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ORIGINAL ARTICLE

Elevation of IL-32 and TNF-α and *23S rRNA* Gene Mutations in *Helicobacter pylori* Infected Iraqi Patients

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ABSTRACT

Helicobacter pylori infection is a common bacterial infection of the stomach that often causes no symptoms, but can lead to gastritis, peptic ulcers, and in some cases, stomach cancer. In this study, (50) blood specimens were taken from patients who had *H. pylori* infection, and from (40) healthy individuals as a control groups in Baghdad teaching Hospital during the period from January to October 2025. The results of the current study showed that the mean level of *H. pylori* IgM antibodies was significantly higher in patients (2.14±0.17) compared to controls (0.10±0.03), and similarly, the mean IgG level was significantly higher in patients (14.31±0.94) compared to controls (0.11±0.03) (P≤0.01). Moreover, the mean level of IL-32 among the *H. pylori* infected patients was (27.06±2.23) compared to the healthy persons (1.25±0.32), also, the mean level of TNF-α among patients was (18.06±0.79) compared to the healthy persons (1.76±0.39), with highly significant differences (P≤0.01) respectively. In the *23S rRNA* gene, the A2143G locus mutated from GG to GA and AA, while the A2142G locus mutated from AA to AG and GG.

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1- INTRODUCTION

Helicobacter pylori infection is a common bacterial infection of the stomach that often causes no symptoms, but can lead to gastritis, peptic ulcers, and in some cases, stomach cancer [1]. This bacterium is thought to spread via water or contaminated foods, or through contact with infected persons' feces or saliva. The infection is treated by an antibiotic and acid-reducing drug combination [2]. The transmission of *H. pylori* occurs mainly via mouth to mouth contacts or contacts with stools or vomits of infected people, most usually during childhood [3]. The risk of infection is elevated by factors such as living in unsanitary or crowded conditions, as well as the lack of clean water. as do other factors such as absence of clean water supplement [4]. The main cause of this bacterial resistance to clarithromycin antibiotic is the mutation in its *23S rRNA* genes, especially at the A2142G and A2143G positions [5]. The binding sites of clarithromycin on the ribosomes are altered by such mutation, which will result in its prevention from protein synthesis inhibition and failure of treatment [6]. The DNA of *23S rRNA* gene can be used to identify *H. pylori* infections, and this gene's sequencing is essential to detect antibiotic resistance, especially to clarithromycin [7]. There is a strong linkage to resistance between the specific point mutation, most frequently A2142G and A2143G, within domain V of this gene, resulting in guidance of more active treatment plans. Using specimens such as gastric juice or stool samples allows for non-invasive genetic analysis [8]. In the gastric cells, infection with *H. pylori* results in highly pro-inflammatory cytokine interleukin-32 (IL-32) expression,

which is associated with inflammations and it depends on the virulence factors of *H. pylori* e.g. CagA [9]. The inflammatory response of the host is stimulated by activating pathways such as NF-κB, which causes upregulation of IL-32 as well as other cytokines e.g. IL-1β & TNF-α [10]. The increased production of IL-32 is involved in the gastric inflammations related to *H. pylori* and can be as the disease progression marker. The infection with *H. pylori* is highly associated with the increased productions of the inflammatory cytokine, tumor necrosis factor-alpha (TNF-α) [11]. These associations are results of the virulence factors of *H. pylori* such as the TNF-α inducing protein (Tipα) as well as the immune responses of the host to the infections. The resulting inflammation is a key factor in the development of *H. pylori*-associated diseases, including gastritis and gastric cancer [12].

2- MATERIALS AND METHODS

In this study, 50 blood specimens were collected from patients diagnosed with *H. pylori* infection and 40 blood specimens from healthy individuals as a control group at Baghdad Teaching Hospital during the period from January to October 2025. *H. pylori* IgM, IgG, IL-32, and TNF-α levels were measured using the sandwich ELISA technique. The rRNA 23s of *H. pylori* strain were identified by convictional PCR and sequenced by Sanger sequencer and the primer used was:

rRNA 23s –F: 5′-GATGACTTGTGGATAGGG-3′
rRNA 23s –R: 5′- CTGTTCGGTTGGGACGGTA-3′

PCR Component Calculation

No. of Reaction	**20**	**rxn**	**Annealing temperature of primers**	**55,60**
Reaction Volume /run	25	µl	Length of PCR product (bp)	634

Ethical approval

Before beginning this study, all participants provided written consent. Medical ethics approval certification, ethics committee approved the study on number 145/ 515 on March 12, 2025.

Statistical analysis

Using "± SE" is a common notation where the standard error (SE) is used to show the variability or precision of a sample statistic, most often the mean. The notation "\(mean\pm SE\)" indicates the sample average and the range around it, representing the likely range of the true population mean and helping to construct confidence intervals.

3- RESULTS AND DISCUSSION

The results of the current study showed that infection rate among patients within the age group (10-24) years was 6(12%) compared to the control group 6(15%), and within the age group (25-39) years was 13(26%) compared to the healthy control 14(35%), and within the age group In (40-54) years, the infection rate was 18(36%) in comparison to the healthy individuals 12(30%), while within the age group (>54) years was 13(26%) compared to the control group 8(20%), with non-significant differences (P=0.72), as shown in table (1).

Table (1): Distribution of studied groups according to age (years)

Age range (years)	**Case (n=50)**		**Control (n=40)**		**P-value**
	N	%	N	%	
(10-24)	6	12	6	15	0.72
(25-39)	13	26	14	35	
(40-54)	18	36	12	30	
>54	13	26	8	20	
Total	50	100	40	100	

These findings agree with those of Teng et al. (2024), who reported that the prevalence of *H. pylori* in various age groups was as follows: under 16 years, 62%; 16 to 25 years, 67%; 26-35 years, 69%; 36-45 years, 67%; 46-55 years, 66%; and 56 years and above, 65% . Statistical analysis revealed no significant difference between the groups [13].

Also, Mohammed (2025) stated that it has been proven that there are a large number of infections among children under 16 years of age [14].

The prevalence of *H pylori* infections according to residence was equal in the rural area 25 (50%) compared to the controls 19(47.5%) and the urban area 25 (50%) compared to the controls 21(52.5%) with no significant differences (P=0.81) as shown in table (2).

Table (2): Distribution of studied groups according to residency

	Case (n=50)		Control (n=40)		P-value
Residency	**N**	**%**	**N**	**%**	
Rural	25	50	19	47.5	0.81
Urban	25	50	21	52.5	
Total	50	100	50	100	

These results agreed with (Naqid *et al.,* 2024) who reviewed that the number of infections was similar between rural 49 (40.2%) and urban 55 (40.1%) areas, with no significant differences [15]. On the other hand, Majeed *et al.,(*2020) reported that the high seropositivity 58(60.4%) was observed in rural areas, while the low seropositivity 70(48.6%) was recorded in urban areas, with a non-significant difference between the two groups [16].

Table (3) showed no significant variations in the prevalence of *H. pylori* infections according to sex, as the prevalence rate of males was 26(52%) compared to the controls 21(52.5%) and females was 24(48%) compared to the controls 19(47.5%) with no significant variation (P=0.96).

Table (3): Distribution of studied groups according to gender

	Case (n=50)		Control (n=40)		P-value
Sex	**N**	**%**	**N**	**%**	
Male	26	52	21	52.5	0.96
Female	24	48	19	47.5	
Total	50	100	50	100	

Wu *et al.,* (2022) revealed that the infection rate among men was 59%, compared to 41% among women, which differed from the findings of the current study, possibly due to the sample size used in the test [17]. The current study aligns with Yuqin et al. (2024), who demonstrated that the infection rate was 49.59%, including 49.74% in males and 49.3% in females [18].

Table (4) illustrated that the mean level of *H. pylori* IgM antibodies was (2.14±0.17) compared to the controls (0.10±0.03), with a highly significant difference, and the mean level of *H. pylori* IgG antibodies was (14.31±0.94) compared to the controls (0.11±0.03), with a highly significant difference. Moreover, the mean level of IL-32 among the *H. pylori* infected patients was (27.06±2.23) compared to the healthy persons (1.25±0.32), also, the mean level of TNF-α among patients was (18.06±0.79) compared to the healthy persons (1.76±0.39), with highly significant differences (P≤0.01) respectively.

Table (4): Distribution of mean levels of studied parameters among cases and control

Test	Groups	Mean	SE	t-test	P-value
***H. pylori* IgM**	Case	2.14	0.17	11.42	≤0.01
	Control	0.10	0.03		
***H. pylori* IgG**	Case	14.31	0.94	15.09	≤0.01
	Control	0.11	0.03		
IL-32	Case	27.06	2.23	51.10	≤0.01
	Control	1.25	0.32		
TNF-α	Case	18.12	0.79	70.69	≤0.01
	Control	1.76	0.39		

SE: Standard Error of the Mean

Zaman *et al.,* (2020) revealed that were positive for serum IgM and 5 (13.88%) were positive for HpSA [19]. The difference in HpSA positivity before and after eradication therapy was statistically significant ($P < 0.05$). Also, the results in the current study agreed with (Qasim and Aboud, 2022) who found that the level of IgG Ab revealed highly significant difference in positive group (172.94 ±13.14 U/ml) compared with negative group (56.67 ±12.26) and asymptomatic group (114.05 ±20.95 U/ml) [20]. In addition, (Owaid *et al*. (2024) reported that the concentration of IL-32 increased significantly in the patients compared with the control group with ($p<0.001$) [9]. The results of the present study demonstrated a significant elevation in IL-32 levels in patients with *Helicobacter pylori* infection (27.06 ± 2.23) compared to the healthy individuals (1.25 ± 0.32). This marked increase suggests that IL-32 plays an important role in the inflammatory response associated with *H. pylori* infection. IL-32 is known as a pro-inflammatory cytokine that induces the production of other inflammatory mediators such as TNF-α, IL-6, and IL-1β, which are strongly linked to gastric mucosal damage and the pathogenesis of *H. pylori*-related diseases [21]. The high level of IL-32 in infected people can reflect its contribution in both adaptive and innate immune response.

During infection with *H. pylori*, there will be activation of infiltrating immune cell and gastric epithelial cell which produce IL-32, which causes further inflammation amplification. This cytokine can be involved in neutrophil and macrophage recruitment, leading to local immune response enhancement, as well as to chronic gastritis and tissue injuries. In addition, former studies demonstrated the expression of IL-32 is correlated with the gastritis severity and can participate in gastric ulcer progression or even gastric cancers. In this study, the highly difference in the level of IL-32 between infected and healthy people supports the suggestion that IL-32 can be used as a possible H. pylori infection marker and associated gastric inflammations.The highly significant levels of IL-32 may be attributed to persistent antigenic stimulations by *H. pylori*, which is known to induce chronic inflammations. Persistent high IL-32 concentrations are also involved in the pro-tumorigenic microenvironment development through sustaining of inflammatory signaling.

Our study demonstrated that the levels of TNF-α is highly significantly increased in *Helicobacter pylori*-infected patients (18.06 ± 0.79) in comparison with the controls (1.76 ± 0.39), with ($P \leq 0.01$) [22]. This noticeable elevation suggests that infection with *H. pylori* causes strong induction of TNF-α production, a main pro-inflammatory cytokine contributed to gastric mucosal inflammations and host immune response. T lymphocytes, gastric epithelial cells and activated macrophages mainly produce TNF-α in response to the virulence factors of *H. pylori* like VacA, CagA and lipopolysaccharide. The high levels of TNF-α cause's promotion of inflammations by stimulation of other cytokine release, enhancement of leukocyte recruitments as well as activation of nuclear factor-kappa B (NF-κB), which is important for expression of inflammatory genes. Such actions are involved in gastric mucosal injuries, epithelial damages as well as chronic gastritis.

Moreover, increased levels of TNF-α was shown to be associated with gastric carcinogenesis and *H. pylori*-induced peptic ulcer. Chronic TNF-α overproductions can be involved in the persistent inflammatory state development, which causes oxidative stress promotion, DNA damages and cellular proliferations, which are factors related to precancerous gastric lesion. In the current study, the significant variation between infected and healthy individuals supports the suggestion that TNF-α has the ability to assist as a potential biomarker for gastric inflammations induced by *H. pylori*. It also shows its potential role as a therapeutic target, which blocks or modulates TNF-α activities can help in reducing gastric inflammations and prevent progressions to more severe gastrointestinal complication [23].

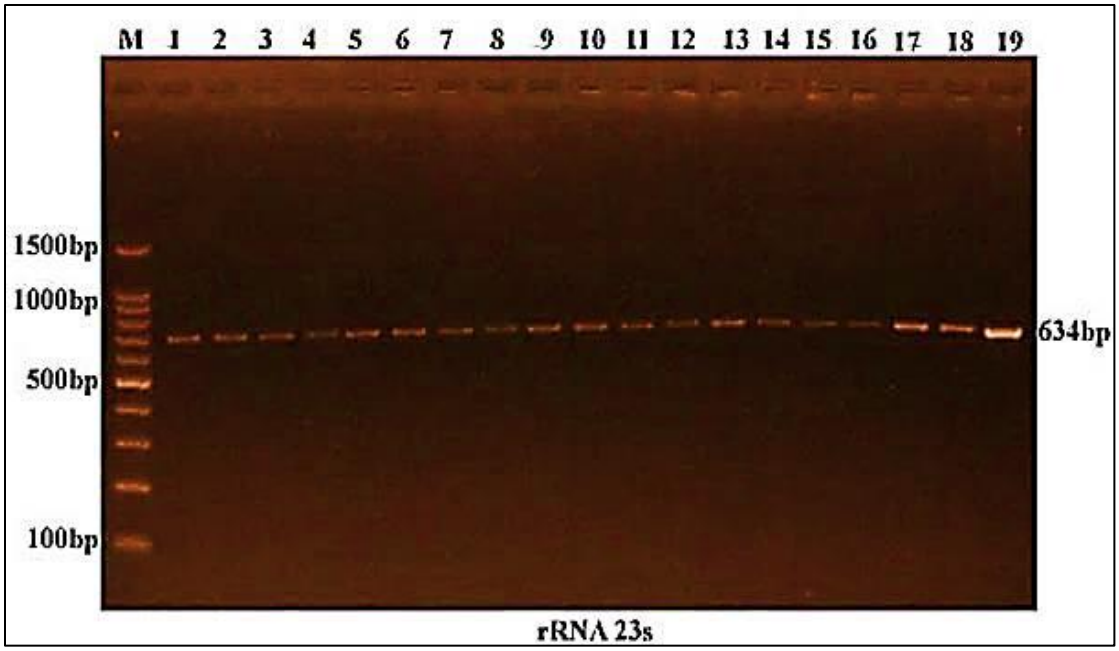

Fig (1): The amplification of *23S rRNA* specific region of human blood samples were fractionated in electrophoresis with ladder marker M:100bp resemble 634bp. Lanes 1-19

In the sequences of 23S rRNA A2142G and A2143G of *H. pylori* strain, the position GG of A2143G gene was changed to GA and AA respectively and the position AA changed to AG and GG respectively as shown in table 5 and figures 2,3.

Table (5): The sequences of 23S rRNA A2142G and A2143G of *H. pylori* strain

SNPs	***23S rRNA* gene A2143G**	***23S rRNA* gene A2142G**
Wild	**GG**	**AA**
Variation	**G>A**	**A>G**
Samples		
1	GA	AG
2	GA	AG
3	GA	AG
4	GG	AG
5	GA	AA
6	GA	AG
7	GA	AG
8	AA	AG
9	GA	AG
10	AA	GG
11	AA	AG
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16	GA	AG
17	GA	GG
18	AA	AG
19	GA	AG

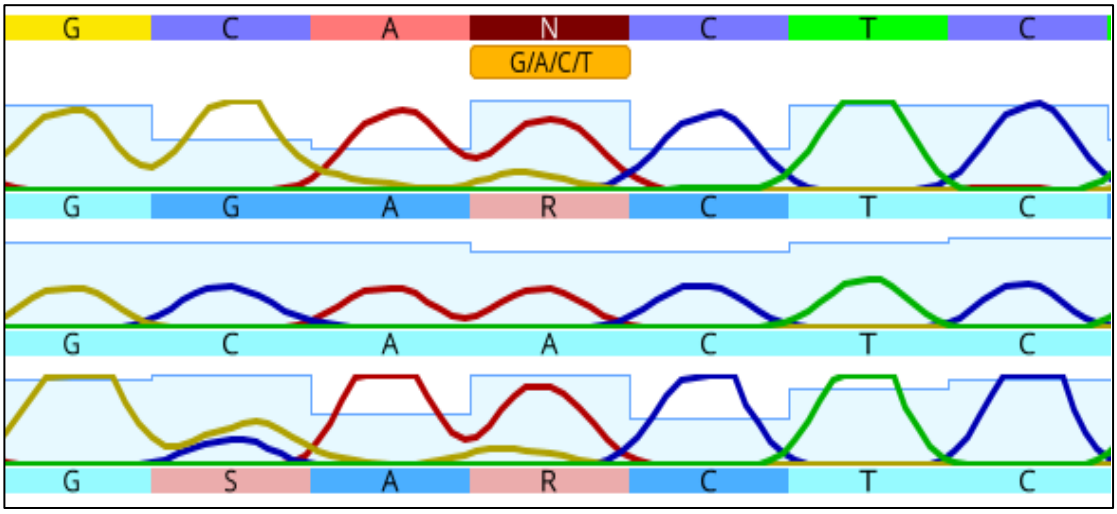

Fig (2): Analysis of rRNA 23s /A2143G gene using Sanger sequencing. Presence of the "G" and "A" peak indicative of G/A heterozygous allele

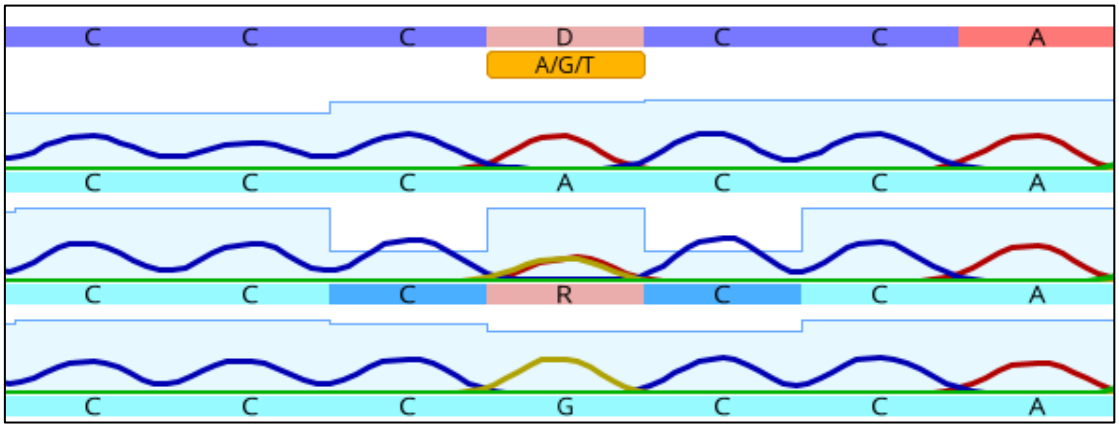

Fig (3): Analysis of rRNA 23s /A2142G gene using Sanger sequencing. Presence of the "A" and "G" peak indicative of A/G heterozygous allele

The mutations in the *23S rRNA* gene (A2142G and A2143G) of *H. pylori* showed that the analysis of the *23S rRNA* gene sequences in *H. pylori* strains revealed nucleotide substitutions at positions A2142 and A2143, which are known key mutation hotspots associated with clarithromycin resistance. In the A2143G mutation, the normal GG genotype was altered to GA and AA, while in the A2142G mutation, the AA genotype changed to AG and GG [24]. These substitutions represent point mutations that lead to purine base transitions (A→G), which are commonly observed in antibiotic resistance mechanisms. These mutations happened in domains V of the *23S rRNA* genes, which is a part of peptidyl transferase regions in the 50S ribosomal subunits, and this region is the macrolide antibiotic binding site, involving clarithromycin. At such positions, the change from adenine (A) to guanine (G) leads to structural changes in ribosomal RNA, lowering the affinity of drug binding and resulting in reduced antibiotic effectiveness [25]. At the position 2143, conversion from GG to GA or AA proposes heterozygous or fully-mutated allele existence, which can result in different resistance levels. There is an association between mutant genotypes (GA and AA) and high and partial resistances, respectively. Likewise, at the position 2142 , alterations from AA to AG or GG suggests progressive mutations, which may be associated with increased levels of resistance. Such genetic difference demonstrates the dynamic nature of resistance development of *H. pylori* and indicates that multiple mutation pattern may coexist within a population, probably because of the selective pressure from exposures to antibiotics. In addition, the existence of mixed genotypes i.e. (GA & AG) may suggest continuing mutation processes or mixed infections with multiple *H. pylori* strains [26].

4- CONCLUSIONS

The results indicated that IL-32 is highly related to infection with *H. pylori* and may be involved in the gastric inflammation pathophysiology. More studies are required to conclude the exact mechanistic roles of IL-32 in the progression of the disease and to find out its capacity in the diagnosis or treatment of the infection. The increased TNF-α level in *H. pylori* infections confirms its significant contribution to the H. pylori pathogenesis gastric disease via its immunomodulatory and pro-inflammatory effect. Moreover, the importance of molecular detections of resistance-associated mutation is underlined by the observed nucleotide substitution at position A2142G and A2143G. Identification of such mutation may help in guiding suitable antibiotic selections and preventing treatment failures in the *H. pylori* infection.

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