Study the Relationship between IL-17 rs 3748067 Gene Polymorphism with H. Pylori Infection in Babylon Province

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Abstract

The objective of the current study is to prove a link between IL17 gene polymorphisms and *H. pylori* infection. We obtained Seventy-eight (78) cases with clinical symptoms associated with *H. pylori* infection. Two tests, fast Ab immunoassay and urea breath testing, located 45 patients with *H. pylori* infection. 45 individuals who appeared to be in good health were used as the control group, and their samples were taken. The urea breath test has been identified as the gold standard technique for non-invasive diagnosis. Additionally, ELISA, sequencing, and traditional PCR techniques were used to find the interleukin 17 gene. In patients compared to controls, the genotypes C/T and C/C were found to be more prevalent, with odd ratios (95% CI) of 2.75 (1.06-7.15) and 0.71 (0.22-2.36) and P = 0.05, respectively. This may suggest that these SNP variants are linked to greater *H. pylori* infection, in contrast to the T/T genotype, which showed no significant difference between patients and controls and may result in reduced *H. pylori* infection for carriers. With a mean concentration of 97.87 ± 39.56 pg/ml in the *H. pylori* infection group as opposed to 75.57 ± 22.75pg/ml in the control group, interleukin 17 levels were elevated. The results of the comparison between the level of IL-17 and the genotypes of the IL-17 gene revealed that the genotype C/T in IL-17 (rs 3748067) showed the highest concentration of IL-17 (119.79 pg/ml) in the serum of these studied groups, while the average serum level of C/C(78.65 pg/ml) and T/T groups (78.68pg/ml).

Keywords: *H.pylori*, IL-17, rs 3748067, *H.pylori* Detection, ELISA.

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INTRODUCTION

According to rRNA sequencing, the Helicobacter genus, a member of the Helicobacteraceae family, has over 35 recognized species. Additionally, *Helicobacter pylori*, a gram-negative, spiral-shaped, microaerophilic bacteria that was once known as Campylobacter pylori, is frequently seen in the stomach (On et al., 2017). The human stomach epithelium is infected and colonized by *H. pylori*, which causes variable degrees of gastric illness (Arachchi et al., 2017). In addition to a range of systemic IgG responses, the bacteria cause innate and adaptive immune reactions in the adjacent gastrointestinal environment (Arachchi et al., 2017). The primary pathophysiological event in *H. pylori* infection is the mobilization of an inflammatory gastric mucosal response, which is regulated and controlled by inflammatory cytokines produced by immune and non-immune cells (Nejati et al., 2018). *H. pylori's* increased pathogenicity is linked to a variety of virulence factors. These bacterial virulence factors result in potent innate and adaptive immune responses (Chang et al., 2018). A recent study showed that Th17 cells are essential for defense against extracellular bacterial and fungal infections. IL-17A, IL-17F, IL-21, and IL-22 are cytokines that Th17 cells produce. Th17 cell proliferation is accelerated by the cytokines IL-23 and IL-21, which are in charge of sustaining the Th-17 cell population (Arachchi et al., 2017). The IL17 family of inflammatory cytokines was founded by interleukin-17 (IL17), the first member to be defined, and today consists of six members (IL17A, IL17B, IL17C, IL17D, IL17E, and IL17F) (Milovanovic et al., 2020). Many immune cells can produce IL-17A, but Th17 cells, a recently discovered subgroup of helper T cells, have drawn the most interest (Kuwabara et al., 2017). Research on the function of Th17 cells in autoimmune disorders has greatly increased our understanding of the biological significance of IL-17. It has also been demonstrated that the local IL-17 levels in the stomach mucosa near the infection site are raised (Serelli-Lee et al., 2012). The cytokine IL-17 is thought to be essential for drawing neutrophils to stomach mucosa that has been infected with *H. pylori.* Additionally, it encourages fibroblasts to produce matrix

metalloproteinases, which aggravates mucosal damage (Arachchi et al., 2017).

Materials and Methods

We gathered seventy-eight (78) cases of *H. pylori* with clinical symptoms. In the lab, a rapid Ab immunoassay and urea breath test were used to confirm *Helicobacter pylori* infection. Clinical sources (whole blood and serum) from patients who were admitted to Murjan Teaching Hospital and AL-Qassim General Hospital between November 2021 and January 2022 were used to get the samples. 45 people who appeared to be normal were chosen as the control group.

Detection of Helicobacter pylori

The H. pylori Ab Combo Quick Test and the urea breath test were used in this experiment as two distinct assays. These tests were carried out in accordance with the manufacturer.

Molecular Detection of IL-17 gene

Interleukins-17 Molecular techniques (PCR and DNA sequencing) were used to find and analyze polymorphisms following the application of a conventional extraction method in accordance with (Favorgen korea, Lot No: CB114121326).

DNA concentration

Using the nano-drop instrument (Nanolyitk/Germany), the concentration and purity of DNA were identified and measured.

PCR Amplifications

Using the traditional PCR technique, the IL-17 gene was discovered in both *H. pylori* infection patients and healthy people. We used a Thermocycler, a conventional PCR device produced by Techne in the US. Table 1 shows the product size and the PCR primer sequences.

Gel electrophoresis

All IL17 gene (Snp Rs 3748067) PCR products were tested in 1.5 percent Agarose.

Table 1: Primer sequences Product size of IL-17 gene rs3748067 used in current study

Reference SNP ID	Product Size (bp)	PCR primer sequence
rs3748067	358	F: 5′-TATGGGGAAAATGAAACC-3′
		R: 5′-GTAGGGCAAGACAGCACA-3′

DNA Sequencing

The PCR products were purified before being forwarded to the Korea-based Macrogen Company for sequencing. The data was then processed to determine the precise position of the SNP.0.

Estimation of IL-17 level by ELISA

According to the instructions provided by the manufacturer, the tests were carried out using the IL-17 ELISA kit (Human Interleukin 17 Elisa Kit, Bioassay Technology Laboratory, Chain, Lot No: E0142Hu).

Results and Discussion

Diagnosis of the H. pylori infections

Out of Seventy-eight (78) cases with clinical symptoms of *H. pylori* infection, there were (45) cases laboratory confirmed by both urea breath test and rapid Ab immunoassay; (8) cases that were only positive for rapid Ab immunoassay; and (25) instances tested negative for *H. pylori* by both (UBT and Rapid Ab test), as shown in the table (2). In addition, 45 samples from individuals who seemed to be in good health were collected as a control.

Table 2: Distribution of H.pyloriresults among combination tests (serologic test and Uerea Breath Test (UBT)).

Diagnostic Method	Posative	Nagative	Total
Serologic test	**53 (67.9%)**	**25(32.05%)**	**78**
Urea Breath Test(UBT)	45 (57.6%)	33(42.3%)	78

Blood samples were taken from patients and individuals who looked to be in good health using EDTA tubes (whole blood) and gel tubes. Samples came from Murjan Teaching Hospital and Al-Qassim General Hospital. The *H. pylori* infections in the 45 cases (19 (42.3%) male and 26 (57.7%) female) were verified using these non-invasive methods. Instances of non-infected *H. pylori* were found in 20 (43.47%) women and 13 (40.62%) men.

Table 3: Number and percentage of infected and non-infected persons included in this study.

Gender	Total	Infected	Non-infected
Male	32 (41%)	19(42.3 %)	13(40.62%)
Female	46 (59%)	26(57.7%)	20(43.47%)
Total	78 (100%)	45(57.69%)	33(42.3%)
$\mathbf{X^2}$	0.063		
P-value	0.802		

The results of the current study showed no statistically significant difference between *H. pylori* infections in males and females.

This study used a rapid Ab test based on a blood serum

sample (a serological test) and a urea breath test, the gold standard for diagnosing *H. pylori* infection, which was used to confirm the rapid Ab test's results. "The urea breath test is the gold standard technique for non-invasively diagnosing *H. pylori*," claims (Stefano et al., 2018). UBT may be suggested in many therapeutic situations because it is straightforward to use and non-invasive.

The results of this study showed that the prevalence of H. pylori infection in the study group was 57.69%, which was less than Jordan's population (88.6%) (Obaidat & Roess, 2019) and Iran's population (83.5%) (Ashtari et al., 2015) but nearly comparable to that of nearby countries like Saudi Arabia (46.5%) (Akeel et al., 2018), Kuwait (49.7%) (Alazmi et al., 2010), and Turkey (66.3%) (Ozbey et al., 2015).

Infection with *H. pylori* was 70% common in the general population of Egypt (Ghaith et al., 2016). *H. pylori* infection is common in Pakistan (74.4%), China (62.2%), and Korea (66.9%). According to (Mungazi et al., 2018), *H. pylori* infection has been connected to a number of contributing factors, including living standards, socioeconomic position, location, and ethnicity. Variations in the prevalence rates of *H. pylori* in different studies conducted around the world may all be due to these reasons. The scale of the study, the exclusion of past antibiotic usage, or the range of *H. pylori* detection methods may be to blame for these disparities. (Aziz et al., 2014).

Human DNA Extraction and PCR Products Detection

The human DNA genome, which had a DNA concentration of 50–150 ng and a purity of 1.8–2.0, was extracted from the whole blood samples of all 45 H. pylori patients and 45 samples from ostensibly healthy controls. Before being submitted for sequencing-based single nucleotide polymorphism (SNP) testing, these DNA samples were subjected to PCR amplification using specialized primers that targeted specific DNA regions.

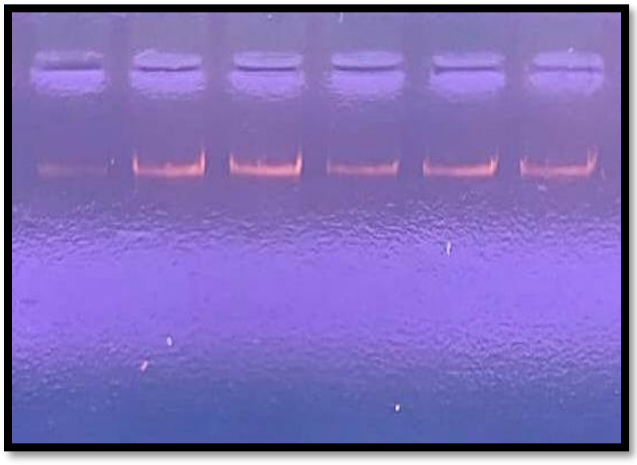

Figure 1: Illustrate the electrophoresis pattern of the extracted DNA

Genotypic Characterization of IL-17 gene SNPs (rs3748067)

According to the figure(2), all study groups' electrophoresis gels showed evidence of gene amplicons due to the IL-17 gene's amplification at region rs3748067.

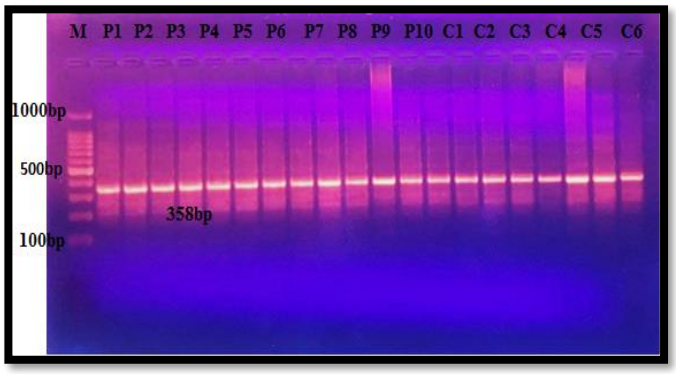

Figure 2: Gel electrophoresis carried on (agarose gel (1.5%), 75% V for 1hr.) for IL-17 gene (rs3748067). PCR products visualized under U.V. light after staining with ethidume bromid. M: 100 bp DNA marker; the lane 1-10: from DNA of H.pyloripatients, 11-20 from DNA of control. The size of product is 358bp.

Table 4: Lists the genotype relationship and allele frequency for patients and controls.

Genotypes	**Control**	**Patients**	**OR (95% CI)**	***P*-value**
T/T	17 (37.7%)	16 (35.6%)	1.00	0.05
C/T	10 (22.2%)	21 (46.7%)	2.75 (1.06-7.15)	
C/C	18 (40.0%)	8 (17.8%)	0.71 (0.22-2.36)	
Allele Frequency				
T	44	53	1.50 (0.83-2.70)	0.23
C	46	37	0.67 (0.37-1.20)	
*(*P*<0.05), OR: odd ratio, CI: confidence interval				

The association between rs3748067 genotypes and H. pylori infection showed that C/T and C/C genotypes were more common in patients than controls, with odd ratios (95% CI) of 2.75 (1.06-7.15) and 0.71 (0.22-2.36) and P=0.05, respectively. This may indicate that these SNP variants are associated with increased H. pylori infection, whereas the T/T genotype has no significant difference between patients and controls.

The findings of the allele frequency analysis revealed that there were no significant differences between the control group and the patients (P = 0.23).

The findings of this study are consistent with those of (Zhang et al., 2014; Zhao et al., 2016), who discovered that

patients with the C/T genotype were more likely to get H. pylori infection than those with the T/T genotype.

Intriguingly, (Zhang et al., 2014) found a significant association between the genotypes rs2275913G > A and rs3748067C > T and gastric cancer brought on by H. pylori infection and also mentioned that genetic variants, which are typically represented as single nucleotide polymorphisms, are a major contributor to this type of cancer susceptibility (SNPs).

In a meta-analysis by (Dai et al., 2016), it was discovered that in Asian (mainly Chinese) populations, IL-17 polymorphisms, particularly the rs2275913G > A and rs3748067C > T genotypes, increase the risk of gastric cancer.

Estimation of IL -17 Serum level

The table(5) shows that patients with *H. pylori* infections had an average IL-17 concentration of 97.87±39.56 pg/ml as opposed to controls, who had an average concentration of 75.57± 22.75 pg/ml. This variation was substantial (P 0.05).

Table 5: IL-17 Serum Level in the Patients and control (Mean± Standard deviation)

Groups	IL-17 mean ±SD
Control	75.57 ± 22.75
Patients	97.87 ± 39.56
T test	3.275
P value	0.002 (S)

S: Significant difference at P<0.05

The results show that interleukin-17 levels are higher in *H. pylori*-infected people than in healthy people. This might be as a result of *H. pylori* bacteria stimulating Th17 cells, which then triggers the production of cytokines by the immune system (IL17). The level of IL17 will consequently increase in affected people.

The results of this study agreed with those of a prior study (Gil et al., 2014), which demonstrated an increase in Th17-associated cytokines in serum samples from patients with *H. pylori* infection.

Recent research (Serelli-Lee et al., 2012), which corroborated our findings in our analysis, revealed that *H. pylori*-infected gastric biopsies from *H. pylori*-infected patients had higher levels of pro-inflammatory cytokines such as IL-17 and Th17 cells than healthy controls.

(Horvath Jr et al., 2012) found that people with *H. pylori* infection had higher levels of circulating Th17 cells, and those who had previously had the illness had even higher levels. After discovering that Th17 cells are elevated in the blood as well as the stomach mucosa, they came to the conclusion that greater IL-17 cytokine serum levels may indicate the response of Th17 cells to *H. pylori* infection.

Correlation of IL-17 Serum Level with IL-17 (rs3748067)

The results showed a significant effect of the C/T genotype in IL-17 (rs 3748067) on the concentration level of IL-17 (119.79 pg/ml) in sera of these studied groups when compared to the median serum level of C/C (78.65 pg/ml) and T/T (78.68 pg/ml) groups. The correlations between IL-17 (rs 3748067) gene polymorphism and the level of IL-17 in the sera of patients were examined.

Table 6: Correlations of IL-7 (rs3748067) gene polymorphism with serum levels of IL-7 in H. Pylori infected patients

Genotype	(Mean± SD)
CC	78.65±10.1
CT	119.79±47.6
TT	78.68±15.6
P value	0.001

Mice with *H. pylori* infection had higher levels of IL-17 in their stomach mucosa (Gu et al., 2013). This proves that Th17 cells, at least in mice, produce *H. pylori* gastritis. For instance, one of the initial cytokines identified in the stomach mucosa of *H. pylori*-infected mice is IL-17 (Bagheri et al., 2015). However, IL-17 promotes the recruitment and activation of polymorphonuclear neutrophils, and it is thought to be a key biological component in the inflammatory lesion associated with H. pylori infection (Tsai et al., 2013).

(Caruso et al., 2007) noted that the Th17 cell signature cytokine IL-17 mediates the host's defense against bacterial infections, particularly at mucosal surfaces. IL-17 is connected to inflammation in a number of autoimmune disorders.

Although the course of an *H. pylori*-driven infection is significantly influenced by bacterial virulence factors, the host's attempt to eradicate the pathogens ultimately leads to an excessively aggressive and incorrectly counter-regulated immune response that may end in tissue damage. Recent experimental data points to a critical role for the IL-23/IL-17 pathway in the persistence of stomach inflammation in *H. pylori*-infected patients. (Shamsdin et al., 2015).

CONCLUSION

Significance Association of the IL-17 gene (**rs3748067**) and *H.pylori* infected in babylon province.

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