMI and smoking status, which may have implications for understanding chronic gastritis. Significantly, the chronic gastritis group exhibited a higher mean BMI compared to the control group, indicating a strong association between obesity and chronic gastritis, which is consistent with the reports of many studies that suggests obesity may contribute to gastric inflammation and the development of gastritis through mechanisms such as increased gastric acid secretion and altered gut microbiota [12, 13]. The relationship between BMI and chronic gastritis underscores the importance of considering weight management as potential therapeutic strategy in managing the condition. The analysis of smoking status revealed a significant disparity, with 30.4% of chronic gastritis patients reporting current smoking compared to only 12.0% in the control group. Smoking has been well-documented as a risk factor for various gastrointestinal disorders, including gastritis, due to its effect on gastric mucosal integrity and inflammatory responses [14]. The high prevalence of smoking among chronic gastritis patients as observed in this study, suggest that smoking cessation programs may be beneficial in the management of the disease. Symptoms associated with chronic gastritis were prevalent among the patients, with abdominal pain, fatigue and weight loss being the most commonly reported. These symptoms are consistent with the clinical presentation of chronic gastritis and highlights the significant impact of the disease in quality of life [15].

We also investigated the prevalence of *H. pylori* infection and associated serum cytokine levels in patients with chronic gastritis compare to the control group. Our findings revealed significant differences in both *H. pylori* detection and cytokine levels, contributing to the understanding of the underlying inflammatory processes in chronic gastritis. The UBT DOB values was significantly elevated in the patients compared to the control, which highlights the high sensitivity of UBT and corroborates previous studies that have shown that a significant proportion of chronic gastritis cases are associated with active *H. pylori* infection [16-18]. Furthermore, the H.P S-Ag test demonstrated similar results, with significantly higher levels in the patients relative to controls. The congruence between both testing methods reinforces the reliability of *H. Pylori* screening in chronic gastritis diagnosis and highlights the role of the bacterium as a primary etiological factor in this condition [2]. In addition to confirming *H. pylori* infection, significant differences were observed in cytokine levels. The patients exhibited elevated levels of IFN-γ compared to the controls. The increased IFN-γ levels suggest a heightened Th1 immune response, which is typically associated with chronic inflammatory conditions and may play a critical role in the immunopathology of gastritis [19]. The presence of IFN-γ in response to *H. pylori* infection is essential, as it can enhance the inflammatory response and contribute to gastric mucosal damage [20]. Similarly, we observed that IL-1β, IL-

10 and GM-CSF levels were significantly elevated among chronic gastritis patients. IL-1β is known to be a pro-inflammatory cytokine that plays a crucial role in the pathogenesis of gastritis by inducing mucosal injury and promoting further inflammation [9]. The significant increase in IL-1β levels emphasizes its involvement in the inflammatory response triggered by *H. pylori* infection. Interestingly, IL-10 is primarily known for its anti-inflammatory properties and its elevation in the context of chronic inflammation may suggest that it is a compensatory response aimed at moderating the excessive pro-inflammatory activity induced by *H. pylori* [21]. The balance between pro-inflammatory and anti-inflammatory cytokines is crucial in determining the outcome of chronic gastritis. The GM-CSF however, is involved in the differentiation and activation of myeloid lineage cells and has been implicated in sustaining inflammatory processes [22]. The increase in GM-CSF levels adds another layer of complexity to the inflammatory processes at play in chronic gastritis.

Investigating the serum cytokines profiles in the subjects who tested positive for UBT in comparison to those who tested positive for H.P S-Ag revealed that the were no statistically significant differences between these two groups, suggesting a shared immunological response to *H. pylori* infection regardless of the detection method. Moreso, our results suggest that chronic gastritis does not always present with differences in cytokine levels based on diagnostic method employed of *H. pylori* detection. Khaiboullina et al. (2016) reported similar findings where cytokine profiles remained largely unchanged in chronic gastritis patients regardless of their *H. pylori* diagnostic status [23]. This strengthens the usefulness of measuring and monitoring systemic cytokine levels in the diagnosis as well as management of chronic gastritis. Nevertheless, additional studies to elucidate the intricate interplay of various cytokines over the course of this chronic infection is necessary.

The diagnostic evaluation of the UBT and H.P S-Ag for chronic gastritis reveals important insights into their clinical utility. Here, we observed that while the UBT demonstrates exceptional sensitivity (99.20%), the H.P S-Ag test shows superior specificity (93.33%). High sensitivity in the UBT indicates its effectiveness in correctly identifying patients with *H. pylori* infection, which is critical for early diagnosis and treatment of chronic gastritis. This is consistent with previous studies that recognizes UBT as a reliable non-invasive method of *H. pylori* detection, reflecting the physiological response of the gut to bacterial colonization [24]. However, while the sensitivity of UBT is outstanding, its relatively low specificity (72.00%) suggests a potential for false positive results, particularly in populations with low prevalence of *H. pylori* infection. This might lead to unnecessary treatments, emphasizing the importance of comprehensive diagnostic approach that integrates clinical judgment and additional testing. Conversely, the higher specificity of H.P S-Ag test signifies its potential to accurately confirm the absence of infection, thus reducing the risk of misdiagnosis. A specificity of 93.33% indicates that the test is robust in identifying true negatives, which is particularly valuable in clinical settings, where ruling out *H. pylori* infection can guide management decisions [25]. The higher PPV for H.P S-Ag test reinforces its reliability when the result is positive. Moreover, the notable difference in NPV favours the UBT, indicating that the patients who test negative using the UBT can be confidently ruled out for active infection. This is crucial in managing chronic gastritis, as it may prevent unnecessary endoscopies or invasive procedures in patients who are most likely free of *H. pylori* infection, and a combination of diagnostic strategies may optimize patient outcome [26].

We also assessed the relationships between various BMI, cytokine levels, age, and their correlation with UBT DOB values and H.P S-Ag levels. Notably, we observed a significant positive correlation with UBT DOB and BMI, suggesting that increased body weight may be associated with higher rates of *H. pylori* infection. This finding is consistent with reports by Baradaran et al. (2021) where obesity was found to be a significant risk factor for various gastrointestinal infections including chronic gastritis [27]. The underlying mechanisms may

involve alterations in the gut microbiota and immune response obese individuals, leading to a more favourable environment for *H. pylori* colonization. We also observed strong positive correlations between UBT DOB and IFN-γ, IL-1β, and IL-10 levels. These results suggest an active immune response associated with *H. pylori* infection, where proinflammatory cytokines like IFN-γ and IL-1β are upregulated in response to the pathogen, as they play crucial roles in mediating immune response and inflammation [5]. The correlation IL-10, an anti-inflammatory cytokine, indicates a complex regulatory mechanism where the body attempts to balance the inflammatory response induced by *H. pylori* while limiting tissue damage. GM-CSF however, showed a weaker correlation with UBT DOB, which may indicate that the role of GM-CSF in the immune response to *H. pylori* is less pronounced compared to the other cytokines assessed. While most studies have identified GM-CSF as a proinflammatory cytokine that stimulates the activation and migration of myeloid cells to the site of inflammation [28, 29], recent studies have reported that the cytokine is indirectly involved in the induction of immunological tolerance and anti-inflammatory response [30, 31]. Regarding H.P S-Ag levels, we found a significant positive correlation with BMI, IFN-γ, IL-1β, and IL-10. This strengthens our findings on the relationship between obesity, immune activation, and *H. pylori* presence. Like UBT, H.P S-Ag levels reflect an underlying inflammatory state and immune response against *H. pylori* infection, thereby affirming the connections established in the UBT group. The absence of significant correlations between GM-CSF and H.P S-Ag levels reiterates the complexities of the immune response in chronic gastritis and how different cytokines may not uniformly influence *H. pylori* diagnosis.

Conclusion

In conclusion, this study highlights significant associations between chronic gastritis and lifestyle factors such as obesity and smoking, emphasizing their role in the pathophysiology of the disease. The elevated cytokine levels (IFN-γ, IL-1β, IL-10, and GM-CSF) in patients indicate a complex immune response related to *H. pylori* infection, underlining the impact of systemic inflammation and metabolic factors. Utilizing both the UBT for sensitivity and the H.P S-Ag for specificity can improve diagnostic accuracy and management of chronic gastritis. Future research should focus on longitudinal analyses of cytokine levels in larger cohorts and investigate the molecular mechanisms behind these associations, while exploring how lifestyle and dietary factors influence *H. pylori* infection and immune responses to develop effective management strategies for those affected by chronic gastritis.

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