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Association of the polymorphism in DNA Repair Enzymes and *E-Cadherin* Gene with Gastric Carcinoma Risk in population from Brazil

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Abstract:

SNPs have been associated with gastric cancer susceptibility. Thus, we evaluated the influence of SNPs in DNA repair genes APE1 2197(T>G) and MLH1 -93(G>A), as well as in CDH1 at positions -160 (C>A) and -347 (G>GA) in gastric cancer risk. Analyses were performed on 264 GC patients and 391 healthy controls matched by age and sex from two regions in Brazil, Ceará and Pará. In the general analysis, a protective was found for the GGA of the CDH1-347 (G>GA). Considering the GC subtypes, the polymorphic allele of both CDH1 SNPs was associated with protection to intestinal subtype. In this subtype, the MLH1 (G>A) allele A was associated with risk, contrast with protection to diffuse subtype. In females, a risk was associated with AA genotype of MLH1 (G>A) and a protection with APE1 (T>G) and CDH1 -347 (G>GA) polymorphic alleles. Considering H. pylori ,

the APE1 (T>G) G allele was associated with low virulence strains, while MLH1 (G>A) GA genotype was associated with more virulence strains. The MLH1 (G>A) A allele in the intestinal subtype was associated with more virulence strains. Considering the two regions studied, we observed that the associations found regarding APE1 (T>G), MLH1 (G>A) and CDH1-347 (G>GA) polymorphic alleles, were only linked to patients from Pará State. Protection was associated with CA of the CDH1-160 (C>A) in males from Ceará State. In summary, our data show that these SNPs are potentials markers of susceptibility to the different subtypes of GC, in which gender is also an important aspect.

Key words: Gastric cancer, *Helicobacter pylori*, *APE-1, MLH1, CDH1*

Introduction

Gastric cancer (GC) is the fourth most frequently diagnosed cancer and the second leading cause of cancer death worldwide, and it has been more prevalent in the Western Pacific and less developed regions (8). In Brazil, this is third in incidence among men and fifth among women. Helicobacter pylori infection and genetic factors have been identified as important in the development of GC. Recent genome-wide association studies have reported that some human single nucleotide polymorphisms (SNPs) are associated with susceptibility to GC, indicating that these genetic alterations are potential targets for CG risk studies (24). Genetic variations in DNA repair enzymes can modulate DNA repair capacity and can consequently be important for GC susceptibility (10),. Additionally, the only gene associated with hereditary diffuse GC is CDH1 (E-cadherin) (2, 20); therefore, polymorphisms in this gene can also contribute to GC development.

There are four major DNA repair pathways, nucleotide excision repair (NER), base excision repair (BER), double-strand break repair (DSBR) and mismatch repair (MMR), and

more than 150 human genes are involved in these pathways (12). Apurinic/apyrimidinic endonuclease 1 (*APE1*) and MutL homolog 1 (MLH1) are key enzymes in the BER and MMR pathways, respectively. APE1 is essential in the repair of oxidized bases and MLH1 repairs base–base mismatches that occur during DNA replication in proliferating cells, which are promoted by some chemical agents (11,13).

Therefore, in this study, we analyzed SNPs in DNA repair genes involved in the BER (*APE1*, T>G) and MMR (*MLH1*) pathways, as well as in *CDH1* at positions -160 (C>A) and -347 (G>GA) to evaluate the role of these SNPs in GC risk.

Materials and Methods

Study Population

Tumoral gastric tissue samples were collected from individuals who underwent gastrectomy in four hospitals: Walter Cantideo Hospital at the Federal University of Ceará. Santa Casa de Misericórdia Hospital in the state of Ceará and Hospital Ofir Loyola, Joao de Barros Barreto in the state Para. DNA samples for the control was obtained from Blood samples were obtained at the Ceará Hematology Center, Santa Casa de Misericórdia Hospital, in the state of Ceará and Pará Hematology Center for control purposes in the polymorphism analysis from cancer-free individuals. The control population was age and sex matched with the gastric cancer patients. All patients signed an informed consent form agreeing with this study and all procedures were approved by the Ethical Committee of the involved hospitals.

DNA Extraction

Genomic DNA was extracted from frozen tumor tissue and whole blood respectively by cetyltrimethyl ammonium bromide (CTAB) method (Foster GD and Tweel D, 1996) and salting-out

procedure (Miller SA et al., 1988) and stored at −20 °C until further use (9,18).

Polymorphism Genotyping

Single nucleotide polymorphisms (SNPs) for DNA repair and E-cadherin genes were determined by a PCR-(restriction fragment length polymorphism— RFLP) based method. PCR products were generated using in each reaction a total volume of 20µl containing PCR MasterMix 1X (Promega Madison WI), 0.4 mM each primer and 100 ng DNA template.

The reactions proceeded under the following conditions: initial denaturation at 94ºC for 4 min. followed by 35 cycles of 94ºC 45 sec, annealing temperature as described in Table 1 and extension at 72ºC for 1 min. An extension period for 5 min followed the final cycle. Negative (water) and positive (DNA containing known each DNA repair enzymes and *E-Cadherin* genes) controls were assayed in each run. The amplified fragments were visualized in 2% agarose gels containing ethidium bromide under UV light and were digested with appropriate restriction endonucleases. The restriction enzymes and the fragment size are described in Table 1. The fragments were resolved by 8% nondenaturing polyacrylamide electrophoretic gels and silver staining.

Genotyping *H. pylori*

The *H. pylori* infection was detected by amplification of the urease C gene using primers for PCR, as described by Lage et al (16). For the H. pylori-positive samples, the presence of the *vacA* and alleles, *cagA*, *cagE* and *virB11* genes were identified using the primer sets from the published literature. These are shown in Table 1. PCR for amplification of *cagA*, *cagE*, *virB11* and *vacA* genes, were prepared as described by Lima et al (17). PCR products were separated on 6% nondenaturing polyacrylamide electrophoretic gels stained with silver.

Statistical Analysis

The statistical analyses were conducted using the EPINFO1 6.0 e UNPHASED 3.1.7. Statistically significant differences were evaluated by the chi-square test (x2) and Fisher's exact test. A *p*-value less than 0.05 was regarded as statistically significant. Hardy-Weinberg equilibrium was also tested. A significance level (<) of 0.05 was adopted for all used tests.

Results

Among the 264 cases analyzed, males were more frequent (174, 65.9%) than females (90, 34.1%). The average age was 62 years old, ranging from 23 to 92. The intestinal subtype was slightly more common than the diffuse subtype (53 *vs* 47%). *H. pylori* infection was detected in 248 out of 264 (93.9%) gastric adenocarcinomas.

The allele and genotype distributions and risk analysis of the DNA repair enzyme and *CDH1* polymorphisms are shown in Table 2. Both groups of GC patients and control were in Hardy-Weinberg equilibrium for all the polymorphisms studied. Protection was statistically associated with *CDH1* -347 (G>GA) polymorphism, where the homozygous wild-type genotype was more frequent in the cases and the heterozygous genotype in the control. However, only a tendency was observed in the dominant model.

Considering the histological subtypes (Table 3), we observed that the *MLH1*-93 (G>A) A allele increased the chance of developing the intestinal subtype, while the heterozygous genotype gave protection against development of the diffuse subtype. On the other hand, the presence of the A (-160) and GA (-347) *CDH1* polymorphic alleles decreased the chance of developing the intestinal subtype. Additionally, with regard to the *CDH1*-347 (G>GA) polymorphism, protection was also observed with the heterozygous genotype and the dominant

model. A tendency for risk of diffuse tumors for this polymorphism was observed associated with the homozygous polymorphic genotype.

Considering risk according to gender (Table 4), significance was only found in females, where risk was associated with the homozygous polymorphic genotype of *MLH1-93 (G>A)* and where protection was associated with polymorphic alleles of *APEI* (T>G) and *CDH1* -347 (G>GA). However, considering a cutoff of 62 years old, no association was found in the total samples (results not shown).

For *H. pylori* tumor analysis, the cases were grouped according to *H. pylori* genotype, using as division criteria for the *vacA* alleles (s1/s2) and the presence of cag pathogenicity island (cag-PAI) integrity, considering the markers studied, *cag*A(+), *cag*E(+) and *vir*B11(+) or at east one on the right and left side of the island. Thus, 2 groups were formed, namely the more virulent strains with *vacA*s1 independent of cag-PAI integrity and *vacA*s2 but with cag-PAI integrity. Low virulence was considered in those with *vacA*s2 without cag- PAI genes or the presence of only one of these genes.

Table 5 shows the analysis of *H. pylori* groups in association with the DNA repair and *CDH1* SNPs. In this analysis, the *APE1* (T>G) polymorphic allele was significantly associated with the low-virulence strain group, while the heterozygous genotype of *MLH1* (G>A) and dominant model were associated with strains of the more virulent group. Considering the histological subtype, patients carrying the A allele of *MLH1* (G>A), with the intestinal subtype, were infected with a more virulent strains (94.5%; 69/73, p=0.00001). No significance was found for the diffuse subtype.

Analysis according to the region studied (Pará and Ceará)

Considering the repair enzymes in the two regions studied, we found that the heterozygous genotype of *MLH1* (GA) was more frequent in the Pará than Ceará population (GA *vs* GG; p=0.005). While the homozygous polymorphic genotype of *MLH1* (AA) was more frequent in the Ceará than Pará population (AA *vs* GG: *p*= 0.022).

Only for Pará State was the *MLH1A* allele found to be associated with risk for the intestinal subtype, while this allele was associated with protection against the diffuse subtype [intestinal (A *vs* G: OR= 1.59; CI:0.92-2.77; p=0.079); diffuse (GA vs GG: OR= 0.47; CI: 0.21-1.04; p=0.042)]. Additionally, in this state, protection against the intestinal subtype was observed for heterozygous genotypes of *APE1* 2197 (T>G) and polymorphic allele for *CDH1*-347 (G>GA) [*APE1* 2197 (TG *vs* TT: OR=0.41; CI: 0.18-0.94; p=0.020); *CDH1*-347 (GGA *vs* GG: OR= 0.38; CI: 0.18-0.80; p=0.005); (GGA+GAGA *vs* GG: OR= 0.40; CI: 0.19-0.83; p=0.007); (GA *vs* G: OR= 0.56; CI: 0.31-0.99; p= 0.031)]. In fact, the frequencies for the enzymes listed above statistically differed in each subtype according to the region. In the intestinal subtype, the presence of the *MLH1-93 (G>A)* allele was statistically more frequent in Pará patients [(A *vs* G; p=0.003), (GA *vs* GG; p= 0.051), (GA+AA *vs* GG; p=0.038)]. In the diffuse subtype, the heterozygous genotypes of *MLH1-93* (G>A) were statistically more frequent in Pará patients (GA *vs* GG: p=0.050).

Considering gender, in Ceará State patients, *CDH1* (-160 C>A) heterozygous genotype was statistically associated with protection in males (CA *vs* CC: OR=0.53; CI: 0.29-0.95; p= 0.043), while in Pará patients, the *CDH1* -347 (G>GA) polymorphic allele was statistically associated with protection in females [(GGA *vs* GG: OR= 0.37; CI: 0.14-0.98; p=0.026);

(GGA+GAGA *vs* GG: OR=0.41; CI: 0.16-1.06; p= 0.041)]. Also, taking into account age, protection associated with the *APE1* 2197 (T>G) polymorphic allele was only observed in patients <62 years old from Pará State [(TG *vs* TT: OR=0.47; CI: 0.23-0.96; p= 0.037); (TG+GG *vs* TT: OR: 0.53; CI: 0.27-1.02)].

Interestingly, infection with low-virulent *H. pylori* strains was significantly associated with the *APE1 (*T>G) polymorphic allele in both states [Ceará (TG *vs* TT: p= 0.013); (GG *vs* TT: p= 0.019); TG+GG *vs* TT: p=0.005); Pará (GG *vs* TT: p= 0.0003); (TG+GG *vs* TT: p= 0.002).

Discussion

Genetic polymorphism can contribute to susceptibility to disease. Extensive analysis of human SNPs has led to the identification SNPs that can be interesting markers for certain disorders such as cancer. The DNA repair gene SNPs *APE1* 2197(T>G) and *MLH1* -93 (G>A) as well as *CDH1* -160 (C>A) and *CDH1* -347 (G>GA) have been poorly studied with regard to GC risk and with inconsistent results (21,28,29).

Our study is the first report showing a case-control study in Brazil considering DNA repair enzyme polymorphisms. In this study, no significant associations with GC risk were found for *APE1* (T>G) and *MLH1* (G>A) SNPs. Similarly, Palli et al. (21), in an Italian population, did not find any association for *APE1* (T>G) SNP, but our data conflict with the study of Canbay et al. (3), who found in Turkish patients a risk for GC associated the with *APE1* (T>G) heterozygous genotype and polymorphic allele. However, in the latter study, the number of patients was small. For *MLH1* (G>A) SNP, our data agreed with two studies in Chinese patients (7,28). The apparent inconsistency of these results may be due to differences in ethnicity and indicate that more investigations are needed to

elucidate the exact role of these polymorphisms in GC susceptibility in different populations.

Different from the repair enzymes, protection was found associated with the *CDH1*-347 (G>GA) heterozygous genotype in this study. This result disagreed with the report by Zhang et al (30), in Chinese patients, who found a risk for GC associated with the homozygous polymorphic genotype. On the hand, the absence of association with *CDH1* -160 (C>A) SNP agrees with the findings of Jenab et al (14), in Korean GC patients. However, previously, Wu et al (27), reported on this SNP in Taiwanese patients, finding protection associated with the homozygous polymorphic genotype. It is hard to understand how the heterozygous genotype for the *CDH1* SNPs reduces the risk of GC, since it is believed that both SNPs are associated with lower protein expression. In this regard, it is important to emphasize that this gene is found methylated in a significant number of cases, and taking into account the results found in the literature (15), these alterations could interfere with risk analysis, and methylation must be determined in polymorphism analysis. So, further work is mandatory to clarify the functional relevance of the inverse association to the polymorphic alleles *CDH1* -160A and -347GA with GC also considering the methylated cases.

Since the intestinal and diffuse GC subtypes have distinct features, we analyzed the risk according to them. The risk found associated with the *MLH1* (G>A) A allele in the intestinal subtype contrasted with the protection this allele conferred against the diffuse subtype, corroborating the notion that these tumors have different pathways and therefore different genetic susceptibility. Recent studies in sporadic colorectal cancer have reported that the A allele of the *MLH1* (G>A) SNP located in the promoter region is significantly associated the *MLH1* promoter methylation followed by loss of MLH1 protein expression (6,19). Thus, the *MLH1* (G>A) SNP

could be an important factor in the susceptibility of the GC intestinal subtype through methylation predisposition.

Interestingly, we found that the polymorphic allele of both *CDH1* SNPs afforded protection against the intestinal subtype. Considering these results, we can argue the possibility that the frequency of intestinal and diffuse subtypes included in studies may influence risk findings, explaining in part the inconsistencies in the literature. Accordingly, the frequencies of intestinal and diffuse subtypes reported by Jenab et al. (14) could be similar as ours, and in the study by Wu et al. (27) the intestinal subtype may be overrepresented.

The risk was also analyzed according to the gender, since this cancer is more frequent in males and since hereditary diffuse GC is more frequent in women (23). In fact, all associations were found only in females, which agrees with the literature. The risk associated with the *MLH1* (G>A) homozygous polymorphic genotype was corroborated in study by Miyakura et al. (19) who reported that this gene in Japanese females carrying the A allele in the *MLH1* (G>A) SNP was more frequently methylated in sporadic colorectal cancers and therefore related to susceptibility to GC. On the other hand, in females, protection was associated with the polymorphic alleles of *APE1* (T>G) and *CDH1* -347 (G>GA). A biological explanation has to be investigated in a large number of cases considering *H. pylori* infection for *APE1* (T>G) and methylation for and *CDH1* -347 (G>GA). However, this data shows that the gender can interfere for GC susceptibility, may be through the physiological or habits differences.

GC is considered a disease of the elderly population with higher occurrence in those over 50 years old, and less than 5% of cases occur under the age of 40 (23). In spite of that, in general analysis, no significant associations for GC risk was found for the SNPs studied considering a cutoff of 62 years old, which was the average age of the cases.

Another important aspect to consider in GC risk studies is *H. pylori* infection. In this analysis, the *APE1* (T>G) polymorphic allele was statistically more frequent in infections with the low-virulence strains than high-virulence strains, corroborating our previous results (25). Accordingly, the decreased activity of APE1 caused by the polymorphic allele seems to explain the finding of GC associated with the less virulent strains. On the other hand, we found an association of the *MLH1* (G>A) heterozygous genotype and polymorphic allele with strains of the high-virulence group. In fact, the risk observed in the intestinal subtype was due to the presence of infection with more virulent strains. These results are in accordance with the hypothesis of breaking the balance between the bacteria and the host in GC development.

Brazilians form one of the most heterogeneous populations in the world, which was formed by Amerindians, Europeans and Africans. There is great diversity even between regions, such as the states of Ceará and Pará, which have a different genetic composition (22). In fact, the associations found in this study regarding *APE1* and *MLH1* and also *CDH1-347* polymorphic alleles were only linked to patients from Pará state. However, the finding of *CDH1-347* mediated protection against the intestinal subtype disagrees with the that of Borges et al (1), in which no association was found with the population from the same state. This disagreement may have due to the small number of cases compared to our study as pointed out by others. On the other hand, in females from Ceará, protection was associated with the *CDH1*-160 heterozygous genotype. These differences could be explained by the existence of a variation in frequencies of the polymorphisms studied between the two states, reinforcing the importance of ethnicity in risk studies.

Interestingly, similar to the general analysis, low-virulence *H. pylori* strains were associated with the *APE1*

polymorphic allele in both states, corroborating our previous results, which showed this association only in patients from Ceará State.

Conclusion

In summary, our results pointed out that DNA repair SNPs *APE1* 2197 (T>G) and *MLH1* -93 (G>A) and *CDH1* SNPs at positions -160 (C>A) and -347 (G>GA) are potential markers of susceptibility to intestinal and diffuse GC, in which gender is also an important aspect. Moreover, we showed a relationship between *H. pylori* genotype and host, where patients carrying the *APE1* polymorphic allele were significantly infected by low-virulence strains, while patients with the heterozygous genotype and polymorphic allele of *MLH1* were significantly infected by high-virulence strains. Additionally, we showed the importance of genetic background in risk studies. More studies of SNPs considering the histological subtype, gender and their combination with *H. pylori* genotypes, in a larger number of cases in different populations, are needed to better understand the importance of these polymorphisms in GC risk.

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Table 1. PCR primer sets, annealing temperature and size of the PCR products used for genotyping Polymorphism and H. pylori . F – follow; R – reverse.

Gene	Primer sequence	Annealing	Size of PCR product	Restriction enzyme	Reference
ureC	F – 5´-AAGCTTTTAGGGGTGTTAGGGGTTT-3´ R – 5`-AAGCTTACTTTCTAACACTAACGC-3`	55 ºC	294 bp	-	Lage AP et al., 1995
vacA s1/s2	F – 5`-ATGGAAATACAACAAACACAC-3´ R – 5´-CTGCTTGAATGCGCCAAAC-3´	55 ºC	259/286 bp	-	Lima et al
m1	F – 5´GGTCAAAATGCGGTCATGG3´ R – 5´-CCATTGGTACCTGTAGAAAC-3´	55 ºC	290 bp	-	
m2	F – 5´-GGAGCCCCAGGAAACATTG-3´ R – 5´-CATAACTAGCGCCTTGCAC-3´	52 ºC	192 bp	-	
cagA	F – 5´-ATAATGCTAAATTAGACAACTTGAGCGA-3´ R – 5´-TTAGAATAATCAACAAACATAACGCCAT-3´	56 ºC	297 bp	-	
cagE	F – 5´-TTGAAAACTTCAAGGATAGGATAGAGC-3´ R – 5´-GCCTAGCGTAATATCACCATTACCC-3´	56 ºC	509 bp	-	
*virB*11	F – 5´-TTAAATCCTCTAAGGCATGCTAC-3´ R – 5´-GATATAAGTCGTTTTACCGCTTC-3´	49 ºC	491 bp	-	
APE-1 2197 (T>G)	F: 5`-CTGTTTCATTTCTATAGGCTA-3` R: 5`-AGGAACTTGCGAAAGGCTTC-3`	48.5ºC	T 164 bp G 144 bp and 20 bp	*BfaI*	Vodicka P et al., 2007
MLH1 -93 (G>A)	F-5`-AGTAGCCGCTTCAGGGA-3` R-5`-CTCGTCCAGCCGCCGAATAA-3`	50ºC	G125 and134bp A259bp	*PvuII*	Chen et al., 2005
CDH1 -160 (C>A)	F- 5'-GCCCCGACTTGTCTCTCTAC-3' R- 5'-GGCCACAGCCAATCAGCA-3'	61°C	C 446 bp A 368 and 68bp	*Hinc*II	Chien et al. (2011)
CDH1 -347 (G>GA)			G-263, 115 e 68 pb GA-331 e 115 pb	*Ban*II	

Table 2. Allelic and genotype frequencies for the polymorphisms of *APE1*. *MLH1* and *CDH1* in the studied population.

Polymorphism	Cases n (%)	Controls n (%)	OR**(IC 95%)**	P
APE1 **2197 (T>G)**				
T/T	100 (38.6)	129 (33.0)	1	-
T/G	118 (45.6)	205 (52.4)	0.74(0.52-1.06)	0.091
G/G	41 (15.8)	57 (14.6)	0.93(0.56-1.54)	0.759
T/T+T/G	218 (84.2)	334 (85.4)	0.84(0.61-1.16)	0.279

T/G+G/G	159 (61.4)	262 (67.0)	0.78(0.56-1.10)	0.142
Alelo T	318 (61.4)	463 (59.2)	0.89(0.65-1.21)	0.425
Alelo G	200 (38.6)	319 (40.8)	0.81(0.58-1.12)	0.186
***MLH1*-93(G>A)**				
G/G	129 (49.8)	194 (49.6)	1	-
G/A	109 (42.1)	178 (45.5)	0.92(0.66-1.29)	0.620
A/A	15 (5.80)	19 (4.9)	1.19(0.55-2.55)	0.636
G/G+G/A	238 (91.9)	372 (95.1)	0.96(0.72-1.28)	0.783
G/A+A/A	124 (47.9)	197 (50.4)	0.95(0.68-1.32)	0.733
Alelo G	367 (72.5)	566 (72.4)	0.98(0.75-1.27)	0.848
Alelo A	139 (27.5)	216 (27.6)	0.97(0.70-1.33)	0.834
***CDH1*-160 (C>A)**				
C/C	128 (49.4)	181(46.3)	1	-
C/A	102 (39.4)	164 (41.9)	0.88(0.62-1.25)	0.452
A/A	28 (10.8)	32 (8.2)	1.24(0.68-2.23)	0.451
C/C+C/A	230 (88.8)	345 (88.2)	0.94(0.70-1.26)	0.680
C/A+A/A	130 (50.2)	196 (50.1)	0.94(0.67-1.3)	0.691
Alelo C	358 (69.4)	526 (69.8)	0.96(0.73-1.26)	0.775
Alelo A	158 (30.6)	228 (30.2)	0.98(0.71-1.34)	0.895
***CDH*1 -347 (G>GA)**				
G/G	163 (62.9)	211 (54.0)	1	-
G/GA	81 (31.3)	152 (38.9)	0.69(0.48-0.98)	0.031*
GA/GA	13 (5.0)	12 (3.1)	1.40(0.58-3.38)	0.411
G/G+G/GA	244 (94.2)	363(92.8)	0.87(0.66-1.14)	0.295
G/GA+GA/GA	94 (36.3)	164 (41.9)	0.74(0.53-1.04)	0.072
Alelo G	407 (79.2)	574 (76.5)	0.92(0.72-1.18)	0.485
Alelo GA	107 (20.8)	176 (23.5)	0.79(0.57-1.09)	0.136

Table 3. Allelic and genotype frequencies for the polymorphisms of *APE1*. *MLH1* and *CDH1* considering the histological subtypes in the studied population.

	Intestinal				Diffuse			
Polymorphism	Cases n (%)	Control n(%)	OR(IC 95%)	p	Cases n (%)	control n(%)	OR(IC 95%)	p
APE1 2197(T>G)								
T/T	52 (37.7)	64 (31.2)	1	-	46 (38.7)	96 (44.7)	1	-
T/G	64 (46.4)	105 (51.2)	0.75 (0.45-1.25)	0.240	54 (45.4)	98 (45.6)	1.15(0.69-1.92)	0.571
G/G	22 (15.9)	36 (17.6)	0.75 (0.38-1.50)	0.385	19 (16)	21 (9.8)	1.89(0.87-4.09)	0.078
T/T+T/G	116 (84.1)	169 (82.4)	0.71 (0.44-1.14)	0.137	100 (84)	158 (73.5)	1.32(0.84-2.08)	0.205
T/G+G/G	86 (62.3)	141 (68.8)	0.75(0.46-1.21)	0.214	73 (61.3)	119 (55.3)	1.28(0.79-2.07)	0.288
T	168 (60.8)	233 (56.8)	1	-	146 (61.3)	218 (60.9)	1	-
G	108 (39.1)	177 (43.1)	0.85(0.61-1.17)	0.292	92 (38.6)	140 (39.1)	0.98(0.69-1.39)	0.911
MLH1 -93 (G>A)								
G/G	63 (45.7)	112 (54.6)	1	-	64 (53.8)	80 (37.2)	1	-
G/A	65 (47.1)	86 (42)	1.134(0.84-2.15)	0.193	44 (37)	89 (41.4)	0.62(0.37-1.04)	0.052*
A/A	6 (4.3)	8 (3.9)	1.33(0.39-4.48)	0.608	9 (7.6)	10 (4.7)	1.13(0.39-3.22)	0.809
G/G+G/A	128 (92.8)	198 (96.6)	1.15(0.77-1.71)	0.473	108 (90.8)	169 (78.6)	0.80(0.52-1.23)	0.280
G/A+A/A	71 (51.4)	94 (45.9)	0.93(0.56-1.54)	0.764	53 (44.5)	96 (44.7)	0.69(0.42-1.13)	0.121
G	191 (56.5)	310 (75.2)	1	-	172 (73.5)	249 (69.5)	1	-
A	147 (43.5)	102 (24.8)	2.56(1.69-3.90)	0.000003*	62 (26.5)	109 (30.4)	0.82(0.56-1.21)	0.299
CDH1 -160 (C>A)								
CC	68 (49.3)	106 (51.7)	1	-	58 (48.7)	76 (35.3)	1	-
CA	52 (37.7)	77 (37.6)	1.05(0.64-1.72)	0.828	49 (41.2)	83 (38.6)	0.77(0.46-1.30)	0.305
AA	16 (11.6)	16 (7.8)	1.56(0.69-3.54)	0.247	12 (10.1)	14 (6.5)	1.12(0.45-2.82)	0.787
CC+CA	120 (87)	183 (89.3)	1.02(0.69-1.53)	0.910	107 (89.9)	159 (74)	0.88(0.57-1.37)	0.557
CA+AA	68 (49.3)	93 (45.4)	1.36(0.93-1.93)	0.979	61 (51.3)	97 (45.1)	0.82(0.50-1.35)	0.417
C	188 (69.1)	216 (55.2)	1	-	165 (69.3)	235 (67.9)	1	-
A	84 (30.9)	175 (44.8)	0.55(0.39-0.77)	0.0003*	73 (30.7)	111 (32.1)	0.94(0.65-1.36)	0.718

CDH1 -347 (G>)GA								
G/G	88 (63.8)	104 (50.7)	1	-	72 (60.5)	106 (49.3)	1	-
G/GA	41 (29.7)	85 (41.5)	0.57(0.35-0.93)	0.018*	40 (33.6)	64 (29.8)	0.92(0.54-1.56)	0.742
GA/GA	7 (5.1)	10 (4.9)	0.83(0.27-2.48)	0.711	06 (5.08)	2 (0.9)	4.42(0.78-32.63)	0.052
G/G+G/GA	129 (93.5)	189 (92.2)	0.81(0.55-1.18)	0.243	112 (94.1)	170 (79.1)	0.97(0.65-1.45)	0.875
G/GA+GA/GA	48 (34.8)	95 (46.3)	0.60(0.37-0.96)	0.023*	46 (38.7)	66 (30.7)	1.03(0.62-1.71)	0.916
G	217 (79.7)	293 (73.6)	1	-	184 (77.9)	276 (80.2)	1	-
GA	55 (20.3)	105 (26.4)	0.62(0.39-0.98)	0.029*	52 (22.1)	68 (19.8)	1.15(0.75-1.76)	0.508

Table 4. Allelic and genotype frequencies for the polymorphisms of *APE1*. *MLH1* and *CDH1* considering the gender in the studied population

Polymorphism	MALE Cases n (%)	Controls n (%)	OR(IC 95%)	P	FEMALE Cases n (%)	Controls n (%)	OR(IC 95%)	P
APE1 2197 (T>G)								
T/T	62 (36.5)	90 (35.2)	1	-	37(41.6)	39(28.8)	1	-
T/G	78 (45.9)	131 (51.2)	0.86(0.55-1.36)	0.504	41(46.1)	74(54.9)	0.58(0.31-1.10)	0.072
G/G	30 (17.6)	35 (13.7)	1.24(0.66-2.33)	0.463	11(12.3)	22(16.3)	0.53(0.21-1.34)	0.137
T/T+T/G	140 (82.4)	221 (86.3)	0.92(0.61-1.38)	0.670	78((88.7)	113(83.7)	0.73(0.41-1.29)	0.242
T/G+G/G	108 (63.5)	166 (64.8)	0.94(0.62-1.44)	0.781	52(58.4)	96(71.1)	0.57(0.31-1.00)	0.049*
T	202 (59.4)	311 (60.7)	1	-	115(64.6)	152((56.3)	1	-
G	138 (40.6)	201 (39.3)	1.06(0.79-1.4)	0.697	63(35.4)	118(43.7)	0.71(0.47-1.06)	0.079
MLH1 -93 (G>A)								
G/G	91(55.2)	132 (51.6)	1	-	38(43.2)	62(46)	1	-
G/A	69 (41.8)	109 (42.6)	0.92(0.60-1.4)	0.678	40(45.5)	69(51.1)	0.95(0.52-1.72)	0.845
A/A	5 (3)	15 (5.9)	0.48(0.15-1.49)	0.165	10(11.3)	4(2.9)	4.08(1.07-16.78)	0.017*
G/G+G/A	160 (97)	241 (94.1)	0.96(0.68-1.36)	0.824	78(88.6)	131(97.03)	0.97(0.58-1.64)	0.908
G/A+A/A	74 (44.8)	124 (48.4)	0.87(0.47-1.31)	0.471	50((56.8)	73(54.1)	1.12(0.63-1.99)	0.687
G	251 (76.1)	373 (72.9)	1	-	116(65.9)	193(71.5)	1	-
A	79 (23.9)	139 (27.1)	0.84(0.61-1.18)	0.299	60(34.1)	77((28.5)	1.3(0.84-199)	0.212
CDH1 -160 (C>A)								
C/C	85 (50.3)	117 (46.8)	1	-	43(48.3)	64 (50.4)	1	-
C/A	63 (37.3)	112 (44.8)	0.77(0.50-1.2)	0.228	39(43.8)	52 (40.9)	1.12(0.61-2.05)	0.703
A/A	21 (12.4)	21 (8.4)	1.38(0.67-2.82)	0.346	7 (7.9)	11 (8.7)	0.95(0.30-2.91)	0.917
C/C+C/A	148 (87.6)	229 (91.6)	0.89(0.62-1.28)	0.509	82 (92.1)	116 (91.3)	1.05(0.63-1.75)	0.835
C/A+A/A	84 (49.7)	133 (53.2)	0.87(0.58-1.31)	0.482	46 (51.7)	63 (49.6)	1.09(0.61-1.94)	0.763
C	233 (68.9)	346 (69.2)	1	-	125 (70.2)	180 (70.9)	1	-
A	105 (31.1)	154 (30.8)	1.01(0.75-1.36)	0.935	53 (29.8)	74 (29.1)	1.07(0.61-1.86)	0.810
C*DH1* *-347 (G>GA)*								
G/G	99 (58.9)	139 (55.8)	1	-	64(71.9)	72(57.2)	1	-
G/GA	59 (35.1)	102 (41)	0.81(0.53-1.25)	0.321	22(24.7)	50(39.7)	0.49(0.26-0.94)	0.021*
GA/GA	10 (6)	8 (3.2)	1.76(0.61-5.09)	0.248	3(3.37)	4(3.2)	0.84(0.14-4.69)	0.570
G/G+G/GA	158 (94)	241 (96.8)	0.91(0.66-1.29)	0.619	86(96.6)	122(96.8)	0.79(0.50-1.25)	0.296
G/GA+GA/GA	69v(41.1)	110 (44.2)	0.88(0.58-1.33)	0.529	25(28.1)	54(42.9)	0.52(0.28-0.97)	0.026*
G	257 (76.5)	380 (76.3)	1	-	150(84.2)	194(77)	1	-
GA	79 (23.5)	118 (23.7)	0.99(0.71-1.39)	0.951	28(15.8)	58(23)	0.62(0.37-1.06)	0.062

Table 5. H. pylori Genotypes in Association With the DNA Repair Enzyme and *CDH1* Polymorphisms.

	Group more virulence)	Group low virulence	p
APE1 2197 (T>G)			
T/T	85 (45,0)	10 (22,20)	1
T/G	79 (41,8)	24 (53.30)	0.017*
G/G	25 (13,2)	11 (24,40)	0.005*
T/T+T/G	164 (86,8)	34 (75,60)	0.136

TG+GG	104 (55,0)	35 (77,80)	0.005*
MLH1 -93 (G>A)			
G/G	87 (47,30)	30 (65,20)	1
G/A	86 (46,70)	15 (32,60)	0.049*
A/A	11 (6,0)	1 (2,20)	0.291
G/G+G/A	173 (94,0)	45 (97,80)	0.295
G/A+A/A	97 (52,70)	16 (34,80)	0.029*
CDH1 -160 (C>A)			
C/C	98 (51,60)	17 (37,80)	1
C/A	70 (36,80)	22 (48,90)	0.095
A/A	22 (11,60)	6 (13,30)	0.396
C/C+C/A	168 (88,40)	39 (86,70)	0.357
C/A+A/A	92 (48,40)	29 (64,40)	0.075
CDH1-347 (G>GA)			
G/G	119 (63,0)	30 (66,70)	1
G/GA	58 (30,70)	14 (31,10)	0.904
GA/GA	12 (6,30)	1 (2,20)	0.465
G/G+G/GA	177 (93,70)	44 (97,80)	0.957
G/GA+GA/GA	70 (37,0)	15 (33,30)	0.642