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Interleukin-8 251- A/T polymorphism related to peptic ulcer disease in *H. pylori* infected patient

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Objectives This study includes the investigation of antifungal activity of the local propolis against dermatophytes and yeast.
Methods A total of 92 tissue samples were taken from patients infected with *H. pylori* and 102 from uninfected individuals has been included. Genetic method used for the detection of *H. pylori* infection by polymerase chain reaction (PCR) for *glM* gene identification. IL-8-251 A/T polymorphism was detected by allele-specific oligonucleotide polymerase chain reaction (ASO- PCR).
Results Among the studied samples, the results improve that genetic polymorphism of IL-8-251 A/T genotype was found in a higher frequency with confidence interval 95% in duodenal ulcer *H. pylori* infected patient than control.
Conclusion IL-8-251 A allele notice that it has been associated with severe inflammation lead to increase severity of disease.
Keywords *Helicobacter pylori*, molecular biology (PCR), allele-specific oligonucleotide.

Introduction

Helicobacter pylori, is a bacterium that infects the stomach of humans, and strongly associated with gastroduodenal diseases such as chronic atrophic gastritis, peptic ulcer,[1,2] and gastric cancer.[3,4] Although this bacterium has been classified as a carcinogen in human, keys of pathophysiological events in *H. pylori* infections is the induction of the inflammatory response in the gastric mucosa, which is mediated and also regulated by inflammatory cytokines produced by gastric epithelial cells.[5,6] Interleukin-8 (IL-8) was a small peptide (chemokine) secreted by a variety of cell types, which serves as a potent inflammatory mediator recruiting and activating neutrophils. Interleukin-8 (IL-8), was a potent chemo attractant for neutrophils and lymphocytes, has been reported as a strong stimulator of angiogenesis in gastric adenocarcinoma.[7,8] Several studies have demonstrated that *H. pylori* strains are capable of inducing IL-8 secretion from gastric carcinoma cells *in vitro*.[9,10] Others researcher noticed that IL-8 is typically secreted by gastrointestinal epithelial cells in response to pathogenic bacteria.[11] Genetic polymorphisms that affect innate immune response genes have also been linked to an increased risk of gastric cancer, and the levels of IL-8 are directly related to the severity of gastritis.[12]

The genetic polymorphism of IL-8-251 A allele notice to be associated with higher IL-8 production, more severe inflammation, mucosal atrophy, and also intestinal metaplasia when compared with the IL-8-251 TT genotype of *H. pylori*-infected patients. The recent studies suggested a possible association of the IL-8-251 A allele with angiogenesis and inflammation in gastric carcinogenesis in *H. pylori*-infected Koreans [13,14]

Materials and Methods

Patients and gastric biopsy samples

194 tissue samples included, 128 women and 66 men with age range from 15 to 75 years (mean age 36 years). They were presented with dyspepsia and referred to the Esophago Gastroduodeno Scope. The endoscopic diagnosis was grouped into three categories: firstly was peptic ulcer disease (PUD) patients who had endoscopic lesions of ulcers, secondly the non-peptic ulcer disease patients (NPUD) were defined as patients who had endoscopic with no lesions of ulcers but they have disease like gastritis or gastric atrophy or gastropathy, and lastly, the control group was defined as they did not have any type of gastric or duodenal disease.

Two tissue biopsies were obtained from antrum. Rapid urease test was performed on one of the antral biopsies at the time of endoscopy. The other biopsy specimens placed in 1 ml of normal saline. The biopsy specimen was preserved immediately at −20°C for molecular analysis.

DNA extraction and PCR amplification conditions

Total DNA extracted directly from gastric biopsy samples using tissue protocol (Geneaid, Korea). The final volume of DNA extraction product was 200 µl, with final concentration 1.25 ng/µl.

Identification of H. pylori glM gene

To confirm the presence of *H. pylori,* DNA in biopsies amplification and melting conditions were optimized for the PCR assay by using specific primer sequences for gene *ureC (glmM)* 294 (bp) PCR selected were as follows: *glmM* – forward primer (5′- AAGCTTTTAGGGGTGTTAGGGGTTT -3′), and *glmM*–reverse primer (5′-AAGCTTACTTTCTAACACTAACGC -3′). PCRpremix™ kit from (Bioneer, Korea) was used to amplify the mentioned gene.

Total reaction volume of 20 µl containing, 3 µl of extracted DNA, 1 µl of 10 pmol/µl of each forward and reverse primers for *glm*M gene in addition to 14 µl of molecular biology grade water then the mixture was added to lyophilized PCRpremix™ formula.

Genotyping of IL-8 251A/T polymorphism gene

(ASO) PCR techniques used for the detection of IL-8 251 polymorphism. It is a simple method to detect of mutation involving single base changes or small deletion. It depends on the sequence of specific primer (specific PCR primer) that

allows the amplification of test DNA only when target allele is contained with samples following reaction in the presence or absence of target allele.

PCR working solution

The master mix component for the detection of IL-8 251 polymorphism gene adopted by ARMS PCR as shown in Table 1.

PCR protocol

PCR was performed in a thermo cycler under the following conditions adopted in Table 2.

PCR amplification analysis

IL-8 - F 1	CCA CCA TTT GGT GAA TTA TCA AT	336	Eskandar et al. 2006
IL-8 - F 2	CCA CCA TTT GGT GAA TTA TCA AA		
IL-8 - R	TGC CCC TTC ACT CTG TTA AC		

Table 1. **The master mix components of PCR used for detection of *IL-8 251 A/T* polymorphism**

Amount (µl)	Concentration	Component
7.0		Deionized water
2.0	2X	PCR Buffer
1.5	10X	PCR F-1 Primers
1.5	10X	PCR R Primers
1	5 U/µl	Taq DNA Polymerase
0.5		dNTP
1.5		$Mgcl_2$
5.0	(200 ng/ µl) 10X	Specimen DNA or Control DNA from kit
20		Total volume

Table 2. **The PCR protocol for detection of gene**

No. of Cycles	Time	Temperature (C°)	Step	No.
1	1 min	94	Initial denaturation	1
	60 sec	57	Initial annealing and extension	
35	60 sec	93	Denaturation	2
	60 sec	72	Annealing and extension	
1	5 min	72	Final extension	3

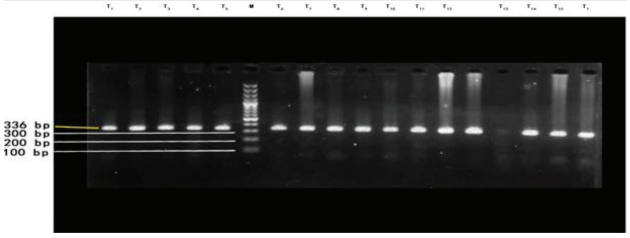

Fig.1 **Representative results relating to the IL-8 genotyping. ASO PCR was used. By means of the allele-specific primers, the homozygote mutant (AA), and the heterozygote (AT) and the homozygote TT variants. The image is from a representative gel electrophoresis of PCR amplification products of IL-8 gene (336 bp) were distinguishable. M: marker of the DNA.**

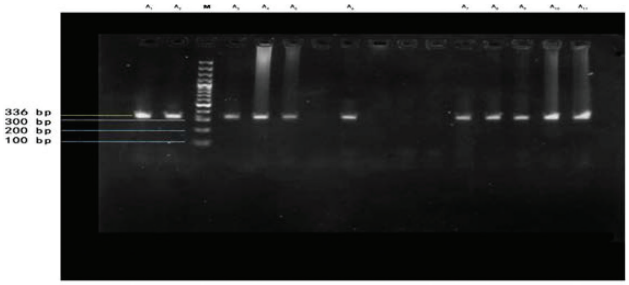

Fig. 2 **Representative results relating to the IL-8 genotyping. ASO PCR was used. By means of the allele-specific primers, the homozygote mutant (AA), and the heterozygote (AT) and the homozygote TT variants. The image is from a representative gel electrophoresis of PCR amplification products of IL-8 gene (336 bp) were distinguishable. M: marker of the DNA.**

Table 3. **Distribution of disease group among positive and negative *H. pylori* infected samples**

Groups	*H. pylori* +ve	*H. pylori* –ve	Total
PUD	29	8	37
NPUD	46	56	102
C.A	0	2	2
Control	17	36	53
Total	92	102	194

Table 4. **Prevalence of IL-8-251 A/T polymorphism among positive and negative *H. pylori* infected samples**

All	*H. pylori* –ve	%	*H. pylori* +ve	%	Total
	(N = 194)		(N = 102)		(N = 92)
AA	4	3.9%	0	0%	4
AT	86	84.3%	92	100%	178
TT	12	11.8%	0	0%	12
Total	102	100%	92	100%	194

Table 5. **The presence of the genotype polymorphisms of IL-8251 A/T gene in *H. pylori*-positive patients among disease groups**

Groups	*H. pylori* +ve N (%)	Total
PUD	29 (78%)	37
NPUD	46 (45%)	102
C.A	0 (0%)	2
control	17 (32%)	53
Total	92 (47%)	194

Statistical analysis

The Chi-square test or Fisher's exact test was used to compare between proportions. The values of $P < 0.05$ were considered statistically significant. A prevalence ratio (PR) with a 95% confidence interval (CI) was calculated to evaluate the relationship between IL-8 251A/T genotyping polymorphisms with gastroduodenal diseases and with *H. pylori* infection.

Results

In this study, a total of 141 patients who were affected with different forms of gastric diseases [37 of them had peptic ulcer diseases (PUD) and 102 had non-peptic ulcer disease (NPUD) and 2 patients had gastric cancer (C.A)]. were compared with 53 who had just epigastric pain while their endoscopic examinations revealed, they were regarded as a control group.

The presence of the genotype polymorphisms of IL-8251 A/T gene in *H. pylori*-positive patients among disease groupe show in Table 5. In this table from total 92 IL-8251 A/T gene in *H. pylori*-positive samples all peptic ulcer disease grouped and all non-peptic ulcer disease grouped show IL-8251 A/T gene, improve that there was significant difference in contrast to the control grouped.

Discussion

Polymorphism in the IL-8 (–251) has been associated with the increased expression of IL-8 (15). Several papers have demonstrated an association between IL-8 (-251) polymorphism and an increased risk of developing gastroduodenal diseases.[16]

A significantly higher frequency of the IL-8 251AT genotype was observed among the *H. pylori*-positive DU patients than among the *H. pylori*-positive healthy subjects without gastrointestinal problems. This genotype reflects a higher IL-8-producing ability.[17] The association with IL-8 was higher reconnoitered at the level of a single SNP (-251 A/T). The higher incidence of the -251 AT genotype with a concomitant higher IL-8-producing potential[18,17] highlights the importance of the genetic determination of IL-8 production in *H. pylori*-induced DU.

We observed a greater frequency in the AA allele genotypes among patients with C.A stomach than in the control group or in other disease group, with the presence of allele A being associated with the risk of developing gastric cancer. Other researcher noted that presence of the A allele in the -251 position of the IL-8 gene was associated with an increase in the risk of stomach cancer in Japanese, Korean, Chinese and Iranian populations.[13] Additionally, some studies have demonstrated that patients carrying the A allele present higher production of IL-8, leading to alteration in the quality and intensity of inflammatory responses produced by the host after exposure to *H. pylori*.[13,19]

Conversely, the frequency of the TT genotype (with a relatively low IL-8-producing potential) was significantly higher among the *H. pylori* negative, healthy individuals. This suggests the possibility that a relative protection from DU disease is observed in association with the TT genotype. This observation is consistent with the results of,[20] who concluded that *H. pylori*-positive healthy individuals with the IL-8-251 TT genotype might display a milder inflammatory reaction.

Conclusion

Regarding IL-8-251 A/T polymorphism was related to associated to the increased expression of IL-8 and increase severity of the disease.

Conflict of Interst

None.

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