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-1β-511*C/T, *TNF-α-308*G/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′-TGGCATTGATCTGGTTCATC-3′ and reverse primer:5′-GTTTAGGAATCTTCCCACTT-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

primer, 5 µl of DNA template and completed to 25 µl by nuclease-free water (Promega, USA). Cyclic conditions consisted of initial denaturation at 94ºC for 1 min followed by 30 cycles of denaturation at 94ºC for 1 min, annealing at 55ºC for 1 min and extension at 72ºC for 1 min, and a final elongation step at 72ºC 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 37ºC 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′-AGGCAATAGGTTTTGAGGGCCAT-3′and reverse: 5′- TCCTCCCTGCTCCGATTCCG-3′primers. The reaction was performed in a total volume of 25µl 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 *NcoI* (New England Biolabs, USA) in the buffer supplied with the kit at 37°C overnight. The wild type; GG was cut by *NcoI* 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′-ATCCAAGACAACACTACTAA-3′ and the reverse:5′-TAAATATCCTCAAAGTTCC-3′ primers. The reaction consisted of 25µl 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^{TM}DNA marker (*iNtRON 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).

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: 2.7, 95% CI 1.7- 4.3), PU (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 1-18.4; 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 1.1-62; 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	PU (116)	P	GC (44)	P
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	19(43.2%)	0.8
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	26(59.1%)	0.06
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	33(75%)	0.06

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.

	H. pylori –ve patients N (%)	H. pylori +ve patients N (%)	OR(95%CI)	P value
IL-1β (-511)				
CC	87(53.4%)	65(33.7%)		
CT	57(35%)	84(43.5%)	1.97(1.2-3.1)	0.004
TT	19(11.6%)	44(22.8%)	3.1(1.7 to 5.8)	<0.001
T allele	95(29.1%)	172(44.6%)	1.95(1.4 - 2.7)	<0.001
TNFα (308)				
GG	127(77.9%)	140(72.5%)		
GA	34(20.9%)	44(22.8%)	0.9(0.5-1.4)	0.5
AA	2(1.2%)	9(4.7%)	0.2(0.05-1.2)	0.08
A allele	38(11.7%)	62(16.1%)	0.7(0.4-1.06)	0.09
IL- 10 (519)				
CC	66(40.5%)	44(22.8%)		
CT	76(46.6%)	101(52.3%)	1.99(1.2-33.2)	0.005
TT	21(12.9%)	48(24.9%)	3.4(1.8-6.5)	<0.001
T allele	118 (36.2%)	197(51%)	1.8(1.4-2.5)	<0.001

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.

	Control	Gastritis	OR(95%CI)	P	PU	OR(95%CI)	P	GC	OR(95%CI)	P
IL-1β										
CC	47	43			47			15		
CT	25	54	2.4(1.3-4.4)	0.007	43	1.7(0.9-3.2)	0.09	19	2.4(1.04-5.5)	0.04
TT	6	21	3.8(1.4-10.4)	0.008	26	4.3(1.6-11.5)	0.003	10	5.2(1.6-16.8)	0.006
T allele	37	96	2.2(1.4-3.5)	<0.001	95	2.2(1.4-3.5)	<0.001	39	2.6(1.5- 4.5)	0.001
TNFα										
GG	60	85			89			30		
GA	17	30	0.8(0.4-1.6)	0.5	23	0.9(0.4-1.8)	0.8	11	0.8(0.3-1.9)	0.6
AA	1	3	0.5(0.04-4.7)	0.5	4	0.4(0.04-3.4)	0.9	3	0.2(0.02-1.7)	0.1
A allele	19	36	0.8(0.4-1.4)	0.4	31	0.9(0.5- 1.7)	0.7	17	0.6(0.3-1.2)	0.1
IL- 10										
CC	33	33			32			12		
CT	35	62	0.6(0.3- 1.1)	0.08	59	0.6(0.3-1.1)	0.09	21	0.6(0.3-1.4)	0.3
TT	10	23	0.4(0.2-1.1)	0.07	25	2.6(1.07-6.2)	0.03	11	3(1.03-8.9)	0.04
T allele	37	108	2.7(1.7- 4.3)	<0.001	109	2.9(1.8-4.5)	<0.001	43	3.1(1.8-5.4)	<0.001

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

Table (4): Frequencies of IL-1β -511, IL-10 -519 and TNFα -308 genotypes in H pylori-positive and H pylori-negative patients with different gastric disorders.

	IL-1β (-511)			TNFα (-308)		IL- 10 (-519)		
	CC	CT	TT	GG	GA+ AA	CC	CT	TT
Gastritis								
H pylori -ve	27	16	5	39	8+0	20	23	4
H pylori +ve	16	38	16	46	22+3	13	39	19
OR (95% CI)		4(1.7-9.4)	5.4(1.7-17.6)		2.6(1.1-6.5)		2.6(1.1-62)	7.3(2-26.4)
P value		0.001	0.005		0.04		0.03	0.002
PU								
H pylori -ve	26	17	6	39	9+1	20	21	8
H pylori +ve	21	26	20	50	14+3	12	38	17
OR (95% CI)		1.8(0.8-4.4)	4.1(1.4-12.1)		0.8(0.3-1.8)		3(1.2-7.4)	3.5(1.1-10.7)
P value		0.1	0.01		0.5		0.01	0.02
GC								
H pylori -ve	10	6	1	13	4+0	9	6	2
H pylori +ve	5	13	9	17	7+3	3	15	9
OR (95% CI)		4.3(1-18.4)	18(1.7-184.7)		0.8(0.3-1.8)		7.5(1.5-37.7)	13.5(1.8-101.1)
P value		0.047	0.02		0.5		0.01	0.01

Statistically significant; P<0.5.

PU: peptic ulcer; GC: gastric cancer; H. pylori: Helicobacter pylori; -ve: negative; +ve: positive.

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-511*C/T, *TNF-α-308*G/A and *IL-10-819*C/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 apoptosis 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 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 Achyut 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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