Evaluation of frequency polymorphism Interleukin 1B gene in patients with peptic ulcer and chronic gastritis

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Original Article

Abstract

Introduction: *Helicobacter pylori* is the main cause of chronic inflammation and peptic ulcer. More than half of the world population is afflicted with Helicobacter, while only 20% of people show the clinical symptoms of the disease. Genes encoding the cytokines interleukin (IL)-1B play a key role in the inflammatory responses of gastric mucus. This study aims to analyze polymorphism in the IL-1B gene.

Methods: 107 individuals who showed the symptoms of chronic gastritis participated in the treatment group. Individuals with no symptoms were considered as the control group. IL-1B+3954C/T polymorphism was analyzed through PCR-RFLP.

Results: There is no association between the presence of the T allele in an IL-1B gene whit peptic ulcer and chronic gastritis as compared with control group (OR=0.66, CI=0.34-1.27, OR=0.75, CI=0.32-1.15) respectively.

Conclusion: Our results suggest that there is no association between IL-1B+3954 C/T polymorphism and peptic ulcer disease and chronic inflammation.

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Introduction:

Gram-negative bacterium Helicobacter pylori is Microaerophilic that is the main cause of chronic gastritis and peptic ulcer (1). More than half of the world population is infected with this bacteria while only 20% of patients show clinical symptoms (2).

While most individuals remain asymptomatic, persistent colonization and the chronic inflammation, increase the risk of gastritis, peptic ulcer and gastric cancer (3). The clinical consequences of Helicobacter infection will be determined by the host and bacteria's genetic features as well as environmental factors (4).

During the past few years, there has been increasing evidence that inflammatory responses are important parts of the pathogenesis of peptic ulcer disease, and it has been suggested that inflammatory cytokines as well as genetic variations in encoding genes of inflammatory mediators may have a fundamental role in the development of peptic ulcer disease (5). Among host factors, interleukin-1 (IL-1) plays an important role in the progression of the disease. The IL-1 gene cluster is located on the long

arm of chromosome 2 and contains three genes linked together (IL-1A, IL-1B, IL-1RN). Respectively, they are encoded inflammatory cytokines IL-1α membrane forms, IL-1βsecreted forms and IL-1ra anti-inflammatory cytokine (6). It was found that the production of IL-1B is increased in the presence of H. pylori infection and the protective molecule is gastric cell and Limited potent gastric acid secretion (7). 3 functional polymorphisms have been reported in the promoter region of the IL-1B gene that increases the production of mucus IL-1B in response to H. pylori infection (8). The aim of this study was to evaluate polymorphism 3954+ in an IL-1B gene in patients with chronic gastritis and peptic ulcer and compared them with normal subjects.

Methods:

This study was conducted as a case-control. In this study, 107 people with symptoms of stomach pain, stomach upset and indigestion, seek medical attention and had necessary indications for endoscopy, and based on endoscopy, they had gastric or duodenal ulcers, or based on pathology tests, they had gastritis, were considered as a patients' group. During sampling, the people who have used nonsteroidal anti-inflammatory drugs (NSAID) and alcoholic and autoimmune gastritis types were excluded. In addition, people, who were healthy in digestive problems and have not consumed stomach medications, including antacids and proton pump inhibitor drugs (PPI), 5cc of blood, were taken. After Pylori IgG serum testing, which represents the previous encounter with H. in person; people with positive IgG were considered as a healthy control group. The study was approved by the ethics committee, and written consent was received of both case and control groups to participate in a research project.

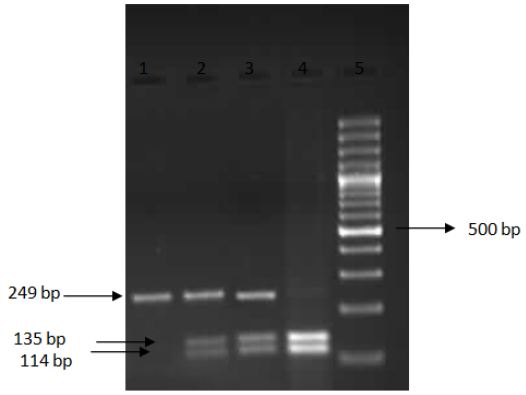
Genomic DNA was extracted from patients' group of biopsy samples and from the control people of their peripheral blood sample by DNA mini kit Qiagene. Polymorphism+3954C/T in IL-1B gene with the method of PCR-RFLP were studied. Primers used are as follows:

PCR reaction was done in a final volume of 25Lμ included KCL buffer 10x, 2.5mM of MgCL2, 0.2mM of each dNTP and 25pmol of primer, 1U polymerase and 100 ng of extracting DNA.

primers	Sequence
IL1-B: F	[5'GTTGTCATCAGACTTTGACC3']
IL1-B: R	[5'TTCAGTTCATATGGACCAGA3']

PCR was performed under the following thermal condition by using PCR-RFLP method:

A step of primary Denaturation 95C° for 5 minutes, then the first phase of multiplication, 3 cycles with temperature conditions 97C° for 90 seconds, Annealing temperature 57C° for 90 seconds and temperature 74C° for 60 seconds in order to do the extension, then 32 cycles with temperature conditions 97C° for 30 seconds, Annealing temperature 57C° for 30 seconds and 74C° temperature for 30, in the end a final amplification stage in 72C° temperature for 10 minutes, were done. The resulting piece had 249Bplengths that were digested with restriction enzyme TaqI. Then, the enzyme product was electrophoresed on agarose gel 3%containing ethidium bromide and was examined. T allele created249bp band and C allele created base bands of 135 and 144 a, while alleles TCcreatedbasetri-band249, 144 and 135.



Allele and genotype frequencies in both groups were controlled and were evaluated and to compare the genotype and allele frequencies between patients and to control as well as to evaluate the Hardy-Weinberg equilibrium, the Chi-square tests were used. Statistical surveys and x^2 analysis were conducted by using Spss version 16 statistical software. Differences in genotype and allele

frequencies of polymorphisms+3954C/T in IL-1B gene were evaluated between the case group and control group by chi-square test with Odds Ratio: OR.

Results:

Allele and genotype frequency of polymorphisms+3954C/T in gene IL-1B is shown in the table 1. T and C allele frequency in patients with peptic ulcers were 28.6% and 71.4%, respectively and in patients with gastric inflammation were respectively, 32.3% and 67.7%, and in the control group were 41.1% and 58.9 respectively. Hardy-Weinberg equilibrium test was done for the SNP examination and the null hypothesis of Hardy-Weinberg equilibrium was not rejected. In Genotypic and allelic frequencies of polymorphisms+3954C/T in IL-1B gene in patients with chronic gastritis and peptic ulcer and control group, there was no significant difference. TT and TC genotype in IL-1B gene did not show a significant difference among patients with peptic ulcer and control group (P=0.15; 0.03-1.66, CI 95%; 0.24, OR and P=0.53; 0.39-1.46, CI 95%; 0.75, OR).

In the study of patients with chronic gastritis, TT and TC genotypes did not show a significant difference between the two groups (P=0.30; 0.38-1.27, CI 95%; 0.70, OR and P=0.18; 0.54-1.09, CI 95%; 0.77, OR).

Table 2. Or and 95% CI for IL-1B Genotypes among gastritis case and control

Polymorphism	Genotype	Gastritis (79)	Control (107)	OR (95% CI)	P-value
IL-1B	CC	36 (45.6)	36 (33.7)	1.0	-
	TC	35 (44.3)	56 (52.3)	0.77 (0.54-1.09)	0.18
	TT	8 (10.1)	14 (14)	0.70 (0.38-1.27)	0.30
	TC+TT	43 (54.4)	71 (66.3)	075 (0.32-1.15)	0.13

Table 3. Or and 95% CI for IL-1B Genotypes among pepotic ulcer case and control

Polymorphism	Genotype	Peptic ulcer(28)	Control (107)	OR (95% CI)	P-value
IL-1B	CC	13 (46.4)	36 (33.7)	1.0	-
	TC	14 (50)	56 (52.3)	0.75 (0.39-1.46)	0.53
	TT	1 (3.6)	15 (14)	0.24 (0.03-1.66)	0.15
	TC+TT	15 (53.6)	71 (66.3)	0.66 (0.34-1.27)	0.30

Conclusion:

Various pathological mechanisms have been suggested in associate with the development of chronic gastritis and peptic ulcer disease (8). Study on cytokine gene polymorphisms has confirmed the previous evidence in supporting the role of inflammation in diseases of the stomach that mediated by inflammatory and anti-inflammatory cytokines. Many studies show that proteins secretion, IL-1β and IL-1Ra varies between individuals that the diversification related with polymorphisms of the IL-1 gene family. The mechanism of gene expression changes associated with these polymorphisms is not well known. Because these polymorphisms are located in the regulatory regions, or in genes encoding regions, may affect the level of IL-1 protein expression and may link with the spread of some diseases. Polymorphisms in these genes are associated with differences in the level of cytokine mRNA in gastric mucosa that have a role in various people clinical outcomes (9). Because of the IL-1B role in the regulation of acid secretion and gastric mucosa protective activity of cells, studies have associated with IL-1B polymorphisms and gastritis and peptic ulcer disease and has also been reported that IL-1B is cell protective molecules and the potent gastric acid secretion limiter (10). According to what was said, we studied the effects of the inflammatory cytokine polymorphisms IL-1B+3954C/T in

connection with the development of peptic ulcer and chronic gastritis. Our results suggest that cytokine allele frequencies had not a significant difference between patients with chronic gastritis and peptic ulcer disease and healthy people. Similar studies with us have conducted in other parts of the world on the other races and countries that in the meantime, some results are consistent with our results and others are inconsistent with this review. For example, in a study by Gonzales and colleagues in 2001 in Spanish patients with peptic ulcers, there was no significant difference in the genotype and allele frequencies IL-1RN and IL-1B+3954 among peptic ulcer patients and the control group (8). Also in the study by Zhang and colleagues that was conducted in 2003 in China, it was found that in areas with high prevalence of gastric cancer, the genotype frequency of IL-1B+3954TT is similar in patients and control group (11).

In another study that was conducted by Mr. Paley and colleagues, there was no significant association between IL-1B+3954T allele carriers and IL-1B-31C allele (12).

In 2003, Gonzales in a study of patients with duodenal ulcer showed that when patients were classified based on gender and age of disease onset, significant differences cannot be seen between IL-1B and IL-1RN genotype, but at the same time carriers of IL-1RN * 2, IL-1B-511 * c, IL-1B-31 * t and IL-1B + 3954 * show a positive genetic risk factor for duodenal ulcer in H. pylori-patients (13).

Among gene polymorphisms IL-1B, two polymorphism of -31C/T and -511C/T have been studied further. So that in Mr. Carrillo and colleagues' study, it was found that IL-1B -511C and -31 T allele and haplotype -511C/-31T and -511T/-31T are associated with increased risk of chronic gastritis and ulcers stomach (14). In another study on the polymorphism of the IL-1B-31 and IL-1RN2 * in the Korean population, there was no association between these polymorphisms and an increased risk of gastric cancer and duodenal ulcer. The examination of haplotypes, there was no significant relation with the disease (7). It was recently reported that people with homozygous genotype TTIL-1B-31 are at increased risk for H. pylori infection and persistent infection (15).

More recently, TNF-308 polymorphism in the TNF promoter region of genes has shown a significant association with susceptibility to duodenal ulcer. In this study, it was found that H. pylori positive patients with TNF-α-308 G/G genotype has an increased risk of duodenal ulcer risk compared to genotype A/A or G/A.

These findings supported the hypothesis that the addition to the IL1 gene cluster, another cytokine gene such as TNF likely plays an important role in peptic ulcer with shifting towards cytokine mucosal (16). These data show that many genetic factors may have a role in clinical outcomes in patients with H. pylori and the pathogenesis of these individuals, that a better understanding of these factors needs more study. In addition, inconsistent conclusions among different studies may be due to a wide range of factors such as the definition of case and control groups, sampling, and study plan. In addition, the small sample size may be the primary cause of inconsistent conclusions.

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