Influence of Cytokine Gene Polymorphisms on the Risk of Gastric Disorders in *H. pylori* Infected Patients

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**Background and study aim:**
Helicobacter pylori (*H. pylori*) is a common organism in developing countries, it causes gastric disorders and cancer. Pathogenesis of these disorders involve cytokine gene polymorphisms that affect cytokine levels and clinical diseases. The aim of the study is to identify the relationship of IL-1β-511, IL-10-519 and TNF-α-308 polymorphisms to the risk of *H. pylori* infection and occurrence of gastric disorders.

**Subjects and methods:** IL-1β-511, IL-10-519 and TNF-α-308 polymorphisms were assessed using polymerase chain reaction (PCR) restriction fragment length polymorphism technique (RFLP) in 356 subjects classified according to *H. pylori* infection and gastric disorders.

**Results:** Carriers of T allele of IL-1β-511 and IL-10-519 had increased risk of *H. pylori* infection (OR:1.95, 95% CI:1.4-2.7, P<0.001 & OR:1.8, 95% CI:1.4-2.5, P<0.001; respectively). The IL-1β-511 and IL-10-519 T allele was associated with gastritis, peptic ulcer (PU) & gastric cancer (GC) (P<0.001). Simultaneous occurrence of either IL-1β-511 TT or IL-10-519 TT genotypes with *H. pylori* significantly augmented the risk for different gastric diseases (gastritis; P=0.005 & 0.002, PU; P=0.01&0.02 and GC; P=0.02&0.01; respectively). While, the copresence of TNF-α-308 GA+ AA genotypes and *H. pylori* was related to gastritis only.

**Conclusion:** This study revealed a significant association of the IL-1β-511C/T and IL-10-819C/T but not TNF-α-308 G/A polymorphisms with risk of *Helicobacter pylori* infection and different gastric diseases in Egyptian patients.

**INTRODUCTION**

*Helicobacter pylori* (*H. pylori*) is a prevalent Gram-negative organism, especially in developing countries. Most infections remain asymptomatic; however, infections can pass from acute with marked gastric inflammation and transient hypochlorhydria to chronic gastritis, peptic ulcer (PU) and gastric cancer (GC) [1]. Moreover, *H. pylori* was accepted by the WHO to be a first group carcinogen in 1994 [2]. Several mechanisms are blamed in the pathogenesis of this infection, the key event of which is the gastric mucosal inflammatory responses, where lymphocytes and macrophages are recruited to the infected mucosa with increased synthesis of proinflammatory as well as anti-inflammatory cytokines that enhance inflammation [3]. Other factors as smoking and food as well as *H. pylori* virulence genes can contribute to disease pathogenesis [4,5]. It is known that cytokine genes have genetic polymorphisms which directly
affect cytokine levels and responses; these in turn influence the clinical outcomes [6].

Interleukin-1β (IL-1β), a proinflammatory cytokine, inhibits gastric secretion and its level is increased with H. pylori infection leading to amplification of the associated inflammatory response [7,8]. The IL-1β gene has three single nucleotide polymorphisms (SNPs) causing upregulation of IL-1β levels in gastric mucosa which is accompanied with an enhanced inflammatory process that can lead to hypochlorhydria, gastric atrophy as well as gastric cancer [9].

Tumor necrosis factor-α (TNF-α), another proinflammatory cytokine, is a principle mediator of host reaction against Gram-negative bacteria. TNF-α regulates gastric acid secretion and is elevated in gastric tissues with H. pylori infection causing cytotoxicity and apoptosis, hence it has an important role in pathogenesis of H. pylori related gastric disorders [10]. Several SNPs in TNF-α gene regulatory regions are associated with increased release of TNF-α and are evaluated for their association with gastric cancer [11].

Interleukin-10 (IL-10) acts as an anti-inflammatory cytokine to downregulate proinflammatory cytokines, including IL-1β and TNF-α [12]. H. pylori can cause an increase in IL-10 levels suppressing the immune response, prematurely, and favoring infection. On the other hand, some SNPs in the IL-10 gene leading to low IL-10 levels are related to increased gastric inflammation and risk of malignancy with H. pylori infection [13-15].

In this context, we conducted this study to assess the frequency of IL-1B-511C/T, TNF-α-308G/A and IL-10-819C/T SNPs in Egyptian patients in order to evaluate their possible relationship to the susceptibility to H. pylori infection and risk of gastritis, peptic ulcer and gastric cancer.

SUBJECTS AND METHODS

2.1. Study subjects and design

This case control study enrolled 356 subjects (209 men and 147 women) complaining of gastric disorders. All participants were recruited from the Specialized Unit for Gastrointestinal Endoscopy, Faculty of Medicine, Zagazig University Hospitals. Smoking habits of the study subjects as well as family history of gastric diseases were recorded. Participants were matched for age and sex.

Inclusion and exclusion criteria

The study included patients suffering from gastric disorders. Subjects who received antimicrobials, proton pump inhibitors, H2 blockers, nonsteroidal anti-inflammatory agents during the 15 days prior to sample collection or subjects who refused the study, were excluded [16].

2.2. Diagnosis of gastric diseases and H. pylori infection

The presence of gastro-duodenal diseases (gastritis, PU or GC; in 278/356 patients) or normal gastric mucosa (78/356 subjects) was confirmed by endoscopic and histological examinations. The 78 participants with normal gastric mucosa represented our control group. H. pylori was diagnosed by Giemsa staining, rapid-urease test upon biopsy or H. pylori stool antigen test using enzyme-linked immunosorbent assay (ELISA) (Cortez diagnostics, USA). A patient was diagnosed with H. pylori infection if at least one test was positive [17]. Hence, subjects were stratified regarding H. pylori infection into 193 H. pylori positive and 163 H. pylori negative patients (with normal gastric mucosa, gastritis, PU, or GC).

2.3. Genotype analysis

2.3.1. Blood sampling and DNA isolation

Two ml of venous blood were aseptically collected from every participant into tubes containing EDTA for DNA extraction. Extraction of DNA was performed using commercial kit (QIA amp Blood Kit; Qiagen GmbH, Hilden, Germany) in accordance with the manufacturer’s protocol, DNA purity and concentrations were spectrophotometrically determined at 260 and 280 nm. The purified DNA was stored at –20 °C till further use.

2.3.2. Genotyping of IL-1B-511 C/T (rs16944) polymorphism

The IL-1B-511 SNP of the promoter region was detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) procedures using; forward: 5'-TGGCATTGATCTGTTCCATC-3' and reverse primer: 5'-GTGGAATCTCCTCCACCACTT-3'. The PCR was carried out in a total volume of 25μl mixtures containing 12.5 μl of PCR master mix (2x), 10 μM of sense primer, 10 μM of antisense

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http://mis.zu.edu.eg/ajied/home.aspx
primer, 5 μl of DNA template and completed to 25 μl by nuclease-free water (Promega, USA). Cyclic conditions consisted of initial denaturation at 94°C for 1 min followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min, and a final elongation step at 72°C for 10 min. Ten μl of the PCR products were digested with 3 units of the restriction enzyme Ava I (MBI) (Fermentas, Vilnius, Lithuania) at 37°C overnight. Three genotypes were observed; the homozygous TT of 305bp, the homozygous CC of 190 and 115bp and the heterozygous CT genotype of 305bp, 190bp and 115bp fragments [18].

2.3.3. Genotyping of TNF-α-308 G/A (rs1800629) polymorphism

Analysis of TNF-α-308 G/A polymorphism was done by PCR-RFLP using the forward: 5'-AGGCAATAGGTTTGAGGGCCAT-3’ and reverse: 5’-TCTTCCTGTCCTCGATTCCG-3’ primers. The reaction was performed in a total volume of 25 μl mixtures of 12.5 μl of PCR master mix (2x), 10 μM of antisense primer, 10 μM of sense primer, 5 μl of DNA template and nuclease-free water to reach 25 μl (Promega, USA). Reaction conditions started with initial denaturation at 94°C for 6 min; then 35 cycles at 94°C for 1 min, at 55°C for 1 min, at 72°C for 1 min; and final extension at 72°C for 5 min. The products were digested in a 10 μl reaction containing 2.5 μl of PCR product and 2 units of Ncol (New England Biolabs, USA) in the buffer supplied with the kit at 37°C overnight. The wild type; GG was cut by Ncol to produce 87 bp and 20 bp fragments, GA genotype produced 107, 87 & 20 bp fragments, while the AA genotype remained uncut as a 107 bp band [19].

2.3.4. Genotyping of IL-10-819 C/T (rs1800871) polymorphism

Another PCR-RFLP test was used for detection of IL-10-819 C/T polymorphism using the forward: 5’-ATCCAGACACACTACTAA-3’ and the reverse: 5’-TAAATATCTCAGGTTCC-3’ primers. The reaction consisted of 25 μl mixtures containing 12.5 μl of PCR master mix (2x), 10 μM of sense primer, 10 μM of antisense primer, 5 μl of DNA template and nuclease-free water up to 25 μl (Promega, USA). The reactions were performed under the conditions of; initial denaturation at 94°C for 6 min; 35 cycles at 94°C for 1 min, at 57°C for 1 min, at 72°C for 1 min, and finally elongation at 72°C for 5 min. The products were then digested with Mae III (Sigma-Aldrich). The produced fragments were 292 bp, 217 bp, & 79 bp for the CC genotype; 509 bp, 292 bp, 217 bp & 79 bp for the CT and 509 bp& 79 bp for TT genotype [20].

The PCR products of all genes were separated by electrophoresis on 2% agarose then examined with ethidium bromide staining under ultraviolet trans-illumination with 100 bp-Sizer™DNA marker (iNiRON Biotechnology).

2.4. Statistical analysis:

Data were processed using the Statistical Package for Social Science version 13 (SPSS Inc., Chicago, IL). The ages of patients and controls were compared by the Student t test. The chi-square test was used to compare gender distribution, to test the association between the genotypes and alleles in relation to cases and controls, and to test for deviation of genotype distribution from the Hardy–Weinberg equilibrium (HWE). The odds ratios (ORs) and their 95% confidence intervals (95% CIs) were calculated to estimate the strength of the association between polymorphism genotype alleles of patients and controls. A value of P<0.05 was considered statistically significant.

RESULTS

3.1. Demographic Characteristics of Patients and Control subjects

Our patients and control subjects were found to be matched in gender and age. Smoking habits and family history of peptic ulcer were significantly associated with gastritis (P=0.004 & P=0.01; respectively) and PU (P=0.04 & P= 0.003; respectively) (Table 1).

3.2. Association of IL-1β-511, TNF-α-308 and IL-10-519 genotypes and H. pylori infection.

Frequencies of IL-1β-511, TNF-α-308 and IL-10-519 genotypes were in Hardy–Weinberg equilibrium for H. pylori infected and noninfected subjects. The frequency of IL-1β CT & TT and IL-10 CT & TT genotypes were statistically significantly higher among H. pylori positive on comparison with H. pylori negative participants (P=0.004, <0.001, 0.005 & <0.001; respectively), with a significant occurrence of the T allele of both polymorphisms among H. pylori infected participants (P=<0.001) (Table 2).

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3.3. Frequencies of IL-1β-511, TNF-α-308 and IL-10-519 genotypes in controls and patients with different gastric diseases.

In comparison to controls, the frequency of IL-1β CT and TT genotypes were significantly higher in gastritis (P=0.007; 0.008), PU (P=0.09; 0.003) and GC patients (P=0.04; 0.006). Accordingly, the T allele carriers had increased risk of gastritis (P<0.001, OR: 2.2, 95%CI: 1.4-3.5), PU (P<0.001, OR: 2.2, 95%CI: 1.4-3.5) and GC (P=0.001, OR: 2.6, 95%CI: 1.5-4.5). The TNFα-308 genotypes showed lack of association with all of the studied gastric disorders (P>0.5). Whilst, the IL-10-519 TT genotype was higher among patients with PU & GC and T allele carriers of IL-10-519 conferred nearly up to two fold risk to develop gastritis (P<0.001, OR: 2.9, 95% CI 1.8-4.5) and GC (P<0.001, OR: 3.1, 95% CI 1.8-5.4) (Table 3).

3.4. Frequencies of the IL-1β-511, TNF-α-308 & IL-10-519 genotypes in H. pylori patients with gastric disorders.

Stratification of patients with gastric disorders according to H. pylori infection, revealed that the simultaneous occurrence of H. pylori infection with IL-1β-511 CT and TT genotypes resulted in higher risk of gastritis (P=0.001, OR =4, 95%CI 1.7-9.4; P=0.005, OR = 5.4, 95%CI =1.7-17.6; respectively), and GC (P=0.047, OR =4.3, 95%CI 1.8-18.4; P=0.02, OR = 18, 95%CI =1.7-184.7; respectively), while the presence of IL-1β-511 TT genotype with H. pylori was significantly associated with higher PU risk (P=0.01, OR = 4.1, 95%CI =1.4-12.1).

In addition, the co-occurrence of both TNF-α-308 GA+ AA genotypes and H. pylori infection elevated risk of gastritis (P=0.04, OR = 2.6, 95%CI 1.1-6.5). Moreover, the presence of H. pylori and IL-10-519 CT and TT genotypes augmented the risk for developing gastritis (P=0.03, OR =2.6, 95%CI 1.1-6.2; P=0.002, OR = 7.3, 95%CI =2-26.4; respectively), PU (P=0.01, OR =3, 95%CI 1.2-7.4; P=0.02, OR = 3.5, 95%CI =1.1-10.7; respectively) and GC (P=0.01, OR =7.5, 95%CI 1.5-37.7; P=0.01, OR = 13.5, 95%CI =1.8-101.1; respectively) (Table 4).

| Table (1): Demographic Characteristics of Patients and Controls. |
|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
|                          | Control (78)             | Gastritis (118)          | P (116)                  | PU (44)                  | P (44)                   |
| Age (years)              | 41.5±14.3                | 39.8±12.3                | 42.1±9.5                 | 43.3±13.4                |
| Sex                      |                          |                          |                          |                          |                          |
| Male                     | 42(53.8%)                | 73(61.9%)                | 69(59.5%)                | 25(56.8%)                |
| Female                   | 36(46.2%)                | 45(38.1%)                | 0.3                     | 47(40.5%)                | 0.4                     |
| Smoking habit            |                          |                          |                          |                          |                          |
| Nonsmoker                | 59(75.6%)                | 65(55.1%)                | 45(38.8%)                | 18(40.9%)                |
| Smoker                   | 19(24.4%)                | 53(44.9%)                | 0.004                   | 71(61.2%)                | 0.04                   |
| Family history of PU     |                          |                          |                          |                          |                          |
| Yes                      | 9(11.5%)                 | 31(26.3%)                | 35(30.2%)                | 11(25%)                  |
| No                       | 69(88.5%)                | 87(73.7%)                | 0.01                    | 81(69.8%)                | 0.003                   |

Statistically significant; P<0.5.

PU: peptic ulcer; GC: gastric cancer
Table (2): Frequencies of the IL-1β -511, IL-10-519 and TNFα-308 genotypes in H. pylori positive and negative patients.

<table>
<thead>
<tr>
<th>IL-1β (−511)</th>
<th>H. pylori –ve patients N (%)</th>
<th>H. pylori +ve patients N (%)</th>
<th>OR(95%CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>87 (53.4%)</td>
<td>65 (33.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>57 (35%)</td>
<td>84 (43.5%)</td>
<td>1.97 (1.2-3.1)</td>
<td>0.004</td>
</tr>
<tr>
<td>TT</td>
<td>19 (11.6%)</td>
<td>44 (22.8%)</td>
<td>3.1 (1.7 to 5.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T allele</td>
<td>95 (29.1%)</td>
<td>172 (44.6%)</td>
<td>1.95 (1.4 - 2.7)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TNFα (308)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>127 (77.9%)</td>
<td>140 (72.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GA</td>
<td>34 (20.9%)</td>
<td>44 (22.8%)</td>
<td>0.9 (0.5-1.4)</td>
<td>0.5</td>
</tr>
<tr>
<td>AA</td>
<td>2 (1.2%)</td>
<td>9 (4.7%)</td>
<td>0.2 (0.05-1.2)</td>
<td>0.08</td>
</tr>
<tr>
<td>A allele</td>
<td>38 (11.7%)</td>
<td>62 (16.1%)</td>
<td>0.7 (0.4-1.06)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IL-10 (519)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>66 (40.5%)</td>
<td>44 (22.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>76 (46.6%)</td>
<td>101 (52.3%)</td>
<td>1.99 (1.2-3.3)</td>
<td>0.005</td>
</tr>
<tr>
<td>TT</td>
<td>21 (12.9%)</td>
<td>48 (24.9%)</td>
<td>3.4 (1.8-6.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T allele</td>
<td>118 (36.2%)</td>
<td>197 (51%)</td>
<td>1.8 (1.4-2.5)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Statistically significant; P<0.5.
H. pylori: Helicobacter pylori; -ve: negative; +ve: positive.

Table (3): Frequencies of IL-1β -511, IL-10-519 and TNFα-308 genotypes in healthy controls and patients with different gastric disorders.

<table>
<thead>
<tr>
<th>IL-1β</th>
<th>Control N (%)</th>
<th>Gastritis N (%)</th>
<th>OR(95%CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>47</td>
<td>43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>25</td>
<td>54</td>
<td>2.4 (1.3-4.4)</td>
<td>0.007</td>
</tr>
<tr>
<td>TT</td>
<td>6</td>
<td>21</td>
<td>3.8 (1.4-10.4)</td>
<td>0.008</td>
</tr>
<tr>
<td>T allele</td>
<td>37</td>
<td>96</td>
<td>2.2 (1.4-3.5)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TNFα</th>
<th>Control N (%)</th>
<th>Gastritis N (%)</th>
<th>OR(95%CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>60</td>
<td>85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GA</td>
<td>17</td>
<td>30</td>
<td>0.8 (0.4-1.6)</td>
<td>0.5</td>
</tr>
<tr>
<td>AA</td>
<td>1</td>
<td>3</td>
<td>0.5 (0.04-4.7)</td>
<td>0.5</td>
</tr>
<tr>
<td>A allele</td>
<td>19</td>
<td>36</td>
<td>0.8 (0.4-1.4)</td>
<td>0.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IL-10</th>
<th>Control N (%)</th>
<th>PU N (%)</th>
<th>OR(95%CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>33</td>
<td>33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>35</td>
<td>62</td>
<td>0.6 (0.3-1.1)</td>
<td>0.08</td>
</tr>
<tr>
<td>TT</td>
<td>10</td>
<td>23</td>
<td>0.4 (0.2-1.1)</td>
<td>0.07</td>
</tr>
<tr>
<td>T allele</td>
<td>37</td>
<td>108</td>
<td>2.7 (1.7-4.3)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Statistically significant; P<0.5.
PU: peptic ulcer; GC: gastric cancer
DISCUSSION

*H. pylori*, a common infection in developing countries, causes a variety of gastric disorders including GC. Disease pathogenesis includes interaction of environmental, host genetic and bacterial factors, one of which is the ability of *H. pylori* to stimulate several cytokines’ production. Gene polymorphisms of these cytokines affect cytokine levels and consequently the clinical outcomes [1,6]. Accordingly, we conducted this work to explore the effect of the *IL-1β*-511T/C, *TNFα*-308G/A and *IL-10*-819C/T SNPs on the risk of *H. pylori* infection and gastric disorders in Egyptian population.

In this study, patients suffering from gastritis and peptic ulcers showed a significant positive family history of PU compared to those with normal gastric mucosa. In addition, there was a significant relation between gastric diseases and smoking. This is accepted as heredity and lifestyles can modify the risks of chronic gastritis and gastric disorders [21].

On studying the possible relation between cytokine SNPs and *H. pylori* infection, the current work showed a significantly increased frequency of *IL-1β*-511 CT and TT genotypes in *H. pylori* cases, revealing a significant presence of the T allele with *H. pylori* infections. This finding is consistent with the knowledge that *IL-1β*-511 T allele is associated with high levels of *IL-1β* production [22]. *IL-1β*, which is overexpressed with *H. pylori* infections, is known to be a strong inhibitor of gastric secretion causing hypochlorhydria, that favors colonization by *H. pylori*, also the decreased flow of gastric secretion causes accumulation of bacterial toxins increasing mucosal damage [23]. Our data is in accordance with Ramis and his coworkers, who found that the *IL-1β* T allele and the TT genotype were associated with *H. pylori* infections [24]. In addition to the meta-analysis of Ren et al., which stated that the *IL-1β*-511 CT genotype is related to *H. pylori* risk [25]. While Takagi et al. stated that the *IL-1β*-511 CC genotype is related to occurrence of *H. pylori* infections [26]. On the other hand, Neto et al. did not observe a significant variation in the genotype distribution of *IL-1β*-511 polymorphism among *H. pylori* infected patients [27].

We analyzed the relationship between the *IL-1β*-511 SNP and gastric disorders, where we found a significantly higher frequency of the *IL-1β*-511 CT & TT genotypes in gastritis and GC and *IL-1β*-511 TT genotype in PU cases. The *IL-1β*-511 T allele was significantly linked to high risk of gastritis, PU and GC. These results are consistent with other researchers who proved the association between *IL-1β*-511 T allele and risk of GC especially with *IL-1β*-511 TT genotype [28,29]. In addition to a meta-analysis which revealed a positive correlation between *IL-1β*-511 T allele and GC in Caucasians [30]. While the occurrence of the *IL-1β*-511 C allele elevated the risk of gastric diseases in Asians [31]. However, other
studies found no relation between IL-1β SNP and risk of atrophic gastritis or GC [32,33].

To evaluate the synergistic effect of IL-1β-511 SNP & H. pylori infection on the risk of gastric disorders, this work showed that the copresence of H. pylori and IL-1β-511 CT & TT genotypes increased the risk for gastritis and GC (4&5 and 4&18 folds; respectively). While the risk of PU was heightened by 4 folds in the presence of IL-1β-511 TT genotype. These genotypes appear to increase IL-1β levels leading to severe inflammatory responses and continuous injury to gastric mucosa [8,22,34]. In agreement with our findings, EL-Omar et al. and Ramis et al., found that the T allele of IL-1β-511 SNP is significantly associated with H. pylori related gastric disorders in Caucasians and Brazilians; respectively [23,24]. Also, previous studies on Chinese and African populations stated that H. pylori patients carrying the IL-1β CT and TT genotypes are more likely to develop GC [35,36]. H. pylori infection is a primary cause of GC by producing harmful oxidative radicals and stimulating the proliferative factor gastrin, in addition, virulent strains of H. pylori were associated with IL-1β-511 TT genotype which appears to increase the risk [34,37].

TNF-α, another important mediator in the inflammatory response, is also increased with H. pylori infection and can inhibit secretion of gastric acid, which is in favor of H. pylori pathogenesis [38]. Noteworthy, H. pylori infection causes elevation of TNF-α which induces pathogenesis of gastric cells [10]. Moreover, increased circulating levels of TNF-α can be influenced by some TNF-α gene polymorphisms at the transcriptional level, which in turn has its effect on H. pylori related gastric diseases [11,39]. The action of TNF-α on prostaglandin E2 synthesis is enhanced in the presence of IL-1β, leading to a synergistic effect [40]. Carriers of several high producer alleles of proinflammatory cytokines can suffer severe inflammation, atrophic gastritis and GC [11].

Despite the previous data, there was no difference in the frequency of any of TNF-α-308 genotypes among H. pylori infected or free patients. Additionally, the present work found no relation between TNF-α-308 SNP and any of our investigated gastric disorders. While on stratifying our cases in relation to H. pylori infection, our results revealed a significant frequency of TNF-α-308 GA+AA genotypes in H. pylori infected cases with gastritis, but not with PU or GC. However, Kulmambetove et al. found no relation between TNF-α-308 SNP and gastritis in H. Pylori infected cases [41]. Several studies on Moroccan and Brazilian patients found no association between this polymorphism and gastric pathologies, which is consistent with our data [42,43]. On the other hand, TNF-α-308 polymorphism was considered a risk factor for GC in Caucasians and western populations [44,45].

Unlike our two mentioned proinflammatory cytokines, IL-10 functions as an anti-inflammatory cytokine which lowers inflammatory responses and suppresses neoplastic processes [46]. The inflammatory reaction is balanced by the actions of pro- & anti-inflammatory cytokines [47]. The IL-10-819 SNP is accompanied with low levels of IL-10 in H. pylori patients increasing gastric inflammation and risk of GC, where IL-10 was shown to be protective by suppressing H. pylori induced immune response [48,49].

Analysis of IL-10-819 SNP revealed the significant presence of IL-10-819 CT, TT as well as the T allele in our H. pylori infected compared to non-infected individuals suggesting the association between this SNP and risk of H. pylori infection. Our results agreed with the study of Assis et al., contrarily, Cheng et al. found no association between IL-10 SNP and H. pylori infection in their patients [20,50].

According to this investigation, IL-10-819 TT genotype was significantly present in our cases with PU and GC, and the T allele significantly occurred with all of the studied gastric diseases. Moreover, the IL-10-819 CT and TT genotypes were present significantly in our H. pylori patients with gastritis, PU and GC compared to H. pylori free patients with those gastric disorders (with fold increased risk of 2.6 & 7.3 in gastritis; 3 &3.5 in PU; 7.5& 13.5 in GC for CT & TT genotypes; respectively). These results corroborate data provided by Achhyut et al., who reported the significant association between IL-10-819 polymorphism with gastritis in his patients [51]. Also, Ramis and his coworkers, found the carriers of IL-10-819 T allele to be at high risk of developing PU if associated with H. pylori infection [13]. Those patients have lower levels of IL-10, and higher levels of proinflammatory cytokines [14,15]. Moreover, it was reported that IL-10-819 SNP is not only associated with high risk of PU and GC but also the risk is increased with virulent H. pylori strains [34]. In contrast,
other researchers could not relate any of the IL-10 SNP alleles to gastritis and eventually GC [52,53]. Our findings regarding the frequency of IL-10-819 T allele in GC patients are similar to other results concerning Caucasians, but opposing data was observed in Asians, as the C allele was related to risk of GC. Additionally, IL-10-819 TT genotype might be protective against GC in Asians and associated with low risk in Mexicans [54-56]. Contradictory findings in different studies can be attributed to ethnicities, genetic differences, environmental factors and lifestyles, in addition to sample sizes and study designs.

The study has the limitations of being performed in single center with limited number of patients, future multi-center studies would give detailed information about the genetic influence of theses SNPs among the community. In addition, including assessment of serum cytokine levels in next studies could clarify their relation to these SNPs.

CONCLUSION

In conclusion, our study reveals a significant association between the T alleles of IL-1β-511 and IL-10-819 and the risk of gastritis, PU and GC especially with H. pylori infection. The co-presence of these two SNPs in H. pylori patients significantly increased the risk of gastric pathology in our patients. We could not relate TNF-α-308 SNP to the studied disorders, except for gastritis in H. pylori infected patients. Further analysis on larger samples would clarify the exact roles of these cytokine gene polymorphisms in gastric diseases.

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