RESEARCH ARTICLE

The -160 (C>A) CDH1 Gene Promoter Polymorphism and Its Relationship with Survival of Patients with Gastric Cancer in Kurdistan

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Abstract

Introduction: Gastric cancer (GC) is the fourth most common type of neoplasm and the second cause of malignancy-related death across much of the world. Complex multi-factorial processes are involved in its genesis, classified in two determinant clusters: non-genetic and genetic . Variation in CDH1 gene expression may play an important role in increasing risk of diffuse and intestinal subtypes of GC. This tumor suppressor gene, located on chromosome 16q22.1, encodes a trans membrane glycoprotein called epithelial cadherin (E-cadherin). **Materials and Methods:** In this historical cohort study, from June 2004 to Journey 2005 we collected 50 samples from Kurdish patients with stage II pathologically diagnosed gastric cancer that underwent surgery. Tumor tissues were paraffin-embedded along with 54 control samples from non-ulcer dyspepsia (NUD) cases undergoing upper gastrointestinal endoscopy. Three biopsies were captured by endoscopy from each individual's gastric antrum. **Result:** The mean age of the patients was 59.5±2 years. Some 23 cases (53.4%) had the CC genotype, 19 AC and 1 AA. H.pylori infection was noted in 30 patients (69%). Survival rates of gastric cancer patients were 90.7% in the first year, 39.5% in the second year and 6.9% in the third year. Female patients had higher survival rates (P=0.004). **Conclusion:** In this study we found that frequencies of -160(C>A) CDH1 genotypes were not comparable in H.pylori-infected and H.pylori-uninfected subjects in both case and control groups. These findings suggest that -160 (C>A) CDH1 polymorphism is not related with H.pylori infection susceptibility. In addition we found no significant relationship between the CDH1 -160(C/A) promoter polymorphism with predisposition to gastric cancer.

Keywords: CDH1 gene- gastric cancer- survival- Kurdistan

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Introduction

Gastric cancer is the fourth most common cause of cancer and 2th cause of malignancy-related to death around the world (Jemal et al., 2011; Ferro et al., 2014). In Western countries Gastric cancer remains difficult to cure yet, because at the time of diagnosis , The condition of patients reach to advance level. In the United States, stomach malignancy is the 15th most common cancer (Jinek et al., 2012, 2015)

Gastric carcinogenesis is a complex multifactorial process that can present in two clusters contains non genetic and genetic determinants. Several factors such as Helicobacter pylori, smoking, low vegetable regimen and high salt intake are major non-genetic determinants (Yeh et al., 2009; Chen et al., 2011, 2015). inherited component contributes to <3% of gastric cancers; the majority of genetic changes associated with gastric cancer are acquired (McLean and El-Omar, 2014).

In recent decades decline occurred for stomach cancer; in the 1930s cancer deaths accounted for 30% and 20% for male and female ,in 2014 stomach cancer accounted for just 2% of cancer deaths.(Siegel et al., 2014). Worldwide diminish of gastric cancer incidence especially in Western countries occurred, its due to improving in dietary, hygiene, earlier diagnosis, lower prevalence of *Helicobacter pylori*, advances in food preservation techniques and new treatments methods. base of treatment for gastric cancers contain surgical resection with Simultaneous chemotherapy or chemo radiation (Takezaki et al., 1999; Kobayashi et al., 2002; Carcas, 2014; Pasechnikov et al., 2014) unfortunately Gastric cancer is often diagnosed at an advanced stage, Although advanced or metastatic gastric cancer has poor prognosis yet and median overall survival (OS) in this group remains <1 year (Carcas, 2014).

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The survival rate means that the percentage of people who survive at least in certain time with years after the cancer is found. The five-year survival rate of all people with stomach cancer is about 28% in united states (2015). among adult aged 15-99 years old in united kingdom One, five and ten year survival rate to arrange 41.8, 18.9 and 15% (2011). Several studies show different genetic variation in CDH1 gene that play important role in increase risk of GC, diffuse and intestinal subtypes of GC (Chen et al., 2011; Deng et al., 2014; Jing et al., 2014). Cadherin-1 also known as epithelial cadherin (E-cadherin) that in humans encoded by the CDH1 gene (Huntsman and Caldas, 1998; Semb and Christofori, 1998).

This gene located on chromosome 16q22.1 and encodes a trans membrane glycoprotein that mediates intercellular adhesion , cellular polarity and regulating morphogenesis of both normal and neoplastic tissues, but also Its known as a tumor suppressor gene (Beavon, 2000; Fleming et al., 2000).

Several single nucleotide polymorphisms (SNPs) in the CDH1 gene may be associated with the increased risk of GC Such as -160 C>A, -347, +54T>C, -616G>C, -2076C>T and -3159T>C. Two of the SNPs are in the promoter site at positions -160 and -347 (160 and 347 nucleotide number far from start site) (Cavallaro and Christofori, 2001; Wang et al., 2006; Gao et al., 2008; Al-Moundhri, 2010), Moreover, several other SNPs were studied in Caucasians and East Asians, which resulted in the identification of haplotypes associated with GC risk (Yamada et al., 2007) a single nucleotide polymorphism in promoter region on CDH1 gene located in -160 is the most widely studied polymorphism is CDH1 _160C>A (Rs16260), where the A allele decreases transcription efficiency of the CDH1 gene (Chen et al., 2011; Tan et al, 2013; Li et al., 2014). In this study we investigate the associations between patient with diffused GC that have SNP in -160C>A with one-year survival of this patient.

Materials and Methods

Subjects

The study protocol was approved by the local Clinical Research ethics committee and the method of tissue collection including informed consent from all patients. in June 2004 to Journey 2005 we accumulate Patients samples with inclusion criteria consist of 50 patients that underwent surgery Tumor tissues and diagnosed as grade two of gastric cancer with no sign of Metastasis and pathologically approved. Tumor tissues were collected in form of paraffin embedded tissue in cancer center of medical University of Kurdistan, Kurdistan, Iran. Controls samples obtain from 54 Kurdish non ulcer dyspepsia (NUD) who were undergoing upper gastrointestinal endoscopy and have any history of gastric pathological abnormality. Three biopsies were captured by endoscopy from each individual's gastric antrum, the first biopsy for rapid urease test, the second were immediately frozen and stored, the third for paraffin embedded section. Their epidemiological and clinical information were obtained from their clinical records; Exclusion criteria for control group included history of gastric neoplasm or surgery, liver disease, and previous treatment with nonsteroidal anti-inflammatory drugs or bismuth salts exclusion criteria for patients group were all the patients with diagnose of gastric cancer except our inclusion criteria. The followed-up was carried out for 4 years from the time of cancer diagnose.In both groups, cases and controls, *H.pylori* infection was determined by the UBT (Urease Breath Test) and PCR 16srRNA (Chong et al., 1996) on 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.

DNA extraction

Genomic DNA of gastric cancer patients was separated from paraffin embedded tumor tissues by using QIAamp DNA Mini Kit (Qiagen, Germany), following the manufacturer's instructions. DNA of controls was extracted from biopsies taken from the corpus using Biospin Tissue genomic DNA Extraction Kit (Bio Flux, Japan). DNA concentration and purity of each sample were measured by Synergy 2Multi-Mode Reader (BioTek® Instruments, Inc.USA). All extracted DNA was resuspended in UltraPure RNAse/DNAse-Free Distilled water and DNA samples were routinely stored at -20°C until processing for genotyping.

Genotyping for -160 (C>A) CDH1 polymorphism

Analysis of the CDH1 SNPs, -160C>A was performed by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP).extracted Genomic DNA from paraffin embedded tumor tissues was used as a PCR template . 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 was performed as follows: denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s. A final extension was carried out at 72 °C for 5 min and cooling down to 4 °C. PCR products were analyzed on a 3% ethidium bromide added agarose gel, photographs were taken under ultraviolet light transilluminator (Figure 1).

The PCR products were 328 bp. For the RFLP analysis, PCR-amplified products were digested with restriction endonuclease BesEII (Fermentas), according to the manufacturer's instructions, at 37°C overnight and then separated by 3% agarose gel electrophoresis. The amplicon with the homozygous CC allele of CDH1 was cleaved by BesEII, yielding 218 bp And 110 fragments, whereas the amplicon with the homozygous AA allele remained uncut, yielding a 328 bp band. The amplicon with the heterozygous CA allele yielded three fragments of 328, 218, and 110 bp length. The digested products were separated on 3% agarose gel And Ethidium bromide-stained gels were visualized under UV light (Figure 2).

Statistical analysis

Data were analyzed using SPSS 16.0 (SPSS Inc., Chicago, IL). Values of p <0.05 were considered as significant. Kaplan–Meier survival curves and the log-rank test were used to evaluate the relationship between genotypes and the outcome of patients to the end of follow-up.

Results

In this study, 48 patients were evaluated. During the period of the study totally 5 missing and 43 events were happened that 3 cases of them were censored. The mean age of patients was 59.52±2 years (between 45-75 years old). Of all, 27 patients (56.3%) were male, 23 patients (53.4%) had CC genotype, 19 patient AC and 1 patient was AA .30 patients (69%) had H.pylori infection. The survival rate of gastric cancer patients was 90.7% in the first year, 39.5% in the second year and 6.9% in the third year. The median survival time of the studied patients was 23 months (95% CI: 21.16-24.84) and the mean duration of survival was 22.65 months (95% CI: 20.69-24.62). The curve of survival probability per month is plotted in Figure 1. As shown in the graph, the curve hasn't a sharp steep until about 20 months, steep continues but its slope is increased.

Table 1 presents the results of the analysis of the median survival time in terms of the studied variables which were calculated using Kaplan-Meier nonparametric method; additionally it shows the likely differences between the curves of each of the variables which were calculated using the Log-rank test. As the results of Table 1 and Figure 4 show there was significant difference between the survival rate of patients at different sex groups; the female patients had higher survival rates (P=0.004).There was no significant difference between the survival of patients that have or have not *H.pylori* infection (P>0.05). In addition, people with ac genotype

Table 1. Results of Evaluation of the Factors Affecting the Survival of Patients with Gastric Cancer Using Kaplan-Meier Nonparametric Method

Variable	Frequency (percentage)	Median survival per month	Probability
Sex			
Male	24 (55.81)	19	0.004*
Female	19 (44.19)	26	
H.pylori			
Have	30 (69.77)	20	0.357
Have not	13 (30.23)	24	
Genotype			
aa	1 (2.32)	14	0.014*
ac	19 (44.19)	18	
cc	23 (53.49)	26	
Age			
<55	13 (30.23)	23	0.31
56-64	19 (44.19)	24	
>64	11 (25.58)	21	

* Significance level, 0.05

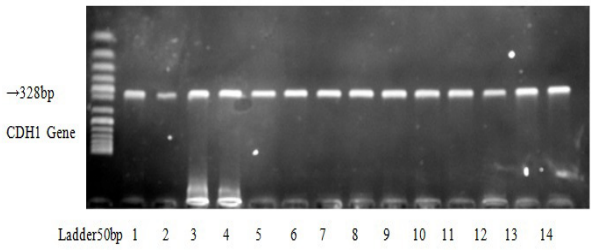

Figure 1. The Electrophoresis of PCR Test Samples

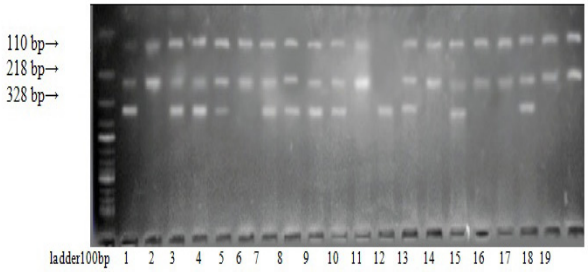

Figure 2. PCR-RFLP 3% Agarose Gel Electrophoresis of the -160(C>A) CDH1 Polymorphism Indicating No.12 (AA = 328 bp) 1, 3, 4, 5, 7, 8, 9, 10, 13, 15, 18 (AC = 328, 218, 110 bp) 2, 6, 11, 14, 16, 17, 19, 20 (CC = 218, 110 bp) genotypes.

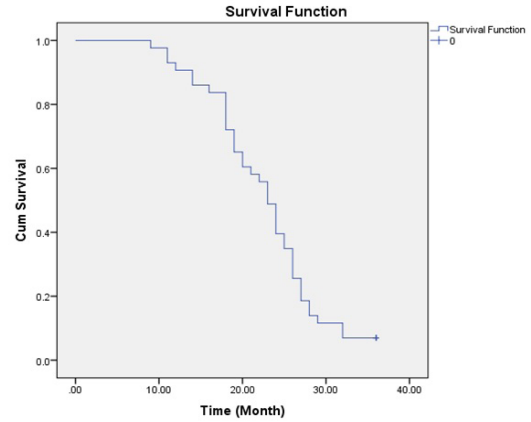

Figure 3. Survival Rate of Patients Calculated Using Kaplan-Meier Nonparametric Method

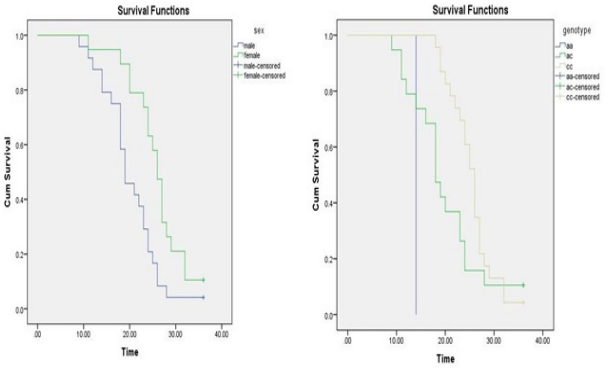

Figure 4. Survival Rate of Patients with Gastric Cancer Calculated for Different Sex and Genotype Groups

had lower survival rates than other (P=0.01). There were no significant differences between the survival rates of patients at different age groups (P>0.05).

Discussion

several diseases in human is not just because of a single genetic change, especially in cancers, complex relationship among genetic and non genetic agents like environmental changes and life style shows cancers is multifactorial complicated situation. In another way genetic alteration has important role in disease intensity, response to treatment and disease progression. In this complicated situation polymorphisms have one of main role in tumorgenesis and increase of cancer affection predisposition (Perera and Weinstein, 2000). Single nucleotide polymorphisms (SNP) are most common genetic alteration and SNP profiling help us to perception dynamics, mechanism and in addition to diagnose and rise up new treatment strategies. CDH1 gene encode E-Catherin protein, a tumor suppressor that recruit epithelial cell adhesion and Located on chromosome 16 (Berx et al., 1998; Machado et al., 2001).Several studies shown that mutations in CDH1 in gastric cancer abundantly exist(Chen et al., 2013, Black et al., 2014).

- 160(C/A)(Singe nucleotide polymorphism (SNP) located in CDH1 promoter and may have effect on transcription process(Yu et al., 2006; Shabnaz et al., 2016). This is no doubt that inactivation of E-Catherin as tumor suppressor gene, have important role in progression of cancer (Zhang et al., 2008; Cui et al., 2011). There is no data about CDH1 Polymorphism among Kurdish population, for studying this aim we perusal Polymorphism of CDH1 gene promoter -160(C/A) and its relationship with the survival rate of this SNP in Kurdish population. In this study we found that frequencies of -160(C>A) CDH1 genotypes were not comparable in H.pylori-infected and H.pylori -uninfected subjects in both of case and control groups. These findings suggest that -160 (C>A) CDH1 polymorphism don't relate with H.pylori infection susceptibility. In addition to we found that it's no significant relationship between CDH1 -160(C/A) promoter Polymorphism with predisposition to affection with gastric cancer, but patients with CC genotype have more survival rate in compare with that's have CA genotype. This result proposed that CC genotype as a good prognosis genotype in the process of cancer but not in gastric cancer susceptibility, in Kurdish population. Molecular epidemiological study about relation of this polymorphism with cancer in various tissue like prostate, colorectal, breast and stomach faced us with different result all around the world. Pharoah et al, In the case-control study reported that no significant relationship among CDH1 -160(C/A) promoter Polymorphism with gastric cancer were seen (Pharoah et al., 2002), as well as lu et al., (2005) have same result among Chinese population and several study have same result too (Jenab et al., 2008; Zhang et al., 2008; Cui et al., 2011). Chen et al., (2011) in meta analysis study among Chinese population report that CDH1 -160(C/A) promoter Polymorphism have any relationship with elevation of stomach cancer risk. The other side in 2011, yodong et al, and alomoundhir et al, showed that -160(C/A) promoter Polymorphism can cause increase the Risk of colorectal cancer and gastric cancer (Wang et al., 2012). Li et al., (2000), showed that A allele in CDH1 gene promoter may lead to decrease transcription state in comparison with CC homozygote genotype, so when the A allele located in -160 position this can related to gastric Cancer susceptibility and prognosis, in result CA genotype can elevate aggressive power of gastric tumor and decrease survivals patient in compare with patient that has CC genotype . Finally, it seems gene alteration in CDH1 -160 (C/A) promoters among Different races and different geographies in different places is different. The relevance of this polymorphism with other factors, including various environmental factors should be examined. It should be noted that CDH1 gene is a tumor suppressor gene and any change that influence in its expression maybe effective in tumor formation, development and progression. Depends on the rate of this genetic modification, we will see Different role on cancer progression and prognosis and Sufficient information about these changes helps to physician for choose better treatment strategy about this patient.

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