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Genetic Polymorphisms of Interleukin-6 Gene Among Patients with *Helicobacter pylori*

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Abstract

Objective: We investigated the connection between IL6 polymorphisms and Helicobacter pylori (H. pylori) infection because both variations in the interleukin-6 (IL6) gene and severe infection of the stomach mucosa caused by *H. pylori* are known to influence inflammation and gastric carcinogenesis.

Methods: Baghdad's Gastrointestinal Tract and Liver Diseases Teaching Hospital was used to collect blood samples from laboratories andfrom laboratories in Al-Karamah Teaching Hospital and in Wasit, that were under monitoring at the time. There were 50 patients with H. pylori infection related gastritis in the first group, which included (23 males and 27 females).The second group (controls) consisted of 50 people who tested negative for H. pylori (39 men and 11 females). Between November 2020 and March 2021, their ages ranged from 12 to 70. All of the samples were sent to the lab and kept at a temperature of -30°C. Blood was immediately drawn into a sterile tube containing EDTA for DNA extraction, which was then applied to IL-6 polymorphisms for molecular analysis employing the (ARMS- PCR) method.

Results: The present work, Detection of IL-6 (rs1800795) SNP, a polymorphism located about -174 in the 5′ flanking domain (G→C) also, the frequency of genotypes C\C was 93 (0.93) as 48(0.96) , 45(0.9) in control and patients, respectively and the frequency of genotype C\G was 7(0.07) as 2(0.04) ,5(0.1) in control and patients, respectively. Finally the frequency of genotypes G\G was 0 (0) for control and patients.

Conclusion: The current study found no link between IL-6 gene polymorphisms and a greater risk of illness in the presence of H. pylori.

Keywords: *H. pylori* infection, cytokine, IL-6, polymorphisms

Introduction:

Helicobacter pylori is one of the most prevalent ethnic pathogens, and it was the first bacterial carcinogen to be clearly recognized [(1)]H. pylori infection affects more than half of the global population. Helicobacter pylori colonizes the stomach and triggers an inflammatory response because of a combination of structural and soluble properties [(2)]The incidence of several digestive system disorders, i.e. gastritis, peptic ulcer disease (10-15 %), gastric cancer (1-3 %) and lymphoma associated with mucosa tissue (MALT) (< 0.1 %)too is associated with colonization with this pathogen[(3)]Furthermore, the stomach epithelial layer secretes chemokines to initiate innate immunity and activate neutrophils, resulting in clinical illnesses including gastritis and ulcers. [(4).]The activation of a gastric mucosal inflammatory response is a criticalH. pylori infection of the stomach mucosa

pathophysiological event. Following infection, H. pylori-activated neutrophils and mononuclear cells enter (5)Theinfected gastric mucosa ofH. pylori- and trigger the transcription and production of various pro-inflammatory cytokines, such as interleukin IL6(6).In the processes of host defense, IL-6 serves as a link between the innate and adaptive immune systems. prompting T cells (Th2) to produce IFN- and stimulating activated B cells to secrete immunoglobulin. [(7)]Because cytokines have such broad and pleiotropic effects on immune cells, as well as epithelial and endothelial cells, they are thought to constitute additional risk factors for the development of gastric cancer (8) during H. pylori-associated gastritis. The goal of recent research has been to identify a set of biomarkers that can become measures of individual susceptibility to and effects of infection, including Polymorphisms in genes that control inflammatory host responses are included[(9)]It is known that genetic polymorphisms exist in cytokine genes,that directly affect cytokine levels and responses, which in turn affect clinical outcomes, other factors such as smoking and food, as well as Helicobacter pylori virulence genes may contribute to disease pathogenesis [(10)]The gene of IL6 is set on chromosome 7p21, and there is a sole nucleotide polymorphism (G/C) in the promoter of the IL6 gene at position -174 in the flanking region of the 5' (rs1800795). At location -174, allele G has been linked to greater IL6 serum levels (Fishman et al., 1998). The impact has also been observed in a Finnish population (11).

Materials and Methods

Patient group and sample collection

A case-control research included two groups, the first of which included 50 patients with H. pylori infection-related gastritis (23 males and 27 female). The second group consisted of 50 people who were negative for H. pylori and served as controls. Their ages ranged from 12 to 70, and they were observed in the Gastrointestinal Tract and Liver Diseases Teaching Hospital in Baghdad,laboratories and from laboratories in Al-Karamah Teaching Hospital and in Wasit, between November 2020 and March 2021.The patients' H. pylori infection was confirmed by tests (one group by urea breath test and the other by stool examination). All samples were sent to the lab and stored at -30oC. Blood for DNA extraction, the samples were collected immediately in a sterile tube containing EDTA, followed by molecular analysis using the (ARMS PCR) technique.

Extraction of Nucleic Acid

The DNA was extracted using a special package (Geneaid, Korea) in accordance with the organization's instructions. Apart from whole blood, the premise of genomic human extraction The PrestoTM Mini g DNA Kit is optimized for genomic and viral DNA purification, as well as organic fluids, chaotropic salt, and Proteinase K, which are all factors that carry However, DNA is redacted in accordance with the composite and glass fiber on the column. Wash stupid ethanol-containing water back because it removes impurities while purifying DNA inside TE and distal water.

Table (1) ARMS PCR PrimersSets Used in the (IL-6) Gene

Gene	Primers 5'→3'	Product Size	Annealing Tempe
IL-6	F-inner Primers (C allele) 481 TCCCCCTAGTTGTGTCTTCCC 501	183	59^0C
	R-inner Primers (G allele) 523 GCAATGTGACGTCCTTTAGCTTC 501	164	59^0C
	F-Outer primer(5'→3') 360 GCTTTACTCTTTGTCAAGACATGCC 348		59^0C
	R-Outerprimer(5'→3') 662TCTCCAAGTCCTATATTTATTGGGGG638		59^0C

Primers of this study were designed by using primer3 software (Untergasser et al., 2007) [12], then provided by Macrogen company (Korea). The Amplification Refractory Mutation System is a mutation that is amplification resistant (ARMS Polymerase Chain Reaction)in this study was used to determining the frequency of SNP of IL-6, Molecular detection of (IL6) genes Polymorphisms were performed by using specific primers.

Amplification Polymerase Chain Reaction Refractory Mutation System

The Amplification Refractory Mutation System (ARMS Polymerase Chain Reaction) was investigated for study the frequency of mutations in this work.,the SNP of IL-6= rs1800895.

Statistical analysis

Statistical package for social science (SPSS 26) was used for all statistical analyses. Chi-square and Fisher's exact tests were employed to assess binomial variables reported as frequency and percentagefor the samples < 0.05.

Result and Discussion

Detection of IL-10 (rs1800896) SNP:

The distribution of IL-6 (rs1800795) SNP was detected by ARMS-PCR technique, at this locus there're three genotype; Product size for C allele: 183bp; Product size for G allele: 164bp; Two outer primers have the following product sizes:303bp Figure (1)

Table (2) IL-6(rs1800795) Frequencies Distribution of SNPs in the Screened Population (Control and Patients).

SNP	Allele	Frequency	Controls	Patients	P Value	OR (95% CI)
rs1800795	C	193 (0.96)	98 (0.98)	95 (0.95)	0.248	1.00 2.579 (0.488-13.6)
	G	7 (0.04)	2 (0.02)	5 (0.05)		
	P value	<0.0001*	<0.0001*	<0.0001*		

	Genotypes					
	C/C	93 (0.93)	48 (0.96)	45 (0.9)	0.28	1.00
	C/G	7 (0.07)	2 (0.04)	5 (0.1)	---	2.65 (0.42-16.56)
	G/G	0 (0)	0 (0)	0 (0)		----
	P value	<0.0001*	<0.0001*	<0.0001*		

represent a significant difference at $p<0.05$.

Genotype analysis revealed 3 genotypes CC, CG and GG when genotype distribution of the IL-6(rs1800795) SNP in study groups, CC genotype was the most frequent in both patients and control groups (45.(0.9), 48 .(0.96) respectively. On the other hand, GG genotype was absent in both groups. There was a statistical significant higher rate of CC and lower CG genotypes in patients and control groups ($p<0.05$). Table (1-1).

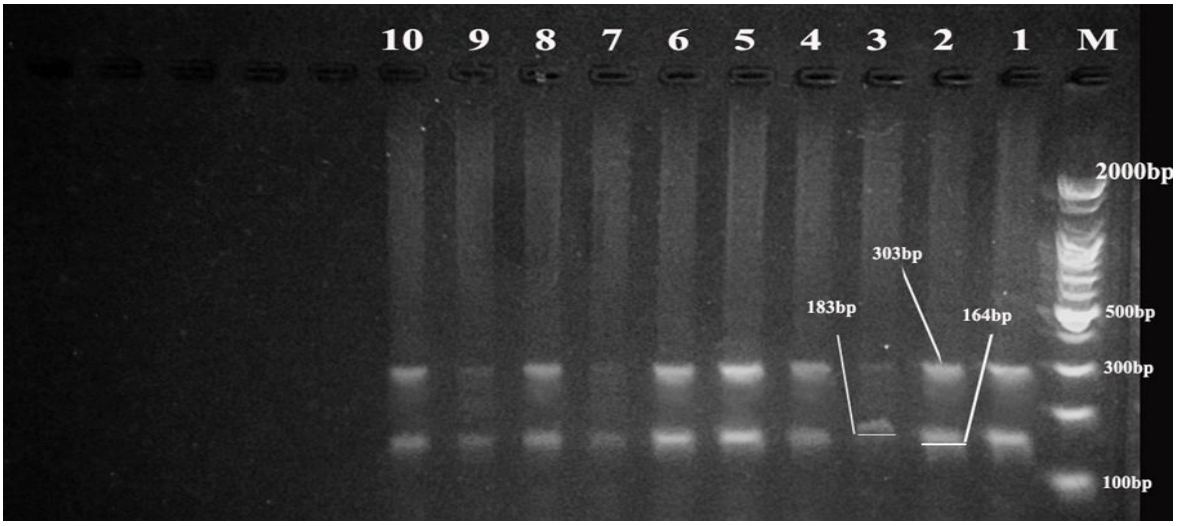

Figure (1): The ARMS-PCR products were electrophoresed on an agarose gel for rs1800795 SNP-specific primer. lanes 1-10 represent the identifieds1800795 SNP products, Lane M represent 100bp DNA ladder. Note: Product size for C allele: 183bp in lane 4; Product size for G allele: 164bp in lane 2; Product size of two outer primers: 303bp in line 2.

The present work, via UBT, dealt with the IL-6 (C/G) polymorphism and found that the rates of induction were 3 (12%) in gastritis patients. While for the gastric ulcer, there was no occurrence of this feature of polymorphism

Table (3) Frequency distribution of *H. pylori* patients (Urea Breath Test) withgastritis according to IL-6 (C/G) genotypes in comparison with control group

IL-6 Genotype (C/G)	Control (*n*=15)		Gastritis (*n*=25)		*p*	OR(95%Cl)	RR (95%Cl)	Chi Sq
	n	%	*n*	%				
C/C	13	86.7	22	88	NS 0.8	0.9 (0.4 to 2.1)	0.99 (0.9 to 1.1)	0.04
C/G	2	13.3	3	12				

n: number of cases; NS: Non-significant at p>0.05.

Table (4) Frequency distribution of H. pylori patients (Urea Breath Test) with gastric ulcer according to IL-6 (C/G) genotypes in comparison with control group.

IL-6 Genotype (C/G)	Control (*n*=15)		Gastric Ulcer (*n*=3)		*p*	OR (95% Cl)	RR (95%Cl)	Chi Sq
	n	%	*n*	%				
C/C	13	86.7	3	100	S 0.0002	0 (0 to 0.3)	0.87 (0.8 to 0.9)	13.9
C/G	2	13.3	0	0				

n: number of cases; S: Significant at p<0.05.

Ramis et al(Ramis *et al*., 2017) have found that patients with *H. pylori* showed a C/G rate of 60 (39.7%) in comparison to that from non-infected people 24 (31.6%).*H. pylori* induces a wide range of illnesses, such as peptic ulcer disease, chronic gastritis, gastric cancer, and mucosa-linked lymphoid tissue are all cases that affect the stomach. [(13)]Gastric mucosa is the target tissue for *H. pylori*, and that target tissue responds to and regulates inflammation through the action of inflammatory cytokines secreted by epithelial cells. Many genetic polymorphisms in cytokines like IL-10, IL-8 andIL-6 affect their levels of secretion and appearance to increase the possibility of developing gastrointestinal disorders like ulcers. IL-6 is a proinflammatory cytokine that has inflammatory and endocrine modulatory properties (5,14). The present work, via SAgT, dealt with the IL-6 (C/G) polymorphism and found that the rates of induction were 2 (8%) in gastritis patients. While for the gastric ulcer, there was no occurrence of this feature of polymorphism.

Table (5) Frequency Distribution of *H. pylori* patients (stool Ag test) with gastritis according to IL-6 (C/G) genotypes in comparison with Control group.

IL-6 Genotype (C/G)	Control (*n*=35)		Gastritis (*n*=25)		*p*	OR (95% Cl)	RR (95% Cl)	Chi Sq
	n	%	*n*	%				
C/C	35	100	23	92	S	Infinity (2.308 to Infinity)	1.087 (1.087-1.087)	8.3
C/G	0	0	2	8	0.004			

n: number of cases; S: Significant at $p<0.05$.

Table (6)Distribution of frequencies of *H. pylori* patients (Stool Ag Test) with gastric ulcer according to IL-6 (C/G) genotypes in Comparison with Control Group.

IL-6 Genotype (C/G)	Control (*n*=35)		Gastric Ulcer (*n*=1)		*p*	OR (95% Cl)	RR (95% Cl)	Chi Sq
	n	%	*n*	%				
C/C	35	100	1	100	- ND	- ND	- ND	- ND
C/G	0	0	0	0				

n: number of cases; ND: Not detected as C/G values are zeros.

Additionally, the interleukin-6 (IL-6) protein acts as a bridge between innate and adaptive defense mechanisms. (15) The IL-6 gene can be found on chromosome 7 of the human genome. A polymorphism in the 5′ flanking domain at -174 (G→C) has been discovered. Inherited polymorphisms may affect vulnerability to a broad variety of illnesses, including Infection with H. pylori, as well as underlying pathophysiology. In gastritis caused by H. pylori infection, IL-6 degrees in the stomach mucosa are elevated, and afterwards return to normal when the infection is eradicated. In addition, the presence of H. pylori increases the levels of IL-6 in the stomach, which is linked to gastric cancer ([16]).Pohjanen et al have found that C/G and C/C rates were not significantly linked to H. pylori[17]

Conclusion:In the findings the current study found no link between IL-6 gene polymorphisms and a greater risk of illness in the presence of H. pylori.

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