RESEARCH ARTICLE

Association between IL-10 Gene Polymorphisms in *Helicobacter pylori* infection and Gastric Illness among Iraq Population

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ABSTRACT

Objective: Because both variants in the interleukin-10 (IL10) gene and the severe infection of the stomach mucosa produced by *Helicobacter pylori* are known to influence inflammation and gastric carcinogenesis, we looked at the link between IL10 polymorphisms and *H. pylori* infection.

Methods: Baghdad's Gastrointestinal Tract and Liver Diseases Teaching Hospital was used to collect blood samples from laboratories 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 50 which them negative *H. pylori* were (40 males and 10females) as controls. Their ages ranged from 12–70 years, in the period from November 2020 to March 2021. All samples were delivered to the lab and stored at -30ºC. Blood was taken immediately into a sterile tube containing EDTA for DNA extraction, then applied to IL-10 polymorphisms using the (ARMS PCR) method for molecular analysis.

Result: The current study, IL-10 (rs1800896) SNP Detection, The frequency of genotypes TT was 81(0.81), as opposed to 45(0.09) and 36(0.72) in the control and patient groups, respectively, respectively; the frequency of genotypes TC was 15 (0.15) as 4(0.08), 11(0.22) in control and patients, respectively; and the frequency of genotypes CC was 4 (0.04) as 1(0.02), 3(0.06) in control and patients. The expression of the IL-10 gene has increased.

Conclusion: Individuals who have IL10 polymorphisms are more likely to develop stomach cancer, according to the study's findings, particularly when associated with *H. pylori* infection.

Keywords: Cytokine, *H. pylori* infection, IL-10, Polymorphisms.

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INTRODUCTION

Helicobacter pylori was the first bacterial carcinogen to be properly recognized, and it is one of the most common ethnic pathogens.[1] More than half of the world's population is infected with *H. pylori*. A combination of structural and soluble properties of Helicobacter pylori allow it to colonize the stomach and trigger an inflammatory response.[2] Although the pathogen is present in mucus and on the surface of the stomach lining, its presence causes persistent inflammation, a major contributing factor to chronic gastritis.[3] Discomfort in the gastrointestinal tract and peptic ulcer disease are caused by this prevalent and possibly curable illness.[4]

It is the human immune system level, the pathogenicity of *H. pylori* strains, and the effect of environmental factors such as nutrition and stress as well as hygiene and presence of co-infections that determine the type and severity of disorders, nutrition, stress, hygiene level and the presence of co-infections are all factors that influence the type and severity of disorders in the host.[5] Patients with dyspepsia can benefit from using a non-invasive test to identify whether *H. pylori* is present and then treating the infection if it is discovered, rather than undergoing endoscopic treatment, which would be more expensive uncomfortable.[4]

The induction of an inflammatory response in the stomach mucosa is mediated and controlled by inflammatory cytokines generated by epithelial cells in the gastrointestinal tract.[6] It is the most important pathophysiological event that occurs during *H. pylori* infection. Interleukin (IL-6, IL-8, and IL-10) secretion levels are regulated by polymorphisms in genes encoding cytokines such as interleukin (IL-6, IL-8, and IL-10) and these polymorphisms appear to increase the risk of gastroduodenal cancer in those with these genetic variations.[7]

Interleukin-10 (IL-10) is an anti-inflammatory cytokine that inhibits cytotoxic inflammatory responses as well as cell-mediated immunity.[8] Helicobacter pylori can employ upregulation of IL-10 to inhibit an effective immune response,

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therefore promoting infection and parasite survival. The IL-10 gene in humans may be located on chromosome 1.[9]

MATERIALS AND METHODS

Patient Group and Sample Collection

A case-control study was done based on two groups. First group was 50 patients with *H. pylori* infection-associated gastritis and included (23 males and 27 females). The second group (50) was negative *(H. pylori*) as controls. Their ages ranged from 12–70 years, who were observed, in Gastrointestinal Tract and Liver diseases Teaching hospital, Baghdad, and Blood samples laboratories from November 2020 to March 2021. The patients were confirmed with *H. pylori* by tests (one group by urea breath test and the second by stool examination). All samples were sent to lab and preserved at -30ºC blood was collected directly in a sterile tube containing EDTA for DNA extraction, then uses (ARMS PCR) technique application to IL-10, gene polymorphisms, for molecular analysis.

Extraction of Nucleic Acid

The DNA was extracted using a special package (Geneaid, Korea) under 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 following 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.

Primer Design

Specific primers tooled then designed through Alpha DNA Company, have been aged to increase to absolute fragments over The IL-10 (rs1800896) is proven of desk 1 (Table 1).

Amplification Refractory Mutation System Polymerase Chain Reaction

In this work, the Amplification Refractory Mutation System (ARMS Polymerase Chain Reaction) was analyzed to evaluate the frequency of the SNP of IL-10= rs1800896.

STATISTICAL ANALYSIS

All statistical analyses were carried out using the Statistical Package for Social Science (SPSS 26). When binomial variables were reported as frequency and percentage, they were evaluated using Chi-square, and when the samples were less than 5, Fisher's exact test was employed. The phylogenetic significance between local isolates and reference isolates and the phylogenetic relevance within local isolates were determined using the MEGA 6 program and 1000 bootstrap repetitions.

RESULT AND DISCUSSION

Detection of IL-10 (rs1800896) SNP

The distribution of IL-10(rs1800896) SNP was detected by ARMS-PCR technique. At this locus, there are three genotypes; Two outside primers have a combined product size of 406bp. The product size for the T allele is 220bp, while the product size for the C allele is 244 (Figure 1).

Genotype analysis revealed 3 genotypes TT, TC and CC when genotype distribution of the IL-10(rs1800896) SNP in study groups, TT genotype was the most frequent in both patients and control groups (45 -0.90, 36-0.72), respectively. On the other hand, TC genotype was predominant in the control group than in the patient group (11 -0.22, 4-0.08). CC genotype was present in nearly in equal form. There was a statistically significant higher rate of TT and lower TC genotypes in patients and control groups ($p < 0.05$) (Table 2).

Interleukin-10 may have a dual role as an anti-inflammatory cytokine and an immuno-inhibitor, which is why it is likely that IL-10 is, associated with the development of gastric cancer in *H. pylori* patients. According to a new study, genetic differences in IL10 are linked to increased IL10 production. The cytokine IL-10 is raised with the presence of *H. pylori* in the stomach

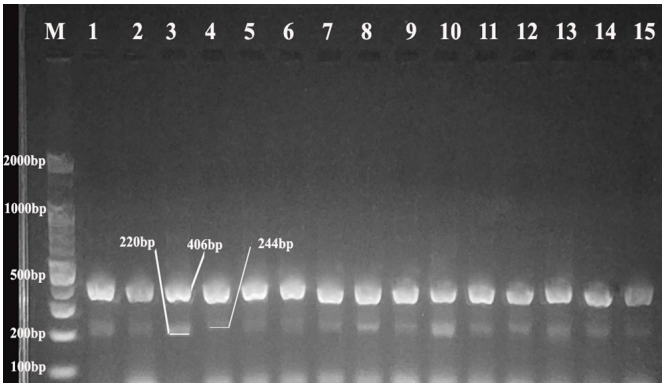

Figure 1: The electrophoresis of ARMS-PCR products produced by employing a primer specific for the rs1800796 SNP was performed on an agarose gel. Lanes 1-15 show the rs1800796 SNP products discovered, and Lane M represents a 100bp DNA ladder. Take note that the product size for the T allele is 220bp, the product size for the C allele is 244bp, and the product size for the two outside primers is 406 bp.

Table 1: Primer sets used in (IL-10) gene.

Gene	*Primers 5′→3′*	*Product size*	*Annealing Temperature °C*
IL-10	F- inner Primers (T allele) 474 TTTCCTCTTACCTATCCCTACTTCCACT 501	220	62
	R- inner Primers (C allele) 530AAGACAACACTACTAAGGCTTCTTTGGTAG501	244	62
	F-Outer primer(5'→3) 287 TCCTCACCCTACTGTACACCATCTC 311		62
	R- Outer primer(5'→3) 692 GATCCCCAGAGACTTTCCAGATATC 668		62

Table 1.2: The frequency of the IL-10 (rs1800896) SNP in the screened population was determined (Control and Patients).

SNP	*Allele*	*Frequency*	*Controls*	*Patients*	*P*	*OR (95% CI)*
(rs1800896) rs	T	177 (0.88)	94 (0.94)	83 (0.83)	0.015*	3.2 (1.209-8.519)
	C	23 (0.12)	6 (0.06)	17 (0.17)		
	P value	< 0.0001*	< 0.0001*	< 0.0001*		
	Genotypes					
	T/T	81 (0.81)	45 (0.90)	36 (0.72)	0.066	1.00
	T/C	15 (0.15)	4 (0.08)	11 (0.22)		3.44 (1.01-11.71)
	C/C	4 (0.04)	1 (0.02)	3 (0.06)		3.75 (0.37-37.60)
	P value	<0.0001*	0.02*	<0.0001*		

* represent a significant difference at $p < 0.05$.

mucosa, and those with severe chronic inflammation have higher levels of IL-10. IL-10 may have a dual function as an anti-inflammatory cytokine and an immuno-inhibitor, which is likely why it is widely used.[10,11] We discovered that IL10 polymorphisms are linked to an elevated risk of *H. pylori* infection and a high risk of other illnesses such as gastric cancer.[12] The number of patients involved, the study design, the age of the patients when they were diagnosed, and the sequencing and analytic technique used all influence clinical research outcomes. Ethnic differences in the prevalence of the IL10 genotypes could also play a role in these variances.[13]

Asians have a significantly lower prevalence of IL10 polymorphisms (relative to other ethnicities). Clinical significance: People who are more likely to develop stomach cancer may be at a higher risk of developing the condition because of this genetic variant.[14] In fact, this could explain the disparity in stomach cancer rates between Caucasians and Asians.[15]

CONCLUSION

In the findings, persons who have the IL10 polymorphisms are more likely to develop gastric cancer, especially if they had an *H. pylori* infection at the same time.

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