The Relationship of Pepsinogen-II Ins/Del Gene Polymorphism in The Genetic Susceptibility to Peptic Ulcer Caused by H. Pylori Infection

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Abstract

Background Polymorphism in the gene of pepsinogen-II (PG-II) and its serum level are effective biomarkers forterminal differentiation of gastric mucosa into gastritis, intestinal metaplasia (IM), peptic ulcer and gastric cancer (GC) inrelationship to Helicobacter pylori infection.
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 PG-II with (PCR) and Gel electrophoresis using 2.25%, 2.5% the concentration of agarose was (respectively) investigated.Results PG-II 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. 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. Conclusions Carriage of the L allele of the PG-II100 bp ins/del polymorphism and elevated levels of PG-IIare associated with peptic ulcer , particularly with H. pylori infection.

Key Words: Pepsinogen II polymorphism _ Helicobacter pylori _ peptic ulcer.
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Introduction

Pepsinogen C (PGC) belongs to the aspartic protease family and is secreted by gastric chief cells. PGC could beactivated to pepsin C and digests polypeptides and amino acids, but as a zymogen PGC's functions is unclear. Innormal physiological conditions, PGC is initially detected in the late embryonic stage and is mainly expressed ingastric mucosa[1].
Pepsinogen is the inactive precursor of pepsin. Two groups have been immunochemically andbiochemically identified. Pepsinogen A (PGA) is localized mainly in the fundus and pepsinogen C (PGC) is found throughout the stomach and in the proximal duodenum [2].
Pepsinogens areconsidered as effective markers of terminal differentiation of the stomach mucosa, and also of preneoplastic and neoplastic changes in this tissue [3]. As far as we are aware, there are few studies of pepsinogen A and C gene polymorphisms and the relationships between polymorphisms and serum pepsinogen I and II levels have not been investigated. This study clarifes the association between the pepsinogen Cgene polymorphism and serum pepsinogens I and II . The three major etiological factors for development of GC are Helicobacter pylori infection, genetic susceptibility, and dietary factors. Recently, severalhost genetic polymorphisms have been regarded as potential factors contributing to development of GC. The human pepsinogen (PG)-II (or C) gene is located in the 6p11–6p21.3 region of chromosome 6 and

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contains a100-bp insertion/deletion (ins/del) polymorphism betweenexons 7 and 8 [4],[5] .
This polymorphism has emerged inrecent years as an important determinant of disease susceptibilityand severity[6]. Different allelic forms of PG-IIgene polymorphism may alter its serum levels, and the changes in PG-II levels are associated with several gastricdiseases such as gastritis, peptic ulcer, and GC.[7]. H. pylori infection is a known factor, influencing interindividual variation in PG-II levelsand the risk of GC [8].

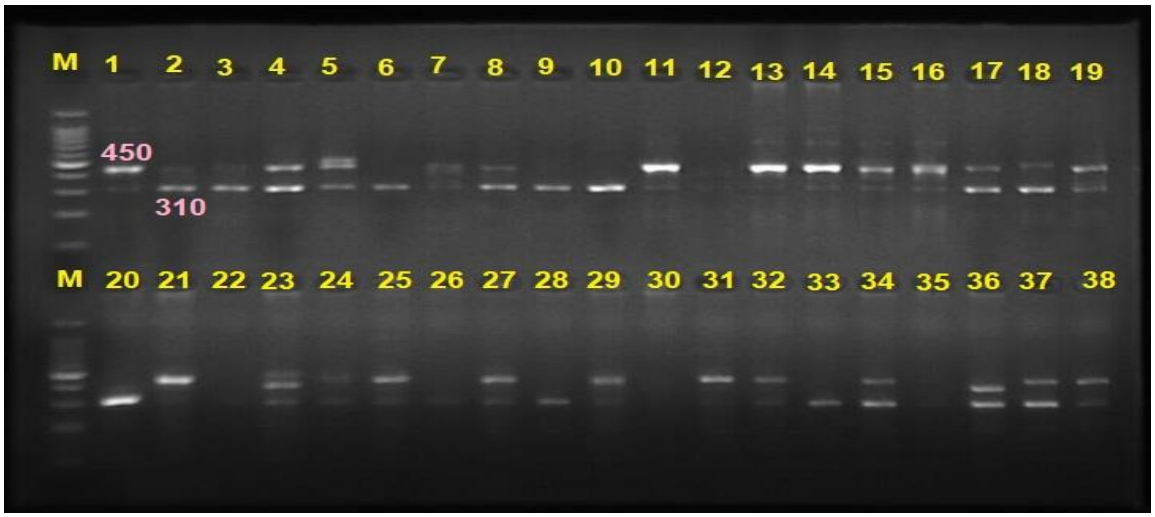

Figure (4-4) Electrophoresis of the Pepsinogen-II gene variant of deletion/addition type Route M represents a volumetric feature of 100 base pairs: Routes 1, 11, 13, 14, 29 and 31 represent homologs of the long alleles: Routes 2, 3, 6, 9, 10, 20, 26, 28, and 33 Represent the genotype of the short allele: pathways 4, 5, 7 and 8 And 15, 16, 17, 18 and 19 represent the heterogeneous genotype containing both the short and long alleles together, pathways 12, 22, 30 and 35 null pathways.

Materials And Methods

We screened 100 people (50 people with chronic stomach ulcers caused by H. pylori and 50 people who did not).
The DNA genome was extracted by kettextraction
Pepsinogen C (PGC) gene polymorphisms were identified by PCR (poloymerase chain reaction), which amplifies the DNA in the region in the intron between exons 7 and 8. Two primers (PGCf: AGCCCTAAGCCTCTTTTTGG,
PGCr:GGCCAGATCTGCGTGTTTTA) were used as described [5]. The PCR reaction proceeded in atotal volume of 30 ll containing 200 ng human genomic DNA , PCR amplifcation consisted of 37cycles of 30 s at 94 °C for denaturing, 40 s at59 °C for annealing and 2 min at 72 °C for extension. The PCR products were resolved by electrophoresis on a 6%polyacrylamide gel.

Analyzing

Gel electrophoresis was performed on 2.25% agarose gel for PG-II gene polymorphism, containing 5 µl red safe. The gel was analyzed and genotypes determined using transilluminator.

Statistical Analyses

Potential associations of PG-II with the risk of H.pylori infection were analyzed by comparing, PG-II 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 PG-II, The PCR results showed the polymorphism of PG-II displayed L and S alleles and three genotypes LL, LS and SS) Fig. 1. The L allele results in an undigested polymerase chain reaction product of bp,450 (hetero),and S allele contributed to a digested PCR product of 310 bp fragment, while the L/S genotype resulted in 450, 310 bp (homo) .

Table 1. Allele and Genotype frequencies of PG-II gene polymorphism and negative H.pylory patients (uninfected controls)

Genotype PG-II	Patients	control	P -value	OR	Ci=95
SS	13(6.5%)	6(3%)			
SL	34(17%)	13(6.5%)	0.48	1.20	(0.37-3.84)
LL	3 (1.5%)	31 (15.5%)	0.0001*	0.04	(0.01-0.20)
Totall	50	50			
Allele					
S	60	25			
L	40	75	0.017*	2.00	(1.09-3.65)

fragments. Revealed that 3 (1.5%)patients infected with H.pylori has LL Allele, and 34(17%) patients infected with H.pylori has SL, while 13(6.5%)patients has SS allele which consider as mutant, compared with the control group, 31 (15.5%) containing the LL allele and 13(6.5%) containing the SL allele,and 6(3%) contain the SS allele (Table 1). There were significant differences between the two Patients groups and the control group, concerning the in PG-II polymorphism. Thecarriage of PG-II L allele was associated with increased risk of peptic ulcers (p =0.017; OR = 2.00; 95% CI: (1.09-3.65)) and, the homozygous PG-II SL genotype was associated with increased risk of peptic ulcers (p = 0.48; OR = 1.20; 95% CI: (0.37-3.84)). Also, PG-II L LL genotype was associated with increased risk of peptic ulcers (p = 0.0001*; OR = 0.04; 95% CI: (0.01-0.20)).

Discussion

H. pylori infection has been associated with a long-term risk of gastroenteritis and carcinogenesis [9]. Pepsinogen (PGC) plays an important role in maintaining cellular differentiation during gastric carcinogenesis. This study aimed to evaluate the role of the PGC tag SNPs and their interactions H. pylori in the development of gastric cancer and its precursor, atrophic gastritis [10]. A series of host genes that respond to H. pylori infection are involved in the process of gastric inflammation and carcinogenesis. Whereas, interactions between H. pylori-related gene polymorphisms, including PGC, have been associated with gastric cancer susceptibility. Moreover, H. pylori infection affected the cumulative effect of the PGC polymorphism (PGC) [11]. Allele L of the PG-II polymorphism H. pylori infection, increased the risk of peptic ulcers Caused by infection H. pylori . 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. These results contradict previous studies, as in the study Sun etal., that the PGC polymorphism was associated with the risk of developing peptic ulcer and patients with gastric carcinoma (GC) [12]. In recent years, Kumar etal., have concluded that PGC may raise the homozygous 1 allele shown in the ss genotype from serum PGC levels in patients with GC, especially in patients with H. pylori infection and intestinal metaplasia [13]. However, Pinto-Correia etal., Explain that the homozygous allele 1 PGC of the SS genotype was related to the up-regulation of PGC expression to serve as a protective factor in the development of gastric disease [14]. In the current study, it was found that the LL genotype is a protective factor from the disease, and this difference may be explained by the fact that the study was limited to a specific geographical spot, and people in this geographical spot are distinguished by this genetic pattern.

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