The Relationship Of IL-1RN 86 VNTR Gene Polymorphism in The Genetic Susceptibility to Peptic Ulcer Caused by H. Pylori Infection

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Abstract

Objective: Interleukin IL-1RN 86bp VNTR upregulates in response to H. pylori infection, playing a central role in gastritis by inducing the production of many other molecules, such as proinflammatory cytokines, mediators, growth factors and adhesion molecules. Material and Methods: Fifty samples of the blood were obtained from patients and a safe control group, then DNA was extracted and analyzed for genotypes IL-86 with (PCR) and Gel electrophoresis using 2,25%, 2% the concentration of agarose was (respectively) investigated. Results: IL-86 mutations were detected in 50 percent of H.pylory infections patients, Although only 25% were observed in the control group, we noticed a substantial correlation between the genotype and the allele frequency and the P<0.05 group of H.pylory infections. Conclusion: IL-86 and H.pylory infections are associated with peptic ulcer.

Key Words: Interleukin 1L- 86, H.pylory, Peptic ulcer, Polymorphism.

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Introduction

H. pylori infection is the most common human infection worldwide, approximately 50% of the world's population is infected and this makes humans the main reservoir of H. pylori bacteria [1] . Helicobacter pylori (H. pylori) infection is considered to be the etiological cause of chronic gastritis and peptic ulcer disease, and it has been associated with gastric cancer [2] . that host genes can be a useful tool for identifying high-risk individuals among dyspepsia patients; Also emphasizing the role of host genetics in gastric carcinogenesis [3] .The presence of a pro-inflammatory response and the level of gastric acid secretion significantly contribute to the development of peptic ulcer disease or atrophic gastritis [4] . Gastric ulcer (GU) disease results from an interplay of environmental, microbial (Helicobacter pylori), pharmacological (non-steroidal anti-inflammatory drugs), excessive gastrin production (Zollinger-Ellison syndrome) and genetic factors [5]. An esophageal ulcer is a distinct break in the margin of the esophageal mucosa . This mucosal damage to the esophagus is often caused by gastroesophageal reflux disease or from severe sustained esophagitis from other causes [6] . Among host factors, the immune system might play an important role in the pathogenesis of duodenal ulcer by controlling the nature and intensity of the inflammatory response to H. pylori infection. The inflammatory reaction is characterized by an infiltration of polymorphonuclear neutrophils, lymphocytes, macrophages and plasma cells into the gastric mucosa [7]. The main biological mediators in this immune inflammatory response are cytokines, low-molecular-weight peptide molecules produced by a large variety of cells that possess a broad range of physiological functions. Among cytokines, interleukin (IL)-1 is a proinflammatory cytokine that plays a key role in initiating and amplifying the immune-inflammatory response to H. pylori infection.

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The interleukin-1 family involves two agonist polypeptides (IL-1a and IL-1b) and the interleukin-1 receptor antagonist (IL-1ra). IL-1a and IL-1b bind to the IL-1 receptor type I, eliciting signal transduction. IL-1ra blocks the binding of IL-1 to receptors acting as a competitive inhibitor of IL-1/IL-1R interactions [8] . the IL-1RN polymorphism associated with susceptibility to H. pylori infection and gastric cancer outcome is a five-allelic 86-bp VNTR (variable number of synonymous repeats) polymorphism in intron 2 [9] . Studies from different ethnic groups have reported that in individuals infected with H. pylori, the IL-1RN*2 allele is associated with increased production of IL-1β leading to severe and persistent inflammation, gastric atrophy, hypochloremia, and eventually the development of gastric cancer [10]. Previous studies aimed to evaluate the association between the 86 bp VNTR polymorphism of the IL-1RN gene and susceptibility to H. pylori infection and gastric cancer [11] .

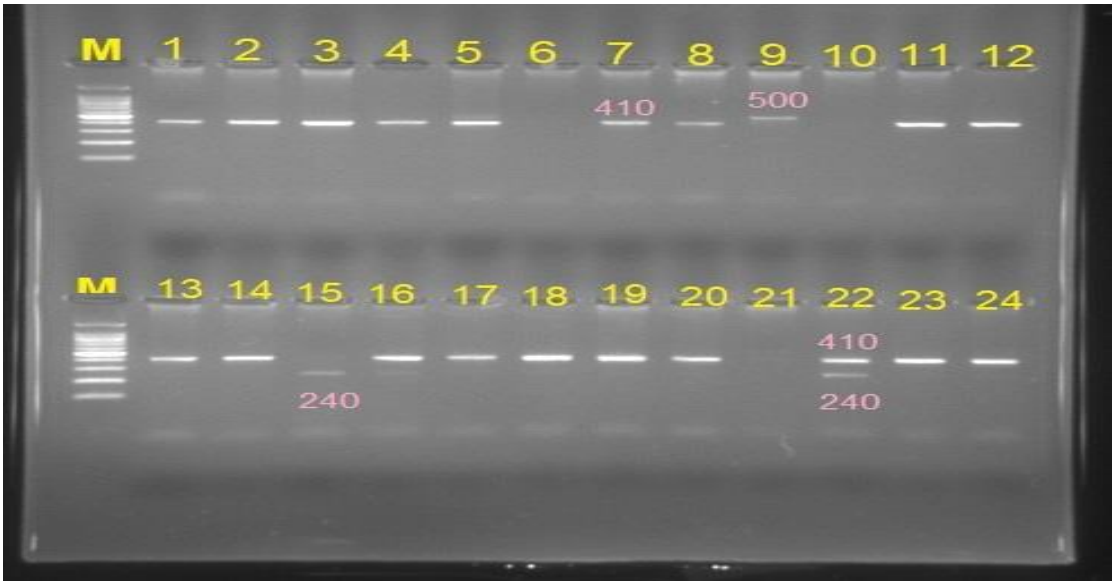

Figure 1: Electrophoresis of the IL-1RN gene heterogeneity. The M pathway represents a size parameter of 100 base pairs: Routes 1, 2, 3, 4, 5, 7, 8, 9, 11, 12, 13, 14, 16, 17, 18, 19, 20, 23 and 24 represent the genotypes For the long alleles: Path 15 represents the genotype of the short allele. Paths 16 and 22 represent the heterogeneous genotype containing both the short and long alleles.

Materials and Methods

Sample collection

The present study is a case-control analysis that involves 100 people divided into two classes, the 50 people with chronic stomach ulcers caused by H. pylori and 50 people who did not). Four ml of venous blood were drawn from all participants using disposable syringe, four ml of blood was obtained for genetic study and slowly pushed into the EDTA tube and given the patient's name at -20 ˚C.

Extraction DNA

In addition to the human DNA extraction kit of Intron Biotechnology, and following the directions of the manufacturer, the DNA was extracted from each patent and control group blood using 200ml of whole blood.

Identification

urease rapid test

The rapid urease test also known as the CLO test is a rapid diagnostic test for the diagnosis of H. pylori. The basis of the test is the ability of H. pylori to secrete the enzyme urease, which catalyzes the conversion of urea to ammonia and carbondioxide. The test was performed at the time of gastroscopy. Where a biopsy is taken from the mucous membrane of the stomach, and it is placed in a medium containing urea and a reagent such as phenol red. The urease produced by Helicobacter pylori breaks down the urea into ammonia, which raises the pH of the medium, and changes the color of the sample from yellow (negative) to red [12] .

Detection of Virulence Factors Genes by PCR:

determinant genes fore helibacterial: were identified via PCR, by specific primes (Table 1).

Table 1: Primer Sequencing and PCR Conditions fore helibacterial

Name	Sequence	Base no.
86-bp VNTRF	CTCAGCAACACTCCTAT	17
86-bp VNTRR	TCCTGGTCTGCAGGTAA	17
Total base no	34	

Genotyping DNA

The IL-1RN 86bp VNTR was analyzes as previously described.[13] Briefly, oligonucleotides primers for PCR amplification were 5'-CTCAGCAACACTCCTAT-3'and 5'-TCCTGGTCTGCAGGTAA-'3. Described by Bioque etal.in 1995[14].The amplification conditions were 94°C for1min, 35 cycles of 94°C for 1min, 60°C for 1min, 72°C for 1min, and a final extension at 72°C for 7 minutes. The PCR products were visualized by electrophoresis on a 2% agarose gel stained with red safe A 1-kilobase DNA ladder was used to standardize alleles' sizes and code conventionally as follows: allele 1 (A1)=410bp (4repeats), allele 2 (A2)=240bp (2 repeats), allele 3 (A3)=500bp (5 repeats), allele 4 (A4)=325bp (3 repeats), and allele 5 (A5)=595bp (6 repeats). For statistical analysis purposes and in accordance with a previous report conducted by Machado et al., these alleles are classified into the short (allele *2=*2) and long alleles (alleles *1, *3, 4, and5=L). The genotyping patterns will classify as L/L, L/*2, and *2/*2.[15] .

Analyzing

Gel electrophoresis was performed on 2% agarose gel for IL-86-bpVNTRF gene polymorphism, containing 5 µl red safe. The gel was analyzed and genotypes determined using transilluminator.

Statistical Analyses

Potential associations of IL-86-bpVNTRF with the risk of H.pylori infection were analyzed by comparing, IL-86 in control group patients use chi-square ($P < 0.05$ find significant) and odd ratio (OD) check CI 95% to measure the effect of this mutation on the infected group relative to the control group.

Results

The genotype of whole 50 patients of H.pylori infection was analyzed for detection the presence of normal or mutant genotype of IL-86, The PCR results showed the polymorphism of IL-86 displayed C and T alleles and three genotypes *L/*L, *L/*2 and *2/*2) Fig. 1. The *L allele results in an undigested polymerase chain reaction product of 410 bp (homo),and *2 allele contributed to a digested PCR product of 240 bp fragment, while the *L/*2 genotype resulted in 410, 240 bp (hetero) .

Table 2. Allele and Genotype frequencies of IL-86 gene polymorphism and H.pylori infection patients (uninfected controls)

Genotype 1L-86	Patients	control	P -value	OR	Ci=95
L/*L	48(24%)	36(18%)			
*L/*2	0	6(3%)	0.008*	0.42	(0.33-0.54)
*2/*2	2(1%)	8(4%)	0.0028*	0.18	(0.03-0.93)
Totall	50	50			
Allele					
*L	96	78			
*2	4	22	0.046*	0.21	0.04-1.08

fragments. Revealed that 48(24%) patients infected with H.pylori has L/*L Allele, and 0 patients infected with H.pylori has *L/*2 , while 2 (1%)patients has *2/*2 allele which consider as mutant, compared with the control group, 36(18%) containing the L/*L allele and 6(3%) containing the *L/*2 allele,and 8(4%)contain the *2/*2 allele (Table 1). There were significant differences between the two Patients groups and the control group, concerning the in IL-1RN VNTR polymorphism. Thecarriage of IL-1RN* 2 allele was associated with increased risk of peptic ulcers (p =0.0046; OR = 0. 21; 95% CI: 0.04-1.08) and, the homozygous ILRN *2/*2 genotype was associated with increased risk of peptic ulcers (p = 0.0028; OR = 0.18; 95% CI: 0.03-0.93). Also, ILRN *L /*2 genotype was associated with increased risk of

peptic ulcers (p = 0.0008; OR = 0. 0.42; 95% CI: 0.33-0.54).

Discussion

Interleukin 1 plays a central role in gastritis by inducing the production of many other molecules, such as inflammatory cytokines, growth factors and adhesion molecules. The results of the PCR polymerase chain reaction after fractionation of the PCR products on agarose gels and staining them with read safe dye showed the presence of L, 2 alleles and three genotypes (*L/*L, *L/*2, *2/*2). Allele 2 of the IL-1RN VNTR polymorphism H. pylori infection, increased the risk of peptic ulcers Caused by infection H. pylori . The results were matched with the study of Furuta and his group, it showed a link between the allele 2 in the IL-1RN*2/*2 gene with a significant protective effect against duodenal ulcer disease .[16]. Many bacteria and host genotypes work together to determine which individuals are most susceptible to disease. The current study indicated that the IL-1RN2/2 genotype is significantly associated with gastric ulcer risk targeting patients with this genotype which has the potential to reduce the increased risk of gastric ulcer to a great extent. Since people who carry this allele have a prolonged and more severe inflammatory immune response than people with other IL-1RA genotypes, it is expected that an increased ratio of homozygous IL1RN*2 may make these people more efficient at fighting microbial infection or colonization due to increased Inflammatory immunity .[17].This difference in result between studies might because difference in sample size, or misinterpretation of PCR result or because difference in race, lifestyle of patient . And living habits may be the source of the contradiction. H. pylori genetic differences (cagA positive or negative) may also influence the association of host IL-1RN VNTR polymorphisms and H. pylori infection.

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