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The role of interleukin-1 gene polymorphisms and *Helicobacter pylori* in gastroesophageal reflux disease

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Original Article

ABSTRACT

Background/Aims: Our aim is to assess the relationship between interleukin 1β (IL-1 β), (-511,-31 alleles), interleukin 1RN (IL-RN), *Helicobacter pylori* (HP) status and gastroesophageal reflux disease (GERD) diagnosed by pH monitoring in the Turkish population.

Materials and Methods: A Total of 100 consecutive patients with GERD were enrolled in the study. Genotypes of IL-1β (-511,-31), IL-1RN gene polymorphisms and HP status of the patients were analyzed.

Results: While thirty-two patients were diagnosed as esophagitis with varying severity the remaining patients had no esophagitis. Seventy six participants were positive for HP and the remaining patients were negative. The difference between erosive and non-erosive groups was statistically significant when we compared IL-1β (-511) but no difference regarding IL-1β (-31) and IL-1RN variations. We also analyzed T/T, C/T and C/C alleles and the difference was significant statistically in T/T allele between patients with and without erosive GERD 1 (3.1%) vs. 12 (17.9%), respectively with a p value <0.05. But C/C, C/T alleles of (-511), (-31) and IL-1RN polymorphisms were not statistically significant between the groups.

Conclusion: IL-1β genetic polymorphisms may take part in the pathophysiology of gastroesophageal reflux disease.

Keywords: Interleukin, gene polymorphisms, GERD, *Helicobacter pylori*

INTRODUCTION

Gastroesophageal reflux disease (GERD) occurs when there is failure in antireflux barriers. GERD produces symptoms due to gastric contents regurgitation. Some studies have shown that environmental, nutritional and patients hereditary differences may play role in pathophysiology of disease (1,2). The importance of the hereditary influences has been shown with regard to erosive esophagitis (EE) among monozygotic twins in comparison with dizygotic twins. Hereditary influences tend to be more essential than environmental ones (3). In addition, ethnic or inter-individual variations could take particular part in the disease outcome.

Interleukin 1 (IL-1), a proinflammatory cytokine, constitutes significant part of immune system and 1 alpha (IL-1α) and 1beta (IL-1β) are two isoforms. Also, IL-1 receptor antagonist (IL-1RN), a non-signaling chemical substance, contests for the receptor binding protein using the functional part of IL-1. Genetics of IL-1α, IL-1ß and IL-1RN form a bunch in the chromosome 2q13 -24. An imbalance between the intensity of IL-1ß and IL-1RN has crucial impact on inflammation in local tissues taking part in numerous illnesses (4). The promoter region of (-511) in the specific IL-1β gene contains bi-allelic polymorphism as a symbol of C/T transition. Participants with IL-1β (-511) T allele have increased values of

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IL-1β. In some researches, an association between IL-1β (-511) T allele and intestinal, digestive and gastrointestinal malignancies has been well established (4,5).

IL-1RN contains intron 2 with five various alleles in which 86-bp variable numbers of tandem repeats (VNTR) polymorphisms take place. The most typical genotypes are the 4-repeat (IL-1RN1) and 2-repeat (IL-1RN2) alleles as well as the remaining alleles are no more than five percent. The specific IL-1RN genotype may be the main determinant related to IL-1β bioactivity together with the IL-1 gene group. The existence of IL-1RN2 is related to higher IL-1RN levels and lower IL-1β allele. This allele has protective effects against duodenal ulcer repetition in older ages (6) and gastric cancer especially the intestinal type when the *Helicobacter pylori* (HP) infection is negative (7). The relationship between these gene polymorphisms and GERD is still unclear. Queiroz et al. (8) showed different effects of these two genotypes in GERD and without HP infection. In the lack of HP, IL-1β (-511) TT was negatively related to GERD (9,10). Chourasia et al. (10) revealed an association regarding IL-1β (-511), IL-1RN-1 and the decreased likelihood of GERD, especially among sufferers with HP infection, most likely due to higher intestinal, digestive and gastrointestinal mucosal IL-1β levels. The results of the study carried out in Japan were very different from the previous published ones. It is concluded that the levels of IL-1β (-511) C were correlated to the higher gastric mucosal IL-1β levels (11). The contradictory results guided the presence of ethnic differences of IL-1gene polymorphisms in patients with GERD.

We carried out this research to evaluate the role of two types of genetic polymorphisms, IL-1β and IL-1RN, in GERD and also investigated the subgroups of these polymorphisms with regard to HP infection in the pathogenesis of GERD.

MATERIALS AND METHODS

Patients

Consecutive adult patients, who had mild heartburn symptoms two or more days a week or moderate to severe symptoms more than one day a week for at least six months duration were prospectively recruited. Individuals were excluded in the presence of gastroenterological surgery, esophageal motility disorders, Barrett's esophagus, benign gastric ulcer, duodenal ulcer, gastroduodenal malignancies and patients on drugs like histamine-2 receptor antagonist (H2RA), prokinetics, proton pump inhibitors (PPI) and anticholinergic agents within the last two weeks of the study Persistent usage of medicines other than those for diabetes mellitus and high blood pressure was also considered as an exclusion criteria.

Ethics

The research was in accordance with the Declaration of Helsinki. All the participants gave written informed consent before the participation and local ethical committee approved the study.

Endoscopic evaluation

From all patients with esophago-gastro-duodenoscopy (EGD), two biopsy samples were gained from the antrum and corpus, and one from each cardia and incisura angularis. From these 6 specimens, 2 of the antrum and 2 of the corpus had been taken in formalin for evaluating the existence of HP (by modified Giemsa staining). The presence of intestinal metaplasia and atrophy was investigated by using Hematoxylin and Eosin (H&E) staining.

While changing the biopsy region, channel of the endoscope was cleaned by 30 cc sterile 0.9% saline. Two skilled pathologists, uninformed about the patients' information, analyzed all biopsies individually and when there was difference, the biopsies had been re-examined by each pathologists until a consensus had been accomplished. Histopathological evaluation was made according to the Advanced Sydney Classification; inflammation, activity, atrophy, density of HP infection, intestinal metaplasia, and dysplasia were determined (12). All these factors except dysplasia were classified as none, mild, moderate and severe. When there were different activity rates, the intense areas of abnormal findings were considered for evaluation. Polymorphisms of IL-1β (-511) and IL-1β (-31) among HP (+) subjects were evaluated depending on the localization. Esophagitis had been rated based on the Los Angeles classification (13).

24-Hours intraesophagial pH monitorization

The drugs that could affect GERD; PPI, H2RA, antacid and prokinetic agents were stopped at least fifteen days before the study. Before pH monitoring, lower esophageal sphincter (LES) level was measured by using water perfused esophageal manometry. A portable pH Monitoring Device (Digitrapper Mark III system; Synetics Medical AB, Stockholm, Sweden) and amonocrystalline antimony pH catheter with 2 sensors (15 cm spaced) were used. Before each test the device was programmed and then calibrated in pH: 7.01 and 1.07 solutions. The catheter was passed trans-nasally into the esophagus and the distal sensor of the pH catheter was placed 5 cm above the lower border of the LES. Patients were sent home and 24 hours pH recordings were obtained during their normal daily life. Patients were advised to sleep only at night, not to take acidic foods, alcohol, and drugs. Reflux episode was defined when pH dropped below 4, and the episode ended when pH increased above 4. Recordings were analyzed by the computer. Total reflux time (Time for pH less than 4.0), count of reflux periods, long reflux episode number (>5min), the duration for the longest reflux episode and DeMeester scores were the parameters calculated during supine position, upright position and both. pH monitoring results were considered abnormal if they were higher than the normal values previously mentioned (14).

Evaluation of gene polymorphisms of IL-1β (-511, -31) and IL-1RN

Blood samples were put into the bottles containing 72 µL 7.5% concentrations of ethylenediaminetetraacetic acid (EDTA)

Table 1. Demographics and clinical features of patients

	GERD	Erosive GERD	Non-Erosive GERD	p
Age (mean ± SD)	40.8± 12.1	41.5±11.9	40.5±12.2	0.87
HP presence	76	23 (71.9%)	53 (77.9%)	0.50
Pyrosis	75	27 (84.4%)	48 (70.6%)	0.36
Chest pain	44	13 (40.6%)	31 (45.6%)	0.94

GERD: gastroesophageal reflux disease; HP: Helicobacter pylorit

(K3EDTA; BD Vacuteiner system, New Jersey, USA). For DNA isolation 200 µl of blood from the samples was used and purificated by DNA isolation kits (Invitek GmbH; Invisorb, Berlin, Germany). After these steps, nucleotides in the alleles of IL-1β (-511 and -31) and repeated oligonucleotide primers of nucleotides in the IL-1RN gene became available. For the determination of polymorphisms of IL-1β and IL-RN, polymerase chain reaction (PCR) consisted of 16mM (NH4) 2SO4, 67 Mm, TrisHCL pH 8.8, 0.01% Tween-20, 1.5 Mm MgCl2, 200 µM for each deoksynucleotide triphosphate, 0.026 U/ µL TaqDNA polymerase enzyme (Taq DNA; Bioron, Ludwigshafen, Germany) and nearly 0.5 µg mold of DNA were put in each tube. All the tubes were kept for 3 minutes of denaturation in the thermal cycler in 94°C, 40 cycles in 95°C for 15 second, in 55°C for 45 second and in 72°C for 60 seconds. For the last elongation, they were kept for 7 minutes in 72°C. Then the obtained PCR and endonuclease degradation products were electrophoresed by 5V/cm potential difference in the agarose of 2% concentration of 90 mM Tris-borat and mM EDTA buffer. After 30 minutes of electrophoresis, the gels were stained in 0.5 µg/mL concentrated ethidiumbromide of 90 Mm Tris-borat and 1Mm EDTA buffer for 15 minutes.

Statistical analyses

Statistical analysis was done by using the SPSS version 17 (Statistical Package for Social Science; Chicago, IL, USA). Categorical data were compared by using ki-square and Fisher's exact tests. The factors were investigated by visual (histogram, probability plots) and analytical methods (Kolomogorov-Smirnov/Shapiro-Wilk'stest) to identify the normality of distribution. Medians and interquartile ranges for non-normally distributed and ordinal variables were used for descriptive analysis. For non-normally distributed variables between groups the Mann-Whitney-U test was performed. P values associated with <0.05 had been regarded as statistically significant.

RESULTS

One hundred consecutive adult patients with gastroesophageal reflux disease diagnosed by pH monitoring were enrolled in the study (42 male and 58 female). The mean age was 40.8±12.1 (17 years-71 years). Thirty two patients were diagnosed as having esophagitis and the other patients didn't have any esophagitis. Twenty patients had grade A esophagitis, 11grade B, and 1 patient had grade C esophagitis. There was no patient with grade D esophagitis. Seventy six patients were positive and 24 were negative regarding the presence of HP. Twenty three patients with esophagitis were positive for HP (71.9%) while 53 patients without esophagitis (77.9%) were positive for HP but difference was not significant statistically (p=0.50). Table 1 represents the demographic features of the patients.

Table 2. Distribution of IL-1β (-511, -31) and IL-1RN genotypes depending on HP status of patients

Polymorphisms		*H. Pylori* (+) (n=76)	*H. Pylori* (-) (n=23)
IL-1β (-511) n (%)			
	C/C	19 (25%)	9 (39.1%)
	C/T	46 (60.5%)	12 (52.2%)
	T/T	11 (14.5%)	2 (8.7%)
IL-1β (-31) n (%)			
	C/C	9 (11.8%)	2 (8.7%)
	C/T	48 (63.2%)	10 (43.5%)
	T/T	19 (25%)	11 (47.8%)
IL-1RN n (%)			
	1/1	50 (65.8%)	15 (65.2%)
	1/2	13 (17.1%)	7 (30.4%)
	2/2	10 (13.2%)	1 (4.3%)
	1/4	2 (2.6%)	0 (0%)
	2/4	1 (1.3%)	0 (0%)

IL: Interleukin; HP: *Helicobacter pylori*

The presence of HP in the antrum and corpus was compared between the patients with and without esophagitis, but not significant statistically (p>0.05). The DeMeester scores of the groups were 26.7±20.1 vs. 13.5±13.3, respectively, p<0.001. Of the 100 patients who participated in the study, gene analysis was done for 99 patients (42 male and 57 female). In Table 2 the comparisons of IL-1β (-511, -31) and IL-1RN between the patients with and without HP groups is shown.

There was no statistically significant difference when we compared IL-1β (-511, -31) in C/C, C/T, T/T genotypes and IL-1RN depending on frequencies between the groups regarding the presence of HP, respectively, p=0.38 and p=0.11. We compared IL-1β (-511) between erosive and non-erosive GERD groups, and the difference was significant statistically (p=0.045), but

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despite low levels of IL-1β (-31) in erosive group it was not significant (p=0.11). We evaluated IL-1RN gene polymorphism between erosive and non-erosive groups but the comparison did not reach statistically significance difference (p=0.53). All the distributions of gene polymorphisms of IL-1β (-511) C/C, C/T, T/T, IL-1β (-31) C/C, C/T, T/T and IL-1RN between groups are shown in Table 3.

Among HP positive patients, the relationship between the corpus and antrum atrophy scores and IL-1β (-511) C/C, C/T, T/T, IL-1β (-31) C/C, C/T, T/T genotypes was investigated with no significant difference as seen in Table 4.

DISCUSSION

By designing this prospective research, we proposed to assess wether there is a correlation between IL-1 gene polymorphisms, HP status and GERD, in the Turkish population. As the result of analysis, a significant relationship between IL-1β (-511) T/T genetic polymorphism and the presence of GERD was found. Our study revealed that IL-1ß (-511) T/T genotype and T allele were related to an increased likelihood of GERD in comparison to the existence of CC genotype and C allele. One of the most important results of our study was the lower existence of IL-1β (-511) T/T genotype frequency in patients with erosive GERD group compared with non-erosive group. Although IL-1β (-31) C/C frequency was lower in erosive group, the difference was not statistically significant between groups. And also there was not any difference in proinflammatory IL-RN frequencies between groups. The lower frequencies of IL-1β (-511) T/T in the erosive group was suggestive that the patients with gastric atrophy might have higher frequencies but in the present research there was not any difference regarding this genotype in HP positive patients with or without gastric atrophy. This shows that IL-1β (-511) T/T frequency might increase predisposition to gastric atrophy due to other factors.

Particular cytokines, IL-1β and IL-1RN, are powerful mediators taking place in the inflammatory process and the imbalance between them may take part in chronic inflammation (15). Hereditary polymorphisms among these inflammatory cytokines might have specific effects on the actual inter-individual variations and GERD. Still the consequences of hereditary aspects upon GERD pathogenesis is unknown. Some recent researches revealed the association between specific proinflammatory polymorphisms in genes and GERD. IL-1β (-31) polymorphic allele and IL-1RN2 are demontrated to be negatively correlated to GERD (9). In a well organized study it has been shown that there is not any difference of IL-1β (-511) polymorphism among patients with Barrett's esophagus, GERD or gastric adenocarcinoma (10). Among healthy persons, important inter-individual variations were seen in pro-inflammatory proteins especially in in-vitro production (9). Numerous reseraches have shown the impact of polymorphisms of IL-1β and IL-1RN on the development of GERD (16). As different patterns are observed among the patients from India (10), Japan (11), it is estimated that there

Table 3. Distribution of IL-1β (-511, -31) and IL-1RN genotypes between patients with erosive and non-erosive GERD

Polymorphism		Erosive GERD (n=32)	Non-Erosive GERD (n=67)	p
IL-1β (-511) n (%)				
	C/C	13 (40.6%)	15 (22.4%)	
	C/T	18 (56.3%)	40 (59.7%)	0.045*
	T/T	1 (3.1%)	12 (17.9%)	
IL-1β (-31) n (%)				
	C/C	1 (3.1%)	10(14.9%)	
	C/T	18 (56.3%)	40 (59.7%)	0.11
	T/T	13 (40.6%)	17 (25.4%)	
IL-1RN n (%)				
	2	10 (31.2%)	22 (32.8%)	0.53
	Others	22 (68.8%)	45 (67.2%)	

(*) p<0.05 was statistically significant
GERD: gastroesophageal reflux disease

Table 4. IL-1β (-511) and IL-1β (-31) gene polymorphisms in HP (+) patients according to localization and inflammation

		IL-1β (-511)				IL-1β (-31)			
		C/C	C/T	T/T	p	C/C	C/T	T/T	p
Corpus	Inflammation (+)	25%	60.7%	14.3%	0.99	10.7%	66.1 %	23.2%	0.67
	Inflammation (-)	25%	60%	15%		15%	55 %	30%	
	Atrophy (+)	38.5%	46.2%	15.4%	0.43	7.7%	53.8 %	38.5%	0.45
	Atrophy (-)	22.2%	63.5%	14.3%		12.7%	65.1 %	22.2%	
Antrum	Inflammation (+)	21.1%	63.2%	15.8%	0.69	10.5%	68.4 %	21.1%	0.80
	Inflammation (-)	26.3%	59.6%	14%		12.3%	61.4 %	26.3%	
	Atrophy (+)	21.1%	63.2%	15.8%	0.89	10.5%	68.4 %	21.1%	0.85
	Atrophy (-)	26.3%	59.6%	14%		12.3%	61.4 %	26.3%	

IL: Interleukin; HP: *Helicobacter pylori*

might be ethnic variations and genetic polymorphisms and their effects on GERD. The results of these studies have claimed an association between IL-1ß (-511), IL-1RN genotypes and the pathogenesis of GERD. The results of the studies about this topic are still in debate therefore, in the future studies among different ethnic populations should be carried out.

Chorousia et al. discovered that the existence of the IL-1ß (-511) T/T allele might be related to improved intestinal, digestive, gastrointestinal mucosal IL-1ß amounts resulting in gastritis (corpus gastritis) and decreased acid-secreting capability of the stomach and a decreased likelihood of GERD (10). Furuta et al. found the patients with IL-1β (-511) T/T genotype had a higher acid liquid pH and also higher score of atrophy in Japanese population (6). But Xuan et al. couldn't find any correlation between IL-1 β (-511) T/T, IL-1 β (-31) and IL-RN genotypes and gastric atrophy (17). In this research, IL-1β (-511) T/T genotype was lower among erosive GERD. Also the results of our study were compatible with the previously published studies.

Between participants with erosive and non-erosive GERD there was no difference depending on IL-1β (-31) C/C and IL-1RN 2/2 genotype frequencies. In the study of Queiroz et al. among HP positive patients, IL-1β (-31) C/C and IL-1RN 2/2 genotype frequencies were lower in erosive group when compared with non-erosive group, but among HP negative patients there was no difference between groups (8). A recent study from Taiwan concluded that, IL-1β (-511) T/T (-31) C/C genotypes, IL-1β (-511) T and (-31) C alleles had been almost all related to an increased likelihood of the presence of reflux esophagitis (18). In addition, it has been often demonstrated that the existence of HP infection together with proinflammatory IL-1ß (-511) T/T allele is correlated to decreased likelihood of GERD by inducing atrophy in the gastric corpus (9,10).

With this particular research we purposed to assess the correlation between IL-1β (-511) C/C, C/T, T/T, IL-1β (-31) C/C, C/T, T/T and IL-RN gene polymorphisms and GERD in the Turkish population. We concluded that IL-1β (-511) T/T genotype may take part in the pathophysiology of the erosive GERD. In the future, population based clinical trials with larger series should be carried out among different ethnicities to confirm this association.

Conflict of interest: No conflict of interest was declared by the authors.

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