Research Article

DNMT1 Gene Expression in Patients with Helicobacter pylori Infection

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DNMT1, as a critical enzyme affecting epigenetics through methylation of DNA cytosine-rich sequences, regulates gene expression. Exterior factors including long-term infections, in this study Helicobacter pylori infection, could change host cells’ epigenetics by affecting DNMT1 gene expression. This study investigated the statistical correlation between H. pylori virulence genes and DNMT1 gene expression in gastric antral epithelial cells of gastric adenocarcinoma and gastritis patients. In a case-control study, 50 and 53 gastritis and gastric adenocarcinoma antral biopsies, including 23 and 21 patients with H. pylori infection, respectively, were collected from hospitals in the west of Iran. Having extracted total RNA from gastric biopsy samples, cDNA was synthesized and virulence genes of H. pylori were detected by using the PCR method. Relative real-time RT PCR was used to detect ΔΔCt fold changes of the DNMT1 gene expression in divided groups of patients based on H. pylori infection and clinical manifestations. The results showed that along with increasing patients’ age, the DNMT1 gene expression will increase in gastric antral epithelial cells of gastric cancer patients (P ≤ 0.05). On the other hand, the biopsy samples with infection of H. pylori cagA, cagY, and cagE genotypes revealed a direct correlation along with increased DNMT1 gene expression. This study revealed the correlations of H. pylori cag pathogenicity island genes with increased DNMT1 gene expression.

1. Introduction

Methylation is a common epigenetic trend in handling cell gene expression [1]. DNMT [DNA (cytosine-5)-methyltransferase] enzymes contribute to the expression of growth-promoting genes and cell differentiation by directly adding and removing methyl groups to cytosine and histones [2]. Mammalian cells’ DNMTs include DNMT1, DNMT3A, and DNMT3B; in the meantime, DNMT1 methylates CpG cytosine-rich sequences in the gene’s promoters of the newly synthesized DNA strand [1, 3]. The results of related studies show that functionally defective DNMT enzymes increase the incidence of adenocarcinoma [4–6]. By means of this, cell proliferation will be increased following the effects of DNMTs inactivating adenocarcinoma suppressor genes through over-methylation [1, 7]. The molecular mechanism of carcinogens is conducted through their effects on DNMT activity; for instance, microbes such as uropathogenic E. coli increase the DNMT1 gene expression in host cells [8–12].

Cell signaling studies show that the APC (Adenomatous Polyposis Coli) as a tumor suppressor gene and a regulator of the Wnt signaling pathway through β-catenin stability and/or degradation controls DNMT1 gene expression [13]. The Wnt (Wingless oncogene INT1) receptors affect the
APC/β-Catenin signaling complex, then releasing β-Catenin and activating the TCF (T-cell factor) transcription factor following attack to the DNMT1 gene promoter block gene expression [13–15]. In this regard, clinical complications are impressed with the direct interaction of DNMT1 protein with the APC/β-Catenin signaling complex that has strongly been reported in colorectal adenocarcinoma mice [15]. In addition, the basic studies show bacteria reduce the expression of Wnt/β-Catenin repressor genes causing an increased expression of the DNMT1 gene [16–18]. Upregulation of Cadherin-1 (CDH1), as an inhibitor of lung cell metastasis, reduces Wnt/β-Catenin activity in non-small-cell lung adenocarcinoma cells [19–21]. Furthermore, methylation of the CpG sequences in the promoter of the CDH1 gene by DNMT1 leads to preventing its expression [21]. Regarding the results of molecular-oriented studies, Helicobacter pylori CagA (Cytotoxin-associated gene A) protein disrupts the Wnt/β-Catenin signaling pathway leading to an increase in DNMT1 gene expression [22–24].

Epstein–Barr virus (EBV) as a well-known carcinogenic viral agent infects B lymphocytes and increases the activity of the DNMT1 enzyme [25, 26]. The relevant studies show that EBV infections have a high prevalence in human societies; in addition, a small percentage of people show H. pylori and EBV co-infection [27–30]. This hypothesis supported that the H. pylori and EBV co-infection could lead to further DNMT1 gene upregulation and accelerates the process of gastric epithelial cell carcinoma [30, 31].

This study aimed to investigate the correlation between H. pylori virulence genes and the DNMT1 gene expression in gastric epithelial cells of patients.

2. Materials and Methods

2.1. Sampling. A case-control study was designed involving gastritis and gastric adenocarcinoma patients as the control and case groups, respectively. 53 and 50 patients with gastric adenocarcinoma and gastritis were enrolled. The cDNA of H. pylori virulence genes in biopsy specimens was detected by conventional PCR (polymerase chain reaction) [32]. The consecutive sampling method was used to collect gastric biopsy samples from patients with gastrointestinal symptoms referred to Tohid and Shahid Ghazi hospitals in Sanandaj city, located in the west of Iran [33–35]. The urea breath test was used to determine the H. pylori active infection [33]. Gastric antral biopsy specimens were obtained from each patient for molecular analysis of H. pylori genotypes, EBV infection, and DNMT1 gene expression. The gastric biopsy specimen was immediately dropped into RNALater (Roche Co.) solution and transferred to the Molecular Microbiology Laboratory of Kurdistan University of Medical Sciences. A clinicopathological biopsy sample was collected from the gastric carcinoma tumor area of patients with gastric adenocarcinoma and transferred to Tohid Hospital Pathology Laboratory. To evaluate the clinicopathological biopsy, the Kaplan–Meier method was used, including stage (TNM system (tumor-node-metastasis)) and tumor grade (including 1, 2, 3, and 4 grades) as macroscopic and microscopic examination methods, respectively [36].

2.2. H. pylori Virulence Gene PCR. Total RNA was extracted from patients’ gastric biopsy specimens by using an RNA extraction kit (Takara Co.) and immediately converted to cDNA. The extracted RNA integrity was evaluated by observing the 28S and 18S rRNA bands on the 1.2% agarose gel [37]. The absorbance ratio at 260 nm and 280 nm was assessed for the purity of RNA by using NanoDrop® 2000 spectrophotometer machine (Thermo Fisher Company, Germany) [37]. Using online primer three software (version 0.4.0), specific primers of H. pylori virulence genes were designed and synthesized (Bioneer, South Korea Co.). First, the H. pylori 16s rDNA gene primer was used to determine the H. pylori genome’s cDNA by PCR (Table 1). Other primers of H. pylori virulence genes were used in the biopsy specimens of patients with positive-H. pylori 16s rDNA gene. The annealing temperature of H. pylori virulence genes’ specific primers is shown in Tables 1 and 2. PCR master mix was included buffer 10X (2.5 microlitre), DNA Taq polymerase 5U/μlitrer (0.25 microlitre), dNTPs 10 mM (0.5 microlitre), MgCl2 50 mM (1 microlitre), cDNA (2 microlitre), forward and reverse specific primers 10 picoliter (each one 0.5 microlitre) and RNase-free water (12.75 microlitre) in final volume 20 microlitre [37]. The Thermal cycling PCR steps involved an initial denaturation at 94°C for 5 minutes, a denaturation at 94°C for 30 seconds, a primer annealing for 45 seconds (temperatures are shown in Table 1), an extension at 72°C for 45 seconds, and a final extension at 72°C for 5 minutes [37]. The PCR product was rounded on 1.5% agarose gel.

2.3. EBER PCR. To detect the EBV virus genome, EBER (Epstein–Barr virus-encoded small RNAs) gene’s cDNA was detected by PCR in the gastric epithelial antral biopsy samples. EBER-specific primers were checked by using the Epstein–Barr virus (Human gamma herpes virus 4) gene sequence in the GenBank and online primer software 3. Table 3 shows the sequence and annealing temperature of the EBER-specific primers. According to the standard PCR protocol, PCR was performed to determine gastric epithelial cells’ infection to the EBER sequence.

2.4. DNMT1 Gene Real-Time PCR. The DNMT1-specific primers as the target gene and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) as internal genes were checked using gene sequence in the GenBank and online primer software 3 (Table 3). The PCR amplification program began with a 10 minute denaturation step at 95°C and then 40 cycles of denaturation at 95°C for 15 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 60 seconds [37]. The relative amounts of the DNMT1 expression gene between gastritis (control group) and gastric adenocarcinoma (case group) patients were evaluated by ΔCt (threshold cycle) = Ct (DNMT1) Ct (GAPDH). Finally, the ΔΔCt expression fold changes were calculated by the 2−ΔΔCt formula [42].

2.5. Statistical Methods. Statistical analysis of the results was performed using SPSS (Statistical Package for the Social Sciences) software version 26. Correlations between
qualitative variables, including sex, disease, and *H. pylori* infection, were calculated using chi-square and crosstab statistical tests [35]. To investigate the *DNMT1* gene ΔCt as a quantitative variable in different groups and subgroups, the distribution of the *DNMT1* gene ΔCt was detected using the Kolmogorov–Smirnov test [35]. The *DNMT1* gene ΔCt values showed normal distribution, so Student’s *T*-tests and ANOVA analysis were used to investigate the correlation

| Table 1: Primers of *H. pylori* T4SS, 16s rDNA, and *vacA* in simple PCR. |
|-----------------------------|--------------------|------------------------|----------------|
| **Specific primers**        | **Sequence**       | **Annealing, Tm °C**   | **Product size, bp** | **Reference** |
| 16s rDNA *H. pylori* F      | CTGGAGAGACTAAGGCCCTCC | 50                     | 446               | This study    |
| 16s rDNA *H. pylori* R      | AGGATCGTGTTTAAGAGATT | 57                     | 108               | This study    |
| *caga* F                    | TGACCAACACACACAAACCCG | 57                     | 188               | This study    |
| *caga* R                    | TCAGGATCCTAGGAGGCGACAG | 57                     | 200               | This study    |
| *caga* EPIYA-C F            | AAGAAAGCAGGAACAGACGC | 55                     | 154               | This study    |
| *caga* EPIYA-C R            | CTAAAGCGATACCCCTACCTT | 56                     | 163               | This study    |
| *cagT* F                    | AGTTGAGCGAAGCCATGAAA | 56                     | 205               | This study    |
| *cagE* R                    | TAGGAAATTCGAGGGCTCAC | 57                     | 205/220*          | This study    |
| *vacA* s1/s2 F              | ATGGAAATCAACACACACAC | 55                     | 259/286*          | [38]          |
| *vacA* s1/s2 R              | CTGGTTAGATGCGGCAACAC | 55                     | 205/220*          | This study    |

*The primers yield various *vacA* depending on a repetitive nucleotide sequence in some *vacA* alleles.

| Table 2: Primers of *H. pylori* adhesins and surface proteins. |
|-----------------------------|--------------------|------------------------|----------------|
| **Specific primers**        | **Sequence**       | **Annealing, Tm °C**   | **Product size, bp** | **Reference** |
| iceA1 F                     | GTTGGTTTAAACCCAAAGTATC | 45                     | 247               | [39]          |
| iceA1 R                     | CTATAGCCASTTCTTTCGCA | 47                     | 229/234           | [39]          |
| iceA2 F                     | GGGTGGTATATCACAATTTAT | 55                     | 187               | [40]          |
| iceA2 R                     | TTRCCCTATTCTCAAGGTT | 55                     | 160               | [40]          |
| hopQ1e F                    | AGTGGAGCGAAGCCATGAAA | 56                     | 205               | This study    |
| hopQ1e R                    | CATCGTGTTATGCGGGCTTAC | 57                     | 205               | This study    |
| hopQII F                    | ACAGCCACTCCTACCGAAGA | 55                     | 248               | This study    |
| hopQII R                    | AAGCCAAGCGTGCTCACTTAC | 56                     | 248/249           | This study    |
| babA2 F                     | CAATTGCCTGGTGCGTAAAATC | 57                     | 248               | This study    |
| babA2 R                     | ATACCCTTGCTGCTTTGTTGA | 56                     | 248               | This study    |
| babB F                      | CATTGCCGCCGGCCGATAAAC | 56                     | 175               | This study    |
| babB R                      | ATTTGAAGTGATGCGTGCTCG | 56                     | 175               | This study    |
| sabA F                      | TCTCTCGGTGCTGATCATAT  | 56                     | 204               | This study    |
| sabA R                      | ATACCCTTGCTGCTTTGTTGA | 56                     | 204               | This study    |
| sabB F                      | GATAGCCCTAAGAGCCGCAAC | 56                     | 248               | This study    |
| sabB R                      | TCTCTGTGCGTATCCTTCCAT | 56                     | 248/249           | This study    |
| alpA F                      | CGCCTCTATCCTACCCCGTC | 55                     | 260               | This study    |
| alpA R                      | TCTCCGAGACCTACCTTGCTA | 56                     | 260               | This study    |
| alpB F                      | TCTCTGGCGACGAGACTGAAC | 56                     | 260               | This study    |
| alpB R                      | ATGCATAGACCCCTACACG  | 56                     | 260               | This study    |
| oipA F                      | CTCCACGTTGAAAGGAAATGG | 55                     | 233               | This study    |
| oipA R                      | CATTTCTGGCTAATCGGTT  | 55                     | 233               | This study    |

| Table 3: Specific primers of the *DNMT1* gene in real-time RT PCR. |
|-----------------------------|--------------------|------------------------|----------------|
| **Specific primers**        | **Sequence**       | **Annealing, Tm °C**   | **Product size, bp** | **Reference** |
| GAPDH F                     | ACCCTGTGCGTGTAGCCA | 56                     | 102               | Universal     |
| GAPDH R                     | CCACCTCCACCGCTTGGCA | 56                     | 81                | [41]          |
| *DNMT1* F                   | AGAACGGTGCTCATGCTTACA | 56                     | 81                | [41]          |
| *DNMT1* R                   | CTCTACGGGCTTCTAAGCTTG | 56                     | 81                | [41]          |
| EBER F                      | AGGACAGCGCTTTGCTCAGGT | 60                     | 173               | This study    |
| EBER R                      | AAAATAAGCGGCTAAGGCGAATACC | 60                     | 173               | This study    |
The results of this study showed that gastric biopsy specimens of patients with the *H. pylori* *cagY*⁺, *cagE*⁺, and *cagA*⁻ genotypes infection showed a negative correlation with the *DNMT1* gene ΔCt (Table 8). These results indicate an increase in *DNMT1* gene expression in gastric biopsy specimens (Table 9). The *DNMT1* gene expression with statistically significant differences in patients’ subgroups was calculated using the ΔΔCt formula (Table 9). The results showed the lowest and highest expression of the *DNMT1* gene in the youngest and oldest patients with a range age of 30–18 and 61–85 years, respectively (Table 9). The *DNMT1* expression genes in the age group of 61–85 years were 5.32-fold higher than in 18–30 years old (Table 9). The *DNMT1* gene expression in gastric adenocarcinoma patients was 3.25-fold higher than in gastritis patients (Table 9). The difference was 3.11-fold higher in the group of patients with *H. pylori* infection than without *H. pylori* infection (Table 9). The *DNMT1* gene expression in the groups of patients with *H. pylori* *cagE*⁺, *cagY*⁺, and *cagA*⁻ genotypes infection compared to patients with *H. pylori* *cagE*⁻, *cagY*⁻, and *cagA*⁺ genotypes infection was 4.09-, 3.08-, and 3.04-fold, respectively (Table 9) (*P* ≤ 0.05).

### Table 4: Demographic characteristics of patients and correlation of demographic variables.

|                        | Gastritis | Gastric adenocarcinoma | Total | *P* value ≤ |
|------------------------|-----------|------------------------|-------|-------------|
| *H. pylori* infection (positive/negative) | 23/27     | 21/32                  | 44/59 | 0.513       |
| Sex (male/female)      | 24/26     | 37/16                  | 50/53 | 0.024       |
| Age group (18–30/31–45/46–60/61–85) | 10/18/17/5 | 0/1/14/38            | 10/19/31/43 | 0.001       |

between *DNMT1* gene expression in different groups [35]. A *P* value less than 0.05 was considered a statistically significant level for measures [35].

### 3. Results

The demographic results showed that gastric adenocarcinoma’s prevalence gradually increased with increasing age (Table 4). The prevalence of gastric adenocarcinoma in men was 2.31-fold higher than in women (Table 4). As adenocarcinoma prevalence, the *H. pylori* infection increased with age increasing (Table 5). The highest *H. pylori* infection rate was observed in the range between 61 and 85 years old (Table 5). The prevalence of *H. pylori* infection in men in the study was 1.75-fold higher than in women (Table 5). The statistical relationship between *DNMT1* gene expression and demographic variables was investigated using the *T*-test. This study showed a gradually increased *DNMT1* gene expression with along increasing age (Table 6). In gastric antral epithelial cells of both groups of patients with *H. pylori* infection and gastric adenocarcinoma, the *DNMT1* gene expression was significantly increased (Table 6). The *DNMT1* gene expression in women was 2.07-fold higher than in men, without statistical correlations (*P* ≤ 0.094) (Table 6).

A survey of cDNA of *H. pylori* virulence genes in gastric biopsy specimens revealed the lowest and highest frequencies for *cagE* and *oipA* (Outer Inflammatory Protein) in gastritis patients and *sabAB* (Sialic Acid Binding Adhesin) (the gastric biopsy samples with both *H. pylori* *sabA* and *sabB* genotypes infection), *oipA*, and *cagT* in gastric adenocarcinoma patients, respectively (Table 7). A significant difference between cDNAs ΔCt of *cag* genes was observed in gastritis biopsy specimens of gastritis patients (*P* ≤ 0.05) (Table 7). The *H. pylori* *cagE*⁺, *cagA* ‘*EPIYAC*’, and *cagA*⁻ genotypes showed a strong correlation with gastritis (Table 7). The results of this study showed that gastric biopsy specimens with *H. pylori* *cagY*⁺ and *cagT*⁺ genotypes infection correlated with gastric adenocarcinoma (Table 7). The *hopQ* (Helicobacter Outer Membrane Protein) and *hopQII* genotypes of *H. pylori* were correlated with gastritis and gastric carcinoma, respectively (*P* ≤ 0.05). Gastric biopsy

### Table 5: Demographic characteristics of patients with *H. pylori* infection.

| *H. pylori* infection (n: 103) | Sex (n) | Age group (n) |
|-----------------------------|---------|---------------|
| Sex (n) | Male | Female | 18–30 | 31–45 | 46–60 | 61–85 |
| *H. pylori* infection (Positive) | 28     | 16     | 2     | 11    | 14    | 17    |
| *H. pylori* infection (Negative) | 33     | 26     | 8     | 8     | 17    | 26    |
| Total | 61     | 42     | 10    | 19    | 31    | 43    |

### 4. Discussion

The patients’ demographic results of the current study complied with related previous studies’ results [33, 43–45]. The prevalence of gastric adenocarcinoma among men was 4 times higher than among women, and the highest adenocarcinoma growth rate was observed among the oldest patients’ group (Table 4). According to other study results, *H. pylori* infection is directly associated with the prevalence of gastric adenocarcinoma, however, our study results showed no significant correlation [44, 45]. In this regard, in the sampling step, we excluded patients with a history of arbitrary use of the anti-*H. pylori* chemotherapy without a medical prescription. So, it could be the possible reason for no significant correlation between *H. pylori* infection and gastric adenocarcinoma prevalence in our patients.

The *DNMT1* enzyme contributes to cell proliferation by methylation and blocking the tumor suppressor genes’ expression [1]. The *DNMT1* gene’s expression is a consequence of cell signaling pathways leading to cell growth and proliferation [1]. According to the results of previous studies, the *DNMT1* gene’s expression in gastric epithelial cells increases with age increasing, gastric adenocarcinoma, and *H. pylori* *cagA* genotype infection [46–51]. Previous studies show that the *H. pylori* CagA oncoprotein interferes with the Wnt/β-Catenin signaling pathway and increases *DNMT1* gene expression and cell proliferation [22, 23]. CagA inhibits GSK3 (Glycogen Synthase Kinase 3) activity by stimulating c-Met via the PI3K-AKT pathway [22]. By inactivating GSK3, the β-Catenin/TCF complex is
Table 7: Frequency of *H. pylori* genotypes in gastric epithelial cells of patients with gastritis and gastric adenocarcinoma.

| *H. pylori* genotype | Gastritis (N: 23) | Gastric adenocarcinoma (N: 21) | P value ≤ |
|----------------------|-------------------|-------------------------------|-----------|
| *vacA* s1m1          | 7                 | 12                            | 0.074     |
| *vacA* s1m2          | 16                | 9                             | 0.001     |
| *cagA*+              | 22                | 10                            |           |
| *cagA*−              | 1                 | 11                            |           |
| CagA−EPIYAC−         | 22                | 10                            |           |
| CagA−EPIYAC+         | 1                 | 9                             |           |
| CagT−                | 17                | 21                            | 0.012     |
| CagT+                | 6                 | 0                             |           |
| CagY−                | 5                 | 17                            | 0.001     |
| CagY+                | 18                | 4                             |           |
| CagE−                | 0                 | 9                             | 0.001     |
| CagE+                | 23                | 12                            |           |
| *Sab*+ (A/B/AB)      | 12/2/1            | 5/9/3                         | 0.022     |
| *Sab*−               | 8                 | 4                             |           |
| *Bab*+ (A2/B/A2B)    | 4/3/1             | 5/5/4                         | 0.155     |
| *Bab*−               | 15                | 7                             |           |
| HopQ+ (I/II)         | 4/5               | 7/11                          | 0.006     |
| HopQ−                | 14                | 3                             |           |
| Alp+ (A/AB)          | 11/6              | 11/7                          | 0.610     |
| Alp−                 | 6                 | 3                             |           |
| OipA+                | 20                | 21                            | 0.086     |
| OipA−                | 3                 | 0                             |           |
| IceA2                | 11                | 10                            | 0.989     |
| iceA1/2              | 12                | 11                            |           |

Table 8: Correlation between *DNMT1* gene ΔCt in gastric antral epithelial cells of gastritis and gastric adenocarcinoma patients using the Spearman test.

| *vacA* s1m1/s1m2 | *cagA* | cagA−EPIYAC− | cagT | CagY | cagE | sabA/B | babA2/B | hopQI/II | alpA/B | oipA | iceA1/2 | DNMT1 correlation | P value ≤ |
|------------------|--------|-------------|------|------|------|--------|---------|----------|---------|-------|------|---------|------------------|-----------|
|                  | 0.116  | −0.388      | −0.293 | 0.209 | −0.446 | 0.517  | 0.313   | 0.176    | 0.155   | −0.040 | 0.196   | −0.366           | 0.001     |

Table 9: Analysis of the relationship between gastric diseases, tumor grade, *vacA* CDNA, and *H. pylori* virulence gene cDNAs with *DNMT1* gene ΔCt (T-test).

| *H. pylori* infection | N  | Mean ΔCt | DNMT1 | Standard deviation | Fold change | P value ≤ |
|----------------------|----|----------|-------|-------------------|-------------|-----------|
| Positive             | 44 | 4.7667   | 6.6035 | 1.2223           | 5.32        | 0.001     |
| Negative             | 59 | 6.1200   | 1.3223 | 1.49580          | 3.11        | 0.001     |
| Positive             | 16 | 6.1200   | 1.3223 | 1.49580          | 3.04        | 0.023     |
| Negative             | 28 | 7.7267   | 1.66526 | 6.6284          | 3.08        | 0.029     |
| Positive             | 23 | 5.9779   | 1.50656 | 1.62487         | 4.09        | 0.002     |
| Negative             | 21 | 7.6030   | 1.62487 | 1.48652         |             |           |
inactivated, and the DNMT1 gene is expressed [13, 15, 16, 18]. The H. pylori VacA inhibits GSK3 activity by influencing EGFR receptors via the PI3K-AKT pathway [22, 52, 53]. Some studies show that H. pylori CagA proteins also increase the DNMT1 gene expression and accelerate the methylation process of tumor suppressor genes by affecting integrin receptors and the JAK/STAT3 signaling pathway [22]. The results of the current study were consistent with the Ma et al. (2017) study that showed an increased DNMT1 gene expression increases the chance of developing gastric adenocarcinoma [54]. However, they showed the increased DNMT1 gene expression was not correlated with the prognosis and clinicopathological including gastric adenocarcinoma area and grade tumor consonant with the present study (Table 6).

As the results of relevant other studies, the present study results showed that the H. pylori cagA, cagE, and cagY genotypes positively correlate with the DNMT1 gene’s expression [8]. The H. pylori vacA genotypes showed no statistical correlation with DNMT1 gene’s expression, which is in contrast with the results of previous studies [8]. In this case, the current study results support the correlation of antitumor VacA effects [55].

Although previous studies have shown the low prevalence of H. pylori and EBV co-infection in gastric epithelial cells, in the current study, no EBER was detected in gastric biopsy samples. So, the possible synergic role of EBV in increasing DNMT1 genes’ expression was waived.

β-Integrins are considered as CagY and CagT proteins’ receptors that stimulate the cell signaling pathway toward cell growth and differentiation [56]. In the current study, the cagY genotype infection was statistically correlated with increased DNMT1 gene expression (Table 8).

5. Conclusion

This study’s results indicate the correlation between DNMT1 gene’s expression in gastric antral epithelial cells and some gastric adenocarcinoma risk factors, including aging and H. pylori genotypes infection, which is consistent with the results of previous studies.

Data Availability

All data are already released in the article results, and the raw datasets used and/or analyzed during the current study are not publicly available due to contracts with research participants but are available from the corresponding authors upon reasonable request.

Ethical Approval

Ethical approval was provided by the Kurdistan University of Medical Science with the ethical committee code number IR.MUK.REC.1397/211. All experiments were performed according to the Helsinki guidelines.

Consent

The institutional review board has approved the study as no published patients’ names were involved in the research project.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

Manouchehr Ahmadi Hedayati, the corresponding author, investigated and designed the study, primers, and real-time PCR; Amjad Ahmadi was responsible for sampling and molecular assistance, Farshad Sheikhmesaemli was responsible for sampling and clinical assistance, and Bijan Nouri was responsible for statistical analysis.

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