

Correlation of $-160C > A$ and $-347GA > G$ polymorphisms in E-cadherin gene and gastric cancer in north of Iran

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Background: E-cadherin (*CDH1* gene) is a protein involved in cell-cell adhesion. There are reports on the association of $-160C > A$ (rs16260) and $-347GA > G$ (rs5030625) polymorphisms in the 5'-promoter region of the *CDH1* gene with tumor development and progression of gastric cancer. This study aimed to examine the potential relationship between these two polymorphisms and gastric cancer in patients from Mazandaran province, Northern Iran. **Materials and Methods:** A case-control study was conducted to test 97 patients and 95 healthy controls. Genomic DNA was extracted from peripheral blood followed by polymerase chain reaction amplification. Genotyping analysis was carried out using restriction fragment length polymorphism analysis for two potentially functional polymorphisms. **Results:** Heterozygous genotype GA/G versus GA/GA of rs5030625 ($-347GA > G$) was found to be associated with increased risk of gastric cancer in the people studied (odds ratio = 5.73, 95% confidence interval = 2.11–15.56, $P = 0.001$). Furthermore, AA or CA genotype in $-160C > A$ polymorphism did not show any increased risk of gastric cancer ($P = 0.559$). **Conclusion:** The present study revealed that GA/G genotype of rs5030625 ($-347GA > G$) polymorphism is associated with gastric cancer in Northern Iran.

Key words: CDH1 protein, E-cadherin, gastric cancer, genotype, polymorphism, restriction fragment length polymorphism

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INTRODUCTION

According to the estimates from the International Agency for Research on Cancer GLOBOCAN project, gastric carcinoma is the fifth most common cancer worldwide (1,033,701 new cases per year in 2018) and remains the third most common cause of death (782,685 death per year in 2018) of all cancers worldwide.^[1] More than two-thirds of gastric cancer occur in developing countries.^[2] High-risk areas are located in East Asia (China and Japan), Eastern Europe, and Central and South America and the low-risk areas are located in Southern Asia, North and East Africa, North America,

Australia, and New Zealand.^[3] Gastric cancer (GC) is the first and the third most common cancer among males and females in Iran, respectively.^[4] The north and northwestern regions of Iran including Guilan, Mazandaran, and Ardabil provinces are high-risk areas for gastric cancer.^[5] A previous study on the E-cadherin gene (*CDH1*) reported mutations in three large Maori families from New Zealand with diffuse early-onset gastric cancer.^[6]

CDH1 gene is located on chromosome 16q22.1 and consists of 16 exons.^[7] It encodes a 120 kDa glycoprotein with a large extracellular domain, a single transmembrane segment, and a short cytoplasmic

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domain, which interacts with the actin cytoskeleton through linker molecules, alpha-, beta-, and gamma-catenin.^[8] E-cadherin is a Ca²⁺-dependent cell–cell adhesion molecule that acts as a tumor suppressor.^[9] Loss of E-cadherin function during tumor progression, associated with *CDH1* mutations, is observed in hereditary diffuse-type gastric cancer, invasive lobular breast cancer,^[10] and colorectal and prostate carcinomas.^[10,11]

Promoter-related polymorphisms of *CDH1* gene (-160C > A and -347GA > G) alter promoter activity, gene transcription, mRNA stability, and translation.^[12] The - 160C > A polymorphism is located upstream of the transcriptional start site of the gene. The - 160A allele is reported to decrease the transcription efficiency of the *CDH1* gene.^[13] Many studies have been conducted to investigate the associations between - 160C > A polymorphism and different including gastric cancer.^[13] -347GA > G is a functional polymorphism, the GA-allele cause downregulation of the *CDH1* gene and low expression of E-cadherin compared with the G-allele.^[14] Furthermore, GA-allele decreases the transcriptional efficiency of the *CDH1* gene and is also reported to be associated with an increased risk of gastric, colon, and esophageal cancers.^[11,13,15,16] This study aimed to examine the potential relationship between these two polymorphisms and gastric cancer in patients from Mazandaran province, Northern Iran.

MATERIALS AND METHODS

The study population

The present study included 97 patients who were diagnosed with gastric cancer (78 males and 19 females, mean age 66.1 ± 9.1) and had blood samples collected from January 2009 to September 2011 in Sari Imam Khomeini Hospital. Furthermore, 95 blood samples were collected as control from healthy individuals (65 males and 30 females, mean age 61.4 ± 10.8) who referred to Sari Cardiology Hospital, Fatemeh Zahra, for other reasons except gastric diseases [Table 1].

Patients were diagnosed with gastric adenocarcinoma by the pathology report of endoscopic samples and surgical resection. Blood samples were taken before any specific oncological intervention (chemotherapy and radiation therapy). Diagnosis of gastric adenocarcinoma and tumor infiltration rate (T), involvement of lymph nodes (nodal status), and determination of tumor grade in all patients were confirmed by two pathologists. To determine the stage of the tumor and lymph nodes (T, N) and the presence of metastasis (M), ultrasound endoscopic imaging techniques (endoscopic ultrasound), spiral chest, and abdomino-pelvic computed tomography scan with contrast were used, respectively.

Tumor characteristics of the patients studied are summarized in Table 2. This research was approved by the Ethics Committee in the Mazandaran University of Medical Sciences (IR.MAZUMS.IMAMHOSPITAL.REC.1398.059). Blood Samples were taken from patients before starting

Table 1: Demographic characteristics of the cases and controls

Variables	Cases (n=97), n (%)	Controls (n=95), n (%)	P
Age (years) (mean±SD)	66.1±9.1	61.4±10.8	0.001
Sex			
Male	78 (80.4)	65 (68.4)	0.069
Female	19 (19.6)	30 (31.6)	

SD=Standard deviation

Table 2: Tumor characteristics, including tumor site, tumor grade, lymphatic invasion, perineural invasion, and tumor stage or tumor type (n=95)

Characteristic	Frequency (%)
Tumor site	
Cardia	12 (12.4)
Fundus	8 (8.2)
Body	21 (21.6)
Antrum	22 (22.7)
Overlapping	34 (35.1)
Grade	
1	6 (6.2)
2	54 (55.7)
3	37 (38.1)
Lymphatic invasion	
Present	61 (62.9)
Absent	36 (37.1)
Perineural invasion	
Present	33 (34.0)
Absent	64 (66.0)
T	
1	1 (1.0)
2	37 (38.1)
3	48 (49.5)
4	11 (11.3)
N	
0	25 (25.8)
1	31 (32.0)
2	34 (35.1)
3	7 (7.2)
M	
0	69 (71.1)
1	28 (28.9)
Stage	
1	17 (17.5)
2	32 (33.0)
3	22 (22.7)
4	26 (26.8)
Tumor type	
Diffuse	60 (62.5)
Intestinal	36 (37.5)

chemotherapy and from controls after age/gender match with patients.

DNA extraction

Peripheral venous blood (5 ml) was collected in an ethylenediaminetetraacetic acid containing tube and stored at -80°C until analysis. Blood samples from patients were taken before initiation of chemotherapy. Total genomic DNA was extracted using a standard salting out methods from blood using DNA extraction kit from Dena Zist company (Denazist, Iran) according to the manufacturer's instructions. The extracted DNA was maintained at -20°C until further study.

Genotyping using polymerase chain reaction-restriction fragment length polymorphism method

The polymerase chain reaction (PCR)-restriction fragment length polymorphism method was applied to analyze $-160\text{C} > \text{A}$ and $-347\text{GA} > \text{G}$ polymorphisms separately. A 447 bp PCR product containing both $-160\text{C} > \text{A}$ and $-347\text{GA} > \text{G}$ polymorphisms was achieved from *CDH1* gene promoter amplification. Two primers: 5'-GCCCCGACTTGTCTCTCTAC-3' (forward) and 5'-TACCGCTGATTGGCTGAGGG-3' (reverse) were applied. The PCR amplification was carried out in a 25 μl reaction mixture containing 2 μl template DNA, 12 μl ready $\times 2$ PCR Master Mix (Amplicon, Denmark), 10 μl distilled water, and 0.5 μl of each forward and reverse primer. The amplification was performed under the following conditions: 1 cycle of 95°C for 2 min; 35 cycles of 95°C for 30 s, 65°C for 30 s, and 72°C for 30 s; and a final cycle of 72°C for 5 min. The PCR product was digested with *HincII* and *BanII* (Thermo Fisher Scientific) at 37°C overnight in two separate tubes, respectively [Table 3].

To detect the $-160\text{C} > \text{A}$ and $-347\text{GA} > \text{G}$ polymorphisms, PCR products were digested with *HincII* restriction enzymes for $-160\text{C} > \text{A}$ (rs16260) and *BanII* restriction enzyme (Thermo Fisher Scientific) for $-347\text{GA} > \text{G}$ (rs5030625) polymorphisms, respectively [Table 3]. After digestion, the products were separated by 3% agarose gel electrophoresis and stained with Green Viewer™ DNA Staining Dye [Figure 1].

Statistical analysis

Chi-square test was used to evaluate differences in the distributions of demographic characteristics. The expected

frequency of control genotypes was tested against the Hardy-Weinberg equilibrium. The odds ratio (OR) and 95% confidence interval (CI) were calculated using a logistic regression model. $P < 0.05$ was considered statistically significant. Otherwise, the pooled ORs and 95% CIs without adjustments were calculated for the $-160\text{C} > \text{A}$ and $-347\text{GA} > \text{G}$ alleles and genotypes frequencies, respectively. The statistical analysis was performed using the SPSS 19 software package (SPSS, Chicago, IL, USA).

RESULTS

Ninety-seven patients with gastric cancer and 95 normal individuals were analyzed in this study. Tumor characteristics of the patients with gastric cancer are summarized in Table 2. Demographic characteristics of the patients and normal control are summarized in Table 3. The cases and controls were sex/age-matched and there were no significant differences in sex or age distribution between the two groups ($P = 0.069$). The genotype and allele frequency of *CDH1* $-160\text{C} > \text{A}$ and $-347\text{GA} > \text{G}$ from patients and healthy controls are shown in Table 4. When homozygous CC genotype (wild type) for $-160\text{C} > \text{A}$ polymorphism

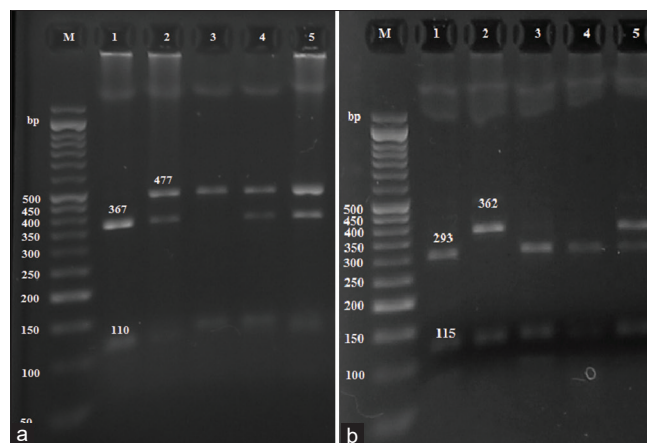


Figure 1: Part (a) 447 bp polymerase chain reaction product from *CDH1* gene promoter digestion for $-160\text{C} > \text{A}$ polymorphism using *HincII* restriction enzyme. Lane M shows 50 bp DNA marker, lane 1 shows homozygous CC (367,110 bp); lanes 2, 4, and 5 show heterozygous AC (477,367,110 bp, respectively) and lane 3 shows homozygous AA genotype (477 bp) respectively. Part (b) Restriction enzyme map of the 477bp polymerase chain reaction fragment for $-347\text{GA} > \text{G}$ polymorphism using *BanII* restriction enzyme. Lane M shows 50bp DNA marker, lanes 1, 3, and 4 show homozygous G/G (293,115,68 bp), lane 2 shows homozygous GA/GA (362, 115 bp) and lane 5 shows heterozygous GA/G genotype (362,293,115,68 bp) respectively. A 3% agarose gel electrophoresis was applied to separate polymerase chain reaction fragments

Table 3: Polymerase chain reaction size and restriction fragment length polymorphism fragments using *HincII* restriction enzymes for $-160\text{C} > \text{A}$ (rs16260) and *BanII* restriction enzyme for $-347\text{GA} > \text{G}$ (rs5030625) polymorphisms, respectively

SNP	PCR size (bp)	Restriction enzyme	Digestion products (bp)		
			Wild-type	Mutant	Heterozygous
$-160\text{C} > \text{A}$ (rs16260)	447	<i>HincII</i>	477	367,110	477,367,110
$-347\text{GA} > \text{G}$ (rs5030625)	447	<i>BanII</i>	362,115	293,115,68	362,293,115,68

PCR=Polymerase chain reaction; SNP=Single-nucleotide polymorphism

was considered as reference, no significant difference was seen between two groups ($P = 0.559$) [Table 4]. Similarly, there was no significant difference in allele frequency for either $-160C > A$ or $-347GA > G$ polymorphisms between patients and controls respectively [Table 4]. Furthermore, the genotype frequency of GA/GA, GA/G, and G/G was 8.2%, 40.2%, and 51.5% in patients and 21.1%, 17.9%, and 61.1% in healthy individuals, respectively.

For $-347GA > G$ polymorphism, when GA/GA (wild type) was considered as reference, a significant difference was revealed between GA/G genotype frequencies in patients and controls ($P = 0.001$). Logistic regression suggested a 5.73-fold higher risk (95% CI: 2.11–15.56) of gastric cancer for GA/G carrier individuals. Furthermore, GA/G carrier showed 5.56-fold higher risk (95% CI: 2.11–15.56) of developing gastric cancer when age and sex were not included [Table 5]. No significant difference was identified when associations of $-160C > A$ or $-347GA > G$ genotypes with sex, tumor size, tumor grade, tumor site, lymphatic invasion, perineural invasion, tumor stage, and tumor type were tested [Table 6].

DISCUSSION

Gastric cancer (GC) is the fourth most common malignancy worldwide, although its incidence and mortality rates have decreased in recent decades. Despite this, patients with GC still have a poor prognosis.^[3] Gastric cancer is a multifactorial disease, and some environmental factors such as high dietary intake of salt, *Helicobacter pylori* infection, smoking, and gastroesophageal reflux disease are reported

as environmental factors for this disease in Iran.^[15,16] Mazandaran, Golestan, and Ardabil provinces are among the high-risk provinces for gastric cancer.^[5] E-cadherin germline mutations were first identified in New Zealand Maori families with early-onset diffuse gastric cancer.^[17] The calcium-dependent cell adhesion molecule (E-cadherin) plays a key role in cell–cell adhesion and maintenance of cell architecture in the epithelium.^[18]

Associations between $CDH1-160C > A$ and $-347GA > G$ polymorphism with various cancers have also been reported previously. Mutant allele A in $-160C/A$ position is found to decrease the transcriptional activity of the $CDH1$ gene by 68% compared with the C allele.^[19] Furthermore, Chen *et al.* showed that the $-347GA > G$ polymorphism in the $CDH1$ promoter decreases the binding affinity for a transcription factor, leading to a 10-fold decrease in the transcriptional efficiency of the GA-allele compared with the G allele, respectively.^[20]

There are some conflicting results regarding the association between the rs16260 ($-160 C > A$) and rs5030625 ($-347GA > G$) polymorphisms with gastric cancer in different ethnicities. Zhang *et al.* in a study about $-347GA > G$ and $-160C > A$ along with three other polymorphisms in the $CDH1$ gene in patients with sporadic gastric carcinoma (SGC) from Northeast China showed that the $-347G/G$ genotype may increase the susceptibility to SGC among males in high-risk areas. They also reported no association between $-160C > A$ and the risk of SGC among subjects either in high-risk or low-risk areas.^[21] In another study, the GA/GA genotypes of $-347GA$ were found increasing the risk of gastric cardia adenocarcinomas (GCA).^[12] Moreover, a significant association of $CDH1-160C/-347G$ and $-160C/-347GA$ haplotypes was reported with the development of GCA, while $-160A$ allele was not found to be associated with an increase risk of gastric cancer.^[12] A meta-analysis that reviewed 17 different studies (3511 GC cases and 4826 normal controls) suggests that $CDH1-160C > A$ polymorphism may be associated with risk of GC among Caucasians, but not among Asians.^[20] Another meta-analysis carried out by Wang *et al.* reported that $-160C > A$ polymorphism may be associated with gastric cancer among Asians but not Europeans.^[22] Other studies from China, young Mexican population, and people from Oman observed that $-160 AA$ genotype is associated with an increased risk of GC.^[19,23,24] On the other hand, a

Table 4: Genotype and allele frequencies of $CDH1-160C>A$ and $-347G>GA$ polymorphisms in gastric cancer patients and healthy controls

Polymorphism	Genotype/Allele	Case	Control	P	
$-160 C>A$ (rs16260)	CC	49 (50.5)	51 (53.7)	0.559	
	CA	39 (40.2)	39 (41.1)		
	AA	9 (9.3)	5 (5.3)		
	C	137 (70.6)	141 (74.2)		-
	A	57 (29.4)	49 (25.8)		0.431
$-347 GA>G$ (rs5030625)	GA/GA	8 (8.2)	20 (21.1)	0.001	
	GA/G	39 (40.2)	17 (17.9)		
	G/G	50 (51.5)	58 (61.1)		
	GA	55 (28.4)	57 (30.0)		-
	G	139 (71.6)	133 (70.0)		0.722

Table 5: Comparison of genotype frequencies of $CDH1 -347GA>G$ gene polymorphisms using Chi-square analysis in gastric cancer patients and normal controls

$-347 GA>G$ (rs5030625)	Odds ratio	95% CI	P	Adjusted odds ratio*	95% CI	Adjusted P
GA/GA	Reference	-	-	Reference	-	-
GA/G	5.73	2.11-15.56	0.001	5.56	1.96-15.76	0.001
G/G	2.15	0.87-5.31	0.096	1.82	0.71-4.69	0.211

*Adjusted age and sex. CI=Confidence interval

Table 6: Distribution of genotypes –160C>A and -347GA>G polymorphisms in the CDH1 gene by sex and tumor characteristics

Variable	rs16260			P	rs5030625			P
	CC	CA	AA		GA/GA	GA/G	G/G	
Sex								
Male	74 (51.7)	57 (39.9)	12 (8.4)	0.6	21 (14.7)	43 (30.1)	79 (55.2)	0.877
Female	26 (53.1)	21 (42.9)	2 (4.1)		7 (14.3)	13 (26.5)	29 (59.2)	
Tumor site								
Cardia	8 (66.7)	2 (16.7)	2 (16.7)	0.565	1 (8.3)	4 (33.3)	7 (58.3)	0.218
Fundus	5 (62.5)	3 (37.5)	-		-	3 (37.5)	5 (62.5)	
Body	11 (52.4)	9 (42.9)	1 (4.8)		2 (9.5)	3 (14.3)	16 (76.2)	
Antrum	8 (36.4)	12 (54.5)	2 (9.1)		2 (9.1)	11 (50.0)	9 (40.9)	
Overlapping	17 (50.0)	13 (38.2)	4 (11.8)		3 (8.8)	18 (52.9)	13 (38.2)	
Grade								
I	5 (83.3)	1 (16.7)	-	0.409	1 (16.7)	2 (33.3)	3 (50.0)	0.166
II	24 (44.4)	25 (46.3)	5 (9.3)		2 (3.7)	19 (35.2)	33 (61.1)	
III	20 (54.1)	13 (35.1)	4 (10.8)		5 (13.5)	18 (48.6)	14 (37.8)	
Lymphatic invasion								
Yes	30 (49.2)	25 (41.0)	6 (9.8)	0.933	6 (9.8)	23 (37.7)	32 (52.5)	0.675
No	19 (52.8)	14 (38.9)	3 (8.3)		2 (5.6)	16 (44.4)	18 (50.0)	
Perineural invasion								
Yes	18 (54.5)	12 (36.4)	3 (9.1)	0.841	5 (15.2)	9 (27.3)	19 (57.6)	0.07
No	31 (48.4)	27 (42.2)	6 (9.4)		3 (4.7)	30 (46.9)	31 (48.4)	
Stage								
I	13 (76.5)	4 (23.5)	-	0.289	2 (11.8)	8 (47.1)	7 (41.2)	0.292
II	14 (43.8)	13 (40.6)	5 (15.6)		-	14 (43.8)	18 (56.2)	
III	10 (45.5)	10 (45.5)	2 (9.1)		4 (18.2)	6 (27.3)	12 (54.5)	
IV	12 (46.2)	12 (46.2)	2 (7.7)		2 (7.7)	11 (42.3)	13 (50.0)	
Tumor type								
Diffuse	33 (55.0)	21 (35.0)	6 (10.0)	0.321	5 (8.3)	26 (43.3)	29 (48.3)	0.619
Intestinal	16 (44.4)	18 (50.0)	2 (5.6)		2 (5.6)	13 (36.1)	21 (58.3)	

meta-analysis of Caucasian and Asian ethnicities failed to confirm any association between the *CDH1*-160C > A polymorphism and risk of gastric cancer.^[25] An analysis in the Iranian Kurdish population showed that gastric cancer susceptibility was not influenced by -160(C > A) *CDH1* polymorphism, regardless of the presence or absence of *H. pylori*.^[26]

In the present study, no difference was observed between -160 C or A allele as well as the different genotypes of -160C > A among GC patients and normal controls ($P = 0.559$) [Table 4], which is in concordance with many results achieved from previous studies. Logistic regression analysis suggested that GA/G genotype carriers had a 5.73-fold higher risk (95% CI: 2.11–15.56) of developing gastric cancer. Our findings did not show any statistical correlation between the genotype distribution of -160C > A or -347GA > G with sex, tumor site, tumor grade, lymphatic invasion, perineural invasion, tumor stage, and tumor type in gastric cancer patients. A significant difference was found in the frequency of E-cadherin-347GA > G genotype between GC patients and normal individuals, instead ($P = 0.001$) [Table 4].

CONCLUSION

Results from this study suggest that -347GA > G polymorphism may increase the chance of developing GC in the population from Northern Iran, and -160C > A polymorphism did not raise the risk of gastric cancer. Our findings support previous researches that reported the -160A allele as an ethnicity-dependent risk factor for GC.

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Conflicts of interest

There are no conflicts of interest.

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