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Modulation of Interleukin-35 In the Immune Response to H. Pylori Infection

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ABSTRACT

Background: Infection with Helicobacter pylori is a common disease of the digestive system, normally associated with several degrees of gastritis as well as chronic inflammation. Interleukin-35 (IL-35) is an emerging anti-inflammatory cytokine that mediates regulatory immunity; however, little research has been directed toward its participation in the pathogenesis of H. pylori. **Objectives**: To assess serum IL-35 levels in patients infected with H. pylori and correlate them with the severity and duration of the disease. **Methods**: This cross-sectional study was done on 80 patients who were clinically suspected to be infected with H. pylori (44 males and 36 females; age range 15–63 years) attending Al-Najaf General Hospital, Iraq, from February 2024 to March 2025. Stool antigen tests confirmed the presence of H. pylori infection. Serum IL-35 concentrations were measured by a sandwich ELISA method. Classification of participants was done according to the status of infection (positive vs. negative), severity of gastritis (mild, moderate, severe), and chronicity of the infection (acute vs. chronic). **Results**: Out of 80 participants, 58 (72.5%) were positive for H. pylori. The levels of IL-35 were significantly decreased in H. pylori-positive individuals (48.37 ± 22.33 pg/ml) as compared to negatives (58.54 ± 11.45 pg/ml, p =0 .0456). Among the infected patients, the levels of IL-35 varied significantly with the severity of gastritis; mild (35.7 ± 8.09), moderate (51.3 ± 11.11), severe (60 .2 ±17 .22) (F=21 .5, p<0 .0001). The concentrations of IL-35 were also significantly higher in chronic cases (62 .54±12 .32 pg/ml) than acute cases (34 .22±8 .54 pg/ml, p=0.000). **Conclusions**: IL-35 is low in early H. pylori infection and seems to go up with more gastritis severity and duration, hinting a modulatory role in the host immune response. These findings support potential involvement of IL-35 in immunopathogenesis and progression of H. pylori related gastric inflammation.

KEYWORDS
Interleukin-35, H. pylori, severity, immune response

INTRODUCTION

Helicobacter pylori is bacterium responds negatively to Gram staining that lives in the gastric epithelium and causes several gastric diseases, such as chronic gastritis, peptic ulcers, and gastric cancer. The epidemiology of H. pylori infection shows very important variations in its prevalence throughout the world (Conteduca et al., 2013). A systematic review done by Hooi et al (2017) showed that places like Africa have higher infection rates which correspond to more disease burden and chronic inflammation. This long-standing inflammation exerts a critical role in the pathophysiology of diseases related to Helicobacter pylori; it enhances immune responses as well as disease progression. Zamani et al (2018) also emphasized that understanding these patterns of prevalence should direct focused immunological studies as well as public health strategies for awareness about H. pylori eradication in those areas where it is most prevalent.

Host immune system interaction with H. pylori has also been an approach toward explaining gastric disease pathophysiology. Malfertheiner et al., (2023) indicated that H. pylori chronically inflames and changes the immunity of the gastric mucosa, hence predisposing to gastric cancer by keeping other conditions in the stomach. In principle, good elimination of H. pylori leads to a considerable decrease in gastric cancer occurrence; this process requires more profound studies on immune mechanisms responsible for such effects when carried out. In reviewing molecular aspects related to carcinogenesis within the stomach resulting from Helicobacter pylori infection, investigation set owners found that microRNAs participate in regulating the immune response and steering inflammation within the gastric mucosa environment, miR-449 among them (Malfertheiner et al., 2023).

Interleukin-35 (IL-35) is a new interleukin of the anti-inflammatory cytokine family belonging to the IL-12 family, which is famed for its effects on immune regulation and tolerance. The anti-inflammatory impacts of IL-35 are more likely to come through (Menam et al., 2024). It has been demonstrated that it induces regulatory T cell populations necessary for the preservation of immune homeostasis (Collison et al.,2010). These T cells generate IL-35 in response to an inflammatory signal which in turn suppresses pro-inflammatory cytokines, therefore reducing tissue damage under chronic inflammatory conditions (Mills, 2022). The other, too, carries weight as it shows that IL-35 counters the signal from pro-inflammatory cytokines like that driven by IL-17. IL-35 does not only regulate T cells but also regulates Bregs which use IL-35 to regulate immunity and induce tolerance. This dual function of acting on T and B cell populations gives IL-35 a very broad effect on inflammation (Li et al.,2012).

Recent studies bring out the need for understanding the unique pathways through which IL-35 exerts its effects. For example, while IL-10 is a well-described anti-inflammatory cytokine, IL-35 has far different regulatory mechanisms which may be brought into play most critically in specific pathological contexts (Saraiva et al., 2019). Knowing these differences could upgrade therapeutic strategies for targeting inflammatory diseases. High levels of interleukin-35 could be signaling not only that there is an infection presently going on but also to what degree the inflammatory response is intense (Hadi et al., 2022).

This study concerns with the assessment of Interleukin-35 serum levels of (IL-35) in patients with H. pylori infections and its link to progression of the disease.

Patients and Methods

Study Design

This cross-sectional study enrolled 80 subjects (44 males and 36 females) within the age range of 15-63 years, in whom there was a clinical suspicion of H. pylori infection. It was carried out at Al-Najaf General Hospital, Iraq, between February 2024 and March 2025. Patients were selected from the OPD on the basis of clinical symptoms; they presented with complaints regarding abdominal pain, bloating, nausea, and various distressing manifestations related to the GIT and were suspected to have H. pylori infection.

Inclusion and Exclusion

Patients of any sex who were clinically suspected to have H. pylori infection within the stipulated age bracket and who had not been treated with antibiotics for at least four weeks prior to testing shall constitute inclusion criteria. Exclusion criteria comprised patients who had previously undergone peptic ulcer surgery, patients on immunosuppressive treatment, and those diagnosed with chronic inflammatory diseases like inflammatory bowel disease, autoimmune diseases, or malignancies. The other two exclusion categories were also applied to

patients who had received proton pump inhibitors and NSAIDs within two weeks preceding the study since these drugs may alter the accuracy of the tests and levels of immune markers.

Each patient was tested with stool antigen assay to identify H. pylori infection. This is an active infection; a non-invasive test and reliable for detecting the specific H. pylori antigens in feces. Results showed that 58 of 80 individuals tested positive for H. pylori (72.5%) while 22(27.5%) were negative; hence, they combined this latter group as a control to compare them against.

Sample Collection and Measurements

About 3 ml of venous blood was drawn aseptically from both H. pylori positive and negative participants into plain tubes having a clot activator. After collection, the samples were allowed to clot at room temperature; then the samples were subjected to centrifugation at 3000 rpm for 10 minutes to separate the serum. The resultant serum samples were aliquoted and kept at –20°C until analysis.

This is a sandwich ELISA intended for the quantitative measurement of IL-35 in human serum; it is to be used for research only. The detection 15.6 -1000 pg/mL sensitivity ~9.4 pg/mL in serum, and current sample usage is 100 µL with an assay time of about 3.5 hours has set this kit apart because it delivers results with precision as high as <10% intra- and inter-assay CV. Reported reference values for healthy individuals fall approximately between 0–55 pg/mL, based on sample type. Proper storage maintains stability; handling in any other way will compromise safe storage. Results are obtained by measuring absorbance at 450 nm Standard Curve generated from the Standard Sets supplied with this kit.

Ethical Considerations

The hospital admin and the bioethics committee gave approval for this study. Before starting, we got written consent from all participants. The study protocol followed ethical standards and conducted under the guidance of internal medicine and gastroenterology experts at Al-Najaf General Hospital; also ensured complete confidentiality & privacy of all participants during the study period.

Statistical analysis

Analysis of data was done with the use of the Statistical Package for the Social Sciences (SPSS), version 26. Student' t test was utilized to hypothesize the difference of IL-35 between two groups. To compare differences in IL-35 concentrations in patients with different severities of H. pylori ANOVA was applied. Least significant difference (LSD) was employed to find variations in multiple comparisons among groups with unequal frequencies (Al-Fahham, 2018).

RESULTS

Table 1 explains the distribution of H. pylori-positive cases (n = 58) according to the severity and duration of gastritis; a significant number of patients presented mild gastritis at 44.83%, followed by moderate at 37.93% and finally, severe at 17.24%. In most cases, the infection was not associated with advanced mucosal damage. Concerning the duration of infection, a major percentage was acute H. pylori infection at 72.41%; only 27.59% were chronic cases. This data indicates that in this study population, H. pylori-infected patients more commonly present early-stage, mild-to-moderate forms of gastritis.

Table 1. Severity and duration of H. pylori infection

Indicators		`Positive (No. = 58)	
		Freq.	%
Severity of gastritis	Mild	26	44.83
	Moderate	22	37.93
	Severe	10	17.24
Duration of the infection	Acute	42	72.41
	Chronic	16	27.59

Comparison of serum IL-35 levels between H. pylori-antigen positive and H. pylori-antigen negative participants showed statistical significance. More specifically, patients infected with H. pylori had lower concentrations of IL-35 (48.37 ± 22.33 pg/ml) than the negative group (58.54 ± 11.45 pg/ml). The independent t-test gave a t-value of 2.03 at a corresponding p-value of 0.0456; hence, it is statistically significant that infected individuals have reduced levels of IL-35 ($p < 0.05$). This may indicate the immunomodulatory role potential of IL-35 in the pathogenesis of H.pylori and its related infection inflammation response (figure 1).

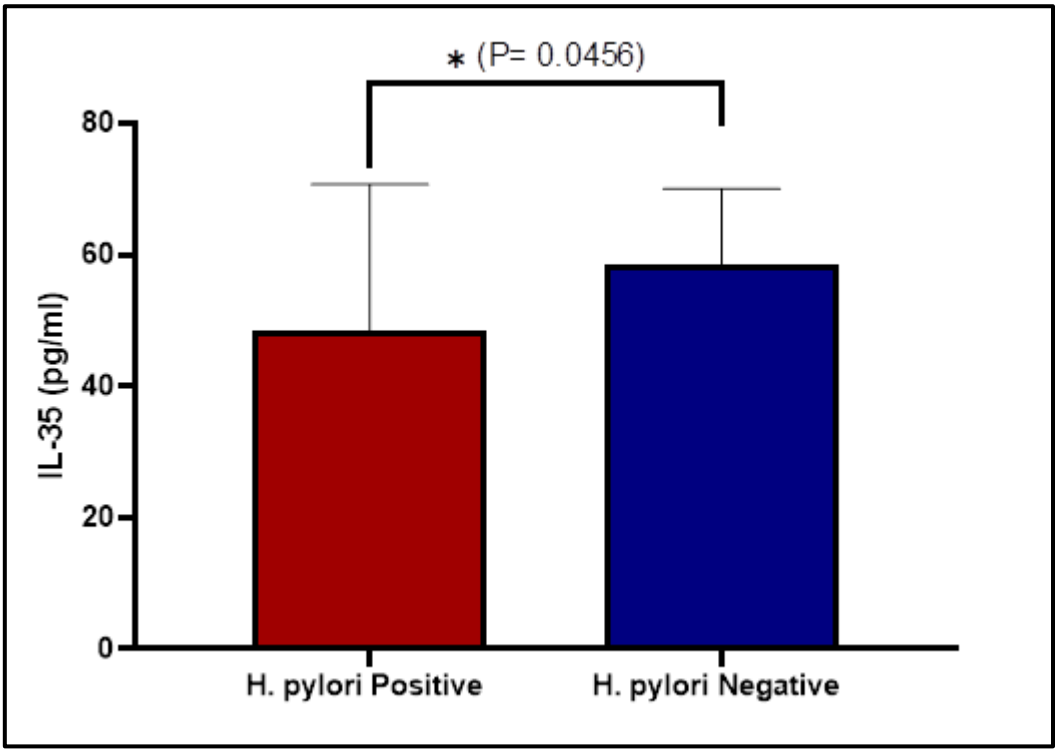

Figure1. Comparison of IL-35 levels between positive and negative H pylori tests

Table 3 shows a very big difference in the concentrations of IL-35 in H. pylori-positive patients when the severity of their gastritis is taken into account. The patients who had mild gastritis also had the lowest concentrations of IL-35 (35.7 ± 8.09 pg/ml), while those with moderate and severe gastritis had higher levels, progressively higher (51.3 ± 11.11 pg/ml and 60.2 ± 17.22 pg/ml, respectively). Different letters (A,B,C) used to denote group means indicate statistically significant variations among the subgroups at $p < 0.05$ level of significance, an F-test result of 21.5 and a highly significant p-value ($p < 0.000$). In other words, it can be said that IL-35 levels increase with severity of infection; therefore, it may play a modulatory role in the strength of immune responses in more advanced cases of gastritis (Table3).

Table 3. Comparison of IL-35 levels in patients' groups according to disease severity

Groups	**Freq.**	**IL-35 (pg/ml) Mean ± S.D**	**F test**	**T test P-value**
Mild	26	A 35.7 ± 8.09	**21.5**	**<0.000 (HS)**
Moderate	22	B 51.3 ± 11.11		
Severe	10	C 60.2± 17.22		

A, B, C refer to significant difference at p <0.05

It shows a statistically significant elevation in the levels of IL-35 in the chronic group as compared to the

acute group, which may indicate the involvement of IL-35 in the enhancement or control of chronic inflammatory responses. The patients from the chronic group had much higher mean concentrations (62.54 ± 12.32 pg/ml) than those in the acute group (34.22 ± 8.54 pg/ml), with a highly significant p-value (p = 0.000) and t-test value of 9.92; that is, it may prove that IL-35 is upregulated during prolonged immune activation and perhaps acts in immune modulation or resolution mechanisms during chronic infectious conditions (table 4).

Table 4. Differences in IL-35 levels in patients' groups according to duration of infections

Groups	Freq.	IL-35 (pg/ml) Mean ± SD	T test P-value
Acute	42	34.22 ± 8.54	**9.92** **(0.000)** **HS**
Chronic	16	62.54 ± 12.32	

DISCUSSION

Results from the current study have suggested that early H. pylori infection might suppress IL-35 production, so that it has been significantly decreased in H. pylori positive participants in comparison to those with negative results. Helicobacter pylori is a bacterium responds negatively to Gram staining, it infects the mucosa of the stomach and causes various diseases associated with the upper intestinal canal, such as peptic ulcer disease gastritis and, carcinoma, etc. Prevalence of H. pylori infection in the world is quite high still robust evidence approximately 50% of the world population is infected systematic reviews indicated it (Hooi et al., 2017; Zamani et al., 2018). The host immune response against H. pylori is involved in several cytokines and immune cells, particularly it raises the level of the regulatory T cell and associated cytokines, which includes IL-35. Previously published articled revealed that in early Helicobacter pylori infections the levels of interleukin-35 (IL-35) are mostly found to be decreased indicating that the initial anti-inflammatory response was not adequate. IL-35 is an immunosuppressive cytokine product primarily secreted by regulatory T cells; lowering its production during the initial stages of H. pylori infection can result in sustained local inflammation within the gastric mucosa (Bassagh et al., 2018). Thus, it paves the way for larger pro-inflammatory cytokines like IL-6, IL-1β and TNF-α to dominate and further promote neutrophil as well as macrophage infiltration that again enhances tissue damage. This suppressed response of IL-35 may also signify a strategy by which pathogens escape immune regulation thus favoring bacterial colonization and chronicity. Therefore, after prolonged infection, these very same IL-35s may rise more as counter-regulatory molecules to excessive inflammation and tissue injury (Ding et al., 2012).

IL-35, a member of the IL-12 cytokine family, has emerged as a crucial factor in the immunoregulation of T cells during H. pylori infection. Its production by Tregs is associated with enhanced immune evasion capabilities of H. pylori, as IL-35 can suppress pro-inflammatory responses that are necessary for the effective clearance of the bacterium (Kao et al., 2010; Zamani et al., 2018). By inhibiting Th17 responses, IL-35 may contribute to maintaining a state of chronic infection, further complicating treatment strategies. Furthermore, the interplay between IL-35 and other cytokines, such as IL-21, also exerts a crucial role in shaping the immune response. IL-21 is essential for sustaining pro-inflammatory T cell actes during H. pylori infection, and its modulation by IL-35 could offer insights into therapeutic strategies (Liebregts et al., 2011). Understanding how these cytokines interact could illuminate pathways for enhancing immune responses against H. pylori and improving patient outcomes.

CONCLUSION

IL-35 is low in early H. pylori infection and seems to go up with more gastritis severity and chronicity, hinting a modulatory role in the host immune response. These findings support potential involvement of IL-35 in immunopathogenesis and progression of H. pylori related gastric inflammation.

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