Relationship Between Polymorphism in Promoter Region of E-Cadherin (Cdh1) Gene and Helicobacter Pylori Infection in Kurdish Population of Iran

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Abstract: Infection with Helicobacter pylori (H. pylori) in addition to inflammation in gastric mucosa and much mortality may progress to gastric cancer. Gastric cancers like many other cancers have a multistage process and cause obvious changes in environmental, genetic and epigenetic factors. Polymorphism in promoter region of CDH1 gene has been associated with reduced E-cadherin protein expression. It has been proposed that H. pylori infection may cause multiple nucleotide changes in CDH1 gene. **Aimed**: We evaluated the association between -160 (C>A) CDH1 gene polymorphism with H. pylori infection in Kurdish population. **Methods**: A total of 162 biopsies taken from non-ulcer dyspepsia patients were classified as H.pylori infected and H.pylori uninfected. All previous diagnoses confirmed pathologically and molecularly. Polymorphism in -160(C>A) CDH1 was evaluated by PCR-RFLP. **Results:** Infection with H. pylori was found in 67% of patients. H. pylori infection was more frequently found in biopsies with the -160C/A genotype than those with the -160C/C (*P* value = 0.01). **Conclusion:** 160C/A genotype might require H. pylori infection to promote the inactivation of CDH1. This suggests that H. pylori infection might affect gastric cancer (GC) in an initial stage.

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Introduction

E-cadherin (E-cad, also known as CDH1) is a member of a family of transmembrane glycoproteins expressed on epithelial cells and is responsible for calcium-dependent cell-to-cell adhesion[1]. E-cad forms complexes and connects actin filaments with α-, β-, and γ-catenins[2,3], which is crucially involved in neoplastic transformation and metastasis[4,5]. Loss of cell adhesion may contribute to loss of contact inhibition of growth, which is an early step in the neoplastic process. Furthermore, loss of cadherin activity may result in cancer cell detachment and metastasis [6,7]. Gastric carcinogenesis is a multistep process with morphological progression involving multiple genetic and epigenetic events, whereas CDH1 is an important putative tumor suppressor gene. In gastric carcinoma (GC), the percentage reduction in E-cad expression varies from 17% to 92%, and is more frequent in diffuse type carcinomas than in intestinal types[8-13]. Germ-line mutation of the CDH1 gene is found in familial GCs[14,15]. Somatic mutations of E-cad are found in more than 50% of gastric carcinomas of the diffuse type[16-18]. In addition to the classic two-hit inactivation mechanism, CDH1 can be silenced by cytosine-guanosine (CpG) sequence methylation in GC[12,17,18]. Moreover, Li et al., reported that -160C/A polymorphism has a direct effect on the transcriptional regulation of CDH1[21,19]. Helicobacter pylori (H. pylori) is the single most important etiological factor for gastric cancer development. The strongest evidence comes from three independent, nested case-control studies in which pre-existing infection markedly increased the risk of GC[20-22]. It is estimated that approximately 60% of all GC cases can be attributed to H. pylori infection[20]. However, there are few data in the molecular profiles of E-cad of H. pylori-positive and H. pylori-negative GCs[23]. El-Omar et al.[24], reported that interleukin-1 polymorphisms that cause the up-regulation of interleukin-1β with H. pylori infection are associated with an increased risk of GC. Moreover, Hmadcha et al.[25], found that interleukin-1β might induce gene methylation

through the production of nitric oxide and the subsequent activation of DNA methyltransferase. It is possible that H. pylori infection induces methylation through the production of interleukin-1β. In this study, we investigated the relationship between H. pylori infection and -160C/A polymorphism is germ line, we also investigated the relationship between H. pylori infection and polymorphism, to find if H. pylori infection affects tumorigenesis in the initial stage.

Subjects and methods

162 Kurdish patients with nonulcer dyspepsia patients who underwent endoscopy in Hospital and cancer center of Kurdistan University of medical sciences, Kurdistan, Iran, were consecutively recruited from 2004 to 2009. All diagnoses were pathologically confirmed. In patients group *H.pylori* infected and uninfected subjects were determined by the RUT, PCR 16srRNA (1) examination of biopsies taken from the corpus. Patients were classified as *H.pylori* -infected only if the two tests were positive and *H.pylori*-uninfected if the two tests were negative, respectively. Demographic and clinical data were obtained from subjects through interview using a standard clinical pro forma. Exclusion criteria included history of nonulcer dyspepsia patients, liver disease, and previous treatment with nonsteroidal anti-inflammatory drugs, proton pump inhibitors, antibiotics, or bismuth salts. Informed consents for participation were signed by all subjects. The study protocol was approved by the Clinical Research Ethics Committee of the Kurdistan University of Medical Sciences.

Histological examination

Sections of biopsy specimens were embedded 10 % buffered formalin and stained with hematoxylin and eosin to examine gastritis and with giemsa to detect *H.pylori* (2). The histological examination of gastric mucosa were blindly performed according to the Updated Sydney system (3)***DNA isolation***

DNA of case was extracted from biopsies taken from the corpus using Biospin Tissue genomic DNA Extraction Kit (Bio Flux, Japan). All extracted DNA was resuspended in UltraPure RNAse/DNAse-Free Distilled water.

Genotyping for -160 (C>A) CDH1 polymorphism

Genotyping analysis of -160 (C>A) CDH1 were performed by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP).Primer sequences for -160(C>A) variation of CDH1 gene are as follows: sense 5´-TGATCCCAGGTCTTAGTGAG-3´, anti-sense 5´-AGTCTGAACTGACTTCCGCA-3´. The PCR amplification was performed in a total volume of 25 µL mixture containing: 100 ng genomic DNA, 1.0 mM of each primer, 200 mM of each dNTP, 2.0 mM of MgCl2 and 1.0 U Taq DNA polymerase and 10 X Taq buffer (Fermentas) using the Biometra Tgradient 96 (Biometra, Germany). PCR conditions were as follows: denaturation at 95 oC for 5 min, followed by 35 cycles of 95 oC for 30 s, 58 oC for 30 s, and 72 oC for 30 s. A final extension was carried out at 72 oC for 5 min and cooling down to 4 oC. The PCR products were digested by restriction endonuclease BesEII (Fermentas), according to the manufacturer's instructions, at 37°C overnight and then separated by 3% agarose gel electrophoresis. Gel analysis was performed after staining with ethidium bromide. PCR products were shown to be digested into three types of fragments (Fig. 1). To confirm the genotyping results, selected PCR samples in both groups including samples of each genotype were regenotyped by other laboratory personnel. There was no difference after sequencing the randomly selected samples.

Statistical analysis

Data were analyzed using SPSS 16.0 (SPSS Inc., Chicago, IL). Hardy–Weinberg equilibrium in all subjects was analyzed with the x2 goodness-of-fit test before the ensuing analyses. The confounding

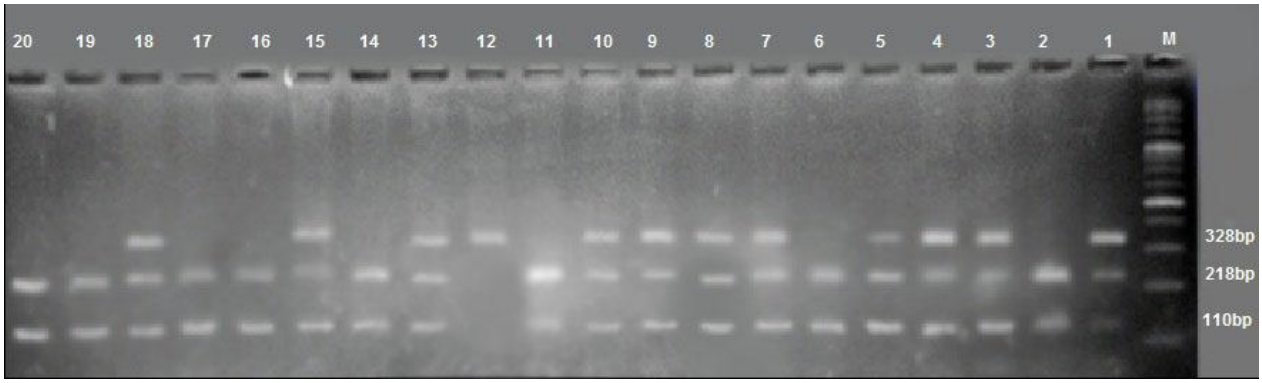

Fig1: PCR-RFLP 3% agarose gel electrophoresis of the -160(C>A) CDH1 polymorphism indicating No.12 (AA = 328 bp) 1, 3, 4, 5, 7, 8, 9, 10, 13, 15, 18 (AC = 328, 218, 110 bp) 2, 6, 11, 14, 16, 17, 19, 20 (CC = 218, 110 bp) genotypes

effects of age and gender were adjusted using conditional logistic regression. Also Statistical analysis was performed by non-paired t-test depending on the data set. Values of p <0.05 were considered as significant

Results

Demographic and clinical characteristics

Genomic DNA was obtained among the 162 nonulcer dyspepsia patients subjects then the DNA all subjects were genotyped. The demographic data of all subjects were demonstrated in Table 1. There was no significant difference between the two groups with respect to the age and gender distribution ($p > 0.05$).

Table 1: Demographic data of study subjects.

Variable	positive (%)	negative (%)
Overall	108 (66.7%)	54(33.3%)
Gender **Male** **Female**	 60(37%) 48(29.6%)	 27(16.7% 27(16.7%
Age **Mean±SD (year)**	59.52 ±15	

p < 0.05 was considered statistically significant

Of these 162 patients, 87 were men, and 75 were women. Their median age was 59.52 ±15 years. H pylori was found in 66.6% of patients and was not found in 33.4% of patients

H. pylori and -160C/A polymorphism

Among the 162 patients, 104 were genotype C/C (64.2%), 56 were genotype A/C (34.6%), and 2 were genotype A/A (1.2%). Interestingly, analyzing 162 available patients of H .pylori infection and H.pylori-uninfected polymorphism status, H. pylori infection was frequently observed in patients with C/A genotype more than in patients CC genotype patients(P = 0.01).

Table 2. 95% confidence intervals (CIs) for -160(C>A) CDH1 polymorphism and susceptibility to *H.pylori* infection

Genotypes of CDH1	H.pylori-infected (%)	H.pylori-uninfected (%)	*#P* value
case **AA*** **AC** **CC**	 1(0.6%) 46(28.8%) 59(36.5%)	 1(0.6%) 9(5.8%) 45(27.7%)	 0.01

***The sample size of **AA** was small so it was not statistically significant.

p < 0.05 was considered statistically significant.

Evaluation of -160(C>A) polymorphism and susceptibility to Helicobacter pylori infection according to sex. -160(C>A) CDH1 polymorphism was evaluated in both sexes for H. pylori infected and non-infected cases (Table 3). Then Polymorphisms of -160(C>A) CDH1 were compared in H. pylori infected and non-infected cases in both groups without any significant (p = 0.504).

Table 3 .Evaluation of -160(C>A) polymorphism and susceptibility to Helicobacter pylori infection according to sex

Variable	H.pylori infected (%)	H.pylori uninfected (%)	*#P* value
Overall	108 (66.7%)	54(33.3%)	
Gender **Male** **Female**	 60(37%) 48(29.6%)	 27(16.7%) 27(16.7%)	 0.504

p < 0.05 was considered statistically significant.

Discussion

CDH1 gene located on chromosome 16 (1/22 q 16). The role of this gene is expression cellular adhesion proteins on epithelial cells. E-cadherin protein that resulting from expression of CDH1 gene, acts as a tumor suppressor (4) . Mutations and impaired function of this gene has been found in gastric cancer[11]. There is no data regarding CDH1 gene polymorphism on Helicobacter pylori (H.pylori) infection among Kurdish population till date. Since the CDH1 gene may play a major role in the development of gastric cancer and nonulcer dyspepsia patients, we studied polymorphism in the region promoter, -160 (C > A), of CDH1 gene to evaluate whether this polymorphism can affect the CDH1 gene in this population with nonulcer dyspepsia patients. In the present study we found that frequencies of -160(C>A) CDH1 genotypes were comparable in H.pylori-infected and H.pylori -uninfected subjects in These findings suggest that -160 (C>A) CDH1 polymorphism have relate with H.pylori infection susceptibility. H. pylori is responsible for the pathogenesis of atrophic gastritis and intestinal metaplasia[30,31]. Epidemiological studies have indicated that infection with H. pylori is a risk factor for GC[20,22,26]. Moreover, Mongolian gerbils develop chronic gastritis, intestinal metaplasia, and adenocarcinoma after inoculation with H. pylori into the stomach. However, the link between H. pylori infection and the molecular mechanism of human gastric carcinoma remains to be investigated. Lim et al., reported that the increased expression of cell adhesion molecules (galectin 1, aldolase A, integrin α5, LMO7) and the decrease in

E-cad expression induced by H. pylori might contribute to cell adhesion, invasion, and possibly cell proliferation in gastric epithelial cells[32,29]. Kitadai et al., reported that coculture with H. pylori increased the expression of interleukin-8, vascular endothelial growth factor (VEGF), angiogenin, uPA, and MMP-9 and increased angiogenic and collagenase activities in gastric carcinoma cells[33]. Sharma et al., reported that the activation of interleukin-8 gene expression by H. pylori is regulated by the transcription factor, nuclear factor-kappa B, in gastric epithelial cells[34]. Akhtar et al., reported that promoter methylation regulates H. pylori-stimulated cyclooxygenase-2 expression in gastric epithelial cells[35, 36]. However, in vivo data on the differences in the molecular profile (ras, MDM2,c-erbB-2, cyclin D1, p53, CDH1) of H. pylori-positive and H pylori-negative gastric carcinomas are almost non-existent[23,27,28]. In this study, H. pylori was found in 67% of patients with nonulcer dyspepsia. This is consistent with the current view that 60% of all GC cases are related to H. pylori infection, irrespective of the histological tumor type[20,23].

H pylori and promoter polymorphism

Li et al., reported that the A allele of the -160C/A promoter polymorphism altered transcriptional binding, resulting in a reduction in transcriptional efficiency of 68% relative to that of the C allele[19]. In this study, H pylori was significantly more frequent in the C/C genotype of CDH1 promoter than in the C/A+A/A genotypes. The C allele shows better transcriptional activity than the A allele, so patients with the C/C type may require H pylori infection to promote the inactivation of CDH1. Since -160C/A polymorphism is germ line, H pylori infection might affect CDH1 inactivation in an early, possibly preneoplastic stage. Therefore, H pylori infection should be considered as a factor that facilitates a multifactorially determined process of gastric carcinogenesis in an initial stage. Finally, we must acknowledge that the CDH1 gene polymorphism at position -160(C-->A) has a various outcomes in different ethnic groups and geographic locations. This polymorphism should be evaluated with other environmental factors simultaneously. Therefore, further studies are needed to confirm our findings.

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