Research Article

Transcriptional Profile of *Helicobacter pylori* Virulence Genes in Patients with Gastritis and Gastric Cancer

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Introduction. Numerous molecular epidemiology studies have been performed about the frequency of *Helicobacter pylori* virulence genes in patients with *H. pylori* infection so far. This study was conducted to detect transcriptional profile by cDNA of *H. pylori* virulence genes in gastric biopsy samples of gastritis and gastric carcinoma patients. *Materials and Methods*. In a case-control study, based on the prevalence of gastritis and gastric cancer in Sanandaj city during 2018 and 2019, 23 and 11 gastric antral biopsy samples with *H. pylori* infection were collected from gastritis and gastric carcinoma patients by the consecutive and available sampling method. Pathological characters, including tumor grades and tumor areas for gastric carcinoma biopsy samples prepared from gastric cancer areas, were determined by the pathologist. Total RNA of gastric antral biopsy samples was extracted, and their cDNA was synthesized by TaKaRa kit. *H. pylori* virulence genes' cDNA using specific primers and PCR was detected. This study's results were analyzed by SPSS version 25 and statics chi-square tests for determination of relationship and correlation between cDNAs of *H. pylori* transcriptional profile and clinical outcomes *of H. pylori* infection, including gastritis, gastric carcinoma, tumor grades, and tumor area. *Results*. The positive statistical correlations were observed between transcripts of *cagA*, *cagA-EPIYAC*, *cagE*, and *cagY* genes and *H. pylori* infection clinical outcomes ($P < 0.05$). *Conclusion*. Detection of the *H. pylori* virulence genes' cDNA in gastric biopsy samples can help provide the prognosis of clinical outcomes.

1. Introduction

Microbe factors, including toxins, enzymes, and structural antigens, induce the host's immune system [1]. The most common cause of human chronic infection is *Helicobacter pylori,* which is considered host-microbe interactions [2–4]. *H. pylori* chronic infection is followed by chronic mild gastritis with or without clinical manifestations, which progresses to gastric cancer in 1-2% of patients with *H. pylori cagA*-positive genotypes infection [5]. The molecular epidemiology studies show a positive correlation between *H. pylori* virulence genes' frequency and the clinical outcomes [6, 7].

The results of previous studies show that the clinical outcomes are related to *H. pylori* virulence genes' expression. In parallel with host immunologic effects on gastritis progression, there are the effects of different ambient conditions on *H. pylori* virulence genes' expression in host cells [8–14]. As the relevant studies, salt and acidic pH change the transcription of the *H. pylori* virulence genes through the bacterial ARS two-components system [9, 13]. Some studies have concluded that the *H. pylori* persistence infection is due to the antigenic phase variations in *H. pylori* outer membrane proteins, including Hop, Bab, Sab, Oip, Dup, and Alp proteins [15–20].

In the early stages of H. pylori infection, Bab adhesin attaches to Lewis B antigens located on gastric epithelial cells' surface by a tight attachment between the bacterial type 4 secretory system and gastric epithelial cells [20]. The babA2 gene, with 60% frequency, encodes bab adhesin in H. pylori strains related to gastric cancer [21].

Besides, the persistence infection is stabilized by attaching Sab antigens to sialyl-Lewis x receptors on gastric epithelial cells [17, 18]. The previous studies show a high frequency of sab genes among H. pylori strains related to severe clinical manifestations [22].

Alp adhesin of H. pylori involves proinflammatory signals in gastric mucosal epithelial cells by attaching to type IV collagen and laminin on gastric epithelial cells (GECs) and activation of MAPK and NFKB cell signaling pathways [23]. The results of relevant studies show that all clinical H. pylori strains express the alpA/B gene [24].

Like Alp adhesin, Oip adhesin in the company with the type 4 secretory system contributes to bacterial colonization and inflammation by increasing IL-8 production [25, 26]. The upregulation of the oip gene induces apoptosis and changes in the GECs cytoskeleton, causing severe clinical manifestations, including gastric cancer [22]. Molecular epidemiology studies show a frequency of oip gene expression up to 70% among H. pylori clinical strains [25].

H. pylori vacuolating cytotoxin A pierces the mitochondrial cytoplasmic membrane and activates the apoptosis cell signaling in GECs [13–15]. H. pylori vacA gene sequence is involved in the s, i, and m variable regions that show statistical correlations with clinical outcomes of H. pylori infection and geographical distribution [27]. The vacA gene regularly expresses because it is located adjacent to the 16s rRNA conserved gene area [13]. Some studies show that vacA gene expression is induced after H. pylori colonization on GECs, and fluctuations in vacA gene expression rate among H. pylori clinical strains can be expectable [28, 29].

The cag pathogenicity island consists of 31 genes that express type 4 secretory system (T4SS) proteins [30, 31]. The T4SS conducts the injection of CagA (cytotoxin-associated gene A) protein into GECs [30, 31]. The CagA protein induces GECs proliferation through JAK/STAT3 and MAPK kinases pathways [30]. The surface connecting complex of T4SS has been comprised of CagY, CagL, CagI, CagC, and CagH proteins [30]. The α5β1 integrin receptor on the gastric epithelial cells is the ligand of CagY protein [32]. The results of relevant studies show a few variable regions with high recombination tendency in the cagY gene [33]. The recombination between forward and middle repeated sequences of the cagY gene changes its expression and, consequently, the cagY protein's affinity to integrin receptors [32]. CagY protein contacts GECs receptors and changes the host mucosal immune response's balance toward reducing mucosal immune and progression of the H. pylori persistent infection [32]. The previous studies show that cagY gene expression is regulated separately from other cag pathogenic island genes [33, 34]. The results of relevant studies state the modulation role of CagY protein in the GECs immune system in the progression of an H. pylori active chronic

infection [32–35]. The previous studies show the positive correlations between CagT and CagE proteins and the H. pylori pathogenicity [36, 37]. CagE, as an NTPase enzyme, involves the secretion of cagA [30]. CagT, as a lipoprotein, connects the T4SS core proteins complex to the bacterial outer membrane [30]. Both CagT and cagE are essential for the injection of CagA into GECs [30].

Various molecular mechanisms of gene expression control have been shown in H. pylori. The detection of bacterial virulence genes alone does not indicate genes' permanent expression [24–28]. In this regard, sab gene expression changes by turning on and off an operator called the switching mechanism [24]. The slipped-strand repair mechanism controls oipA gene expression through repetitive CT sequences at the promotor's 5′ end [22, 23]. hopZ gene has a repeat sequence in the signaling region that shows high recombination frequency and antigenic phase changes [38]. The changes in the stomach acidity flow and the constant contact of H. pylori to the gastric epithelial cells increase the expression of H. pylori adhesin's genes such as hopZ [38]. These statements mean that microbe and host interaction depends on ambient variations on microbes' virulence genes expression.

In the current study, we surveyed H. pylori virulence genes' transcript profile and their relation with clinical outcomes by detecting cDNAs in gastric antral biopsy samples collected from gastritis and gastric cancer patients H. pylori infection referred to hospitals of Sanandaj city.

2. Methods

Cochran's statistical formula was used in a case-control study to determine the number of required samples based on gastritis and gastric cancer patients' prevalence in Sanandaj city [39]. Accordingly, 50 gastritis (control group) and 30 gastric carcinoma biopsy samples (case group) were collected by the consecutive and available sampling method from patients referred to Tohid and Shaheed Ghazi hospitals in Sanandaj city for 18 months from September 2018 to March 2019. 23 (46%) and 11 (66.36%) patients had been infected with H. pylori in gastritis and gastric carcinoma patients, respectively. After obtaining ethical consent for publishing research results without patient details, data of geography for each patient, including age, sex, and results of a urea breath test for rapid diagnosis of H. pylori infection, were recorded in questionnaire forms. The exclusion criteria included the patients with chemotherapy against H. pylori infection, smoking, and alcohol consumption to eliminate study interventions. Endoscopic observations of gastroenterologists diagnosed gastritis and gastric carcinoma. The histological and pathological characters' data for gastric carcinoma samples were collected from the Pathology Laboratory of Tohid Hospital in Sanandaj city. According to the manufacturer's RNALater solution protocol, a gastric antral biopsy sample was obtained from each gastritis and gastric cancer patient with H. pylori infection to detect cDNAs of H. pylori virulence genes by using the PCR method. Gastric antral biopsy samples were dropped into RNALater solution (Roche Co., Germany) immediately. The

total RNA of biopsy samples was extracted according to the manufacturer's protocol (all in one mini preps kit, Bio Basic Canada Inc.). The 28S and 18S rRNA bands had evaluated the RNA integrity on an agarose gel at a concentration of 1.2%. The absorbance ratio at 260 nm and 280 nm was assessed for the purity of RNA by the NanoDrop® 2000 spectrophotometer machine (Thermo Fisher Company, Germany). Total RNA was stored at −70 degree centigrade. At the next step, according to the manufacturer's protocol of PrimeScriptTM RT reagent Kit (TaKaRa Co.), total RNA was converted to cDNA. To assess cDNAs' purity, PCR was performed using *H. pylori* 16s rRNA-specific primers; then, PCR products were run on agarose gel 1.5%. Forward and reverse specific primers for detecting *H. pylori* virulence genes' cDNA were designed using Primer3 online software (version 0.4.0). Table 1 shows the characteristics of all used specific primers in this study that include the sequence of primers, annealing temperature, and product size. The cDNAs of *H. pylori* virulence genes were detected using the gradient thermocycler PCR machine (BioRad Company, Germany). PCR master mix included buffer 10x (2.5 microlitres), DNA Taq polymerase 5 U/microliter (0.25 microlitres), dNTPs 10 mM (0.5 microlitres), $MgCl_2$ 50 mM (1 microlitre), cDNA (2 microlitres), forward and reverse specific primers 10 picoliters (each one 0.5 microlitres), and RNase-free water (17.75 microlitres) in final volume 25 microlitres. The thermal cycling PCR steps were involved an initial denaturation at 94°C for 5 minutes, a denaturation at 94°C for 30 seconds, a primer annealing for 45 seconds (primers temperatures have been shown in Table 1), an extension at 72°C for 45 seconds, and a final extension at 72°C for 5 minutes [27]. The denaturation through the extension step was repeated for 30–35 cycles. The PCR products of *H. pylori* virulence genes' cDNA were run on 1.5% agarose gel. The results were analyzed using SPSS software version 25 and statics chi-square tests. We used the exact Fisher static test and confidence interval 95% to analyze subgroups with a few numbers.

3. Results

3.1. Demographic Characteristics of Patients. Table 2 shows the demographic data of patients. The static results did not show any significant difference between disease (gastric and gastric cancer) and *H. pylori* infection ($P = 0.487$). As the same result, there was no significant static correlation between *H. pylori* infection and sexuality of patients ($P = 0.518$). However, there was a robust static correlation in the prevalence of gastric carcinoma in men ($P = 0.005$). The frequency of gastric carcinoma in men was four times rather than in women (Table 2). The highest and lowest *H. pylori* infection frequency was 61–73 and 18–30 years old, with 11 (32.4%) and 2 (5.9%) patients (Table 3). These results show that there is no significant correlation statistically between *H. pylori* infection and patients' age ($P = 0.314$). On the other hand, there was a robust static correlation between gastric carcinoma and patients' age ($P = 0.000$) (Table 2). Like the other similar study, increasing age in patients' populations increases gastric carcinoma frequency.

3.2. Transcription Profile of H. pylori Virulence Genes. Molecular detection of *H. pylori* infection in gastric biopsy samples was detected using the PCR method and 16s rRNA-specific primers. The vacA s2 gene's partial cDNA was sequenced (Bioneer Company, South Korea) and registered in GenBank with accession number MK642592.1 to confirm the PCR result. Table 4 shows *H. pylori* virulence genes' cDNA's frequency in biopsy samples of gastritis and gastric carcinoma patients with *H. pylori* infection. The frequency of *H. pylori* virulence genes' cDNA in gastric biopsy samples was different. Except for the *cagT* gene's cDNA, the frequency of *cag* pathogenicity island gene's cDNA including *cagA*, *cagA-EPIYAC*, *cagY*, and *cagE* genes had a significant difference statistically with gastritis and gastric carcinoma and *H. pylori* infection (Table 4). The remarkable result was the low frequency of *cagA* gene's cDNA (4.35%) in biopsy samples of gastritis patients. In contrast, the cagA gene's cDNA frequency was 45.5% in patients with gastric carcinoma. 52.2% of gastritis biopsy samples with *H. pylori* infection had *cagT* gene's cDNA ($cagT^+$ cDNA) ($P = 0.001$) (Table 4). In contrast, 36.4% of gastric carcinoma biopsy samples with *H. pylori* infection had simultaneous *cagA*, *cagT*, *cagY,* and *cagE* genes' cDNA ($cagA^+$/$cagT^+$/$cagY^+$/$cagE^+$ cDNA) ($P = 0.001$). Transcript profile of *H. pylori* outer membrane adhesin's genes showed that the frequency of *sab* and *hop* genes' cDNA was different between gastric biopsy samples of gastritis and gastric carcinoma patients. However, this difference was not significant, with an error level of 0.05 (Table 3). Our results show there was not any significant difference statistically in the frequency of *vacAs1m1/s1m2, oipA, alpA/B,* and *IceA1/2* genes' cDNA in two groups of patients with gastritis and gastric carcinoma that had *H. pylori* infection (Table 3).

3.3. Correlation of H. pylori Virulence Genes' Expression. The results of Spearman's statistic test showed the low positive correlations between the frequency of *bab* genes' cDNAs (*babA2* and *babB*) and other genes including *cagA, cagA-EPIYAC, sab, hopQ,* and *alp* genes' cDNAs in gastric biopsy samples (correlations coefficients 0.428, 0.343, 0.435, 0.462, and 0.397 with $P = 0.012$, 0.047, 0.01, 0.02, and 0.006, respectively). There were the low positive correlation coefficients 0.357 and 0.362 (Spearman's static test) between the frequency of the *hopQ* gene's cDNA (*hopQI* and *hopQII*) and the frequency of *cagA* and *cagE* genes' cDNA in gastric biopsy samples, respectively ($P = 0.038$ and 0.035, respectively). There was a negative correlation coefficient −0.344 (Spearman's static test) between the frequency of *sab* gene's cDNA (*sabA* and *sabB*) and the frequency of *vacAs1m2* gene's cDNA ($P = 0.046$). There were positive correlation coefficients 0.432, 0.460, 0.460, 0.440, and 0.406 (Spearman's static test) between the frequency of *sab* gene's cDNA (*sabA* and *sabB*) and frequency of *cagA, cagA-EPIYAC, cagE, alp,* and *oip* genes' cDNA in gastric biopsy samples ($P = 0.011$, 0.006, 0.006, 0.009, and 0.017, respectively). Our study showed that the alpA/B gene's cDNA's frequency had only a positive correlation of 0.440 with the frequency of *sab* gene's cDNA in gastric biopsy samples ($P = 0.009$). In sum, these

TABLE 1: Primers used in this study.

Specific primers	Sequence	Annealing Tm, °C	Product size, bp	Reference
16s rDNA *H. pylori* F	CTGGAGAGACTAAGCCCTCC	50	446	This study
16s rDNA *H. pylori* R	AGGATCAAGGTTTAAGGATT			
cagA F	TGACCAACAACCACAAACCG	57	108	This study
cagA R	TCAGGATCGTATGAAGCGACAG			
cagA EPIYA-C F	AAGAAAGCAGGACAAGCAGC	55	188	This study
cagA EPIYA-C R	CTAACCGATCGCCCTACCTT			
cagT F	AGGGTGTGGTGATGATAGCG	55	154	This study
cagT R	TGCTTGTTGTTTGCTCCACT			
cagE F	GAATGGAGCGAGCGATGAAA	56	163	This study
cagE R	TAGGAATTTGCAGCGCTCAC			
cagY F	AGTTCAAGTGGCGCTAGATTG	57	200	This study
cagY R	ACAAGCCTTTCAAGCATTCGT			
vacA s1/s2 F	ATGGAAATACAACAAACACAC	55	259/286	Atherton et al. [40]
vacA s1/s2 R	CTGCTTGAATGCGCCAAAC			
vacA m1/m2 F	TGGATAGTGCGACTGGGTTT	54	205/220	This study
vacA m1/m2 R	TCCATGCGGTTGTTGTTGTT			
iceA1 F	GTGTTTTTAACCAAAGTATC	45	247	van Doorn et al. [41]
iceA1 R	CTATAGCCASTYTCTTTGCA			
iceA2 F	GTTGGGTATATCACAATTTAT	47	229/234	van Doorn et al. [41]
iceA2 R	TTRCCCTATTTTCTAGTAGGT			
hopQI F	ACGAACGCGCAAAAACTTTA	55	187	Sicinschi et al. [42]
hopQI R	TTGCCATTCTCATCGGTGTA			
hopQII F	ACAGCCACTCCAATCCAGAA	55	160	Sicinschi et al. [42]
hopQII R	AACCCCACCGTGGATTTTAG			
babA2 F	CAATGCGGTGCGTGAAAATC	57	205	This study
babA2 R	ATACCCTGGCTCGTTGTTGA			
babB F	CAATTCCCCGGCGTATCAAG	56	175	This study
babB R	ATTGCAAGTGATGGTCGTCG			
sabA F	TCTCTCGCTTGCGGTATCAT	56	204	This study
sabA R	AGCTCAATGTTGTTGGCGTT			
sabB F	GCATTCAAACGGCGAACAAC	56	248	This study
sabB R	TCCTGTGCAGTTCCCATCTT			
alpA F	CGCTCCTATCAAAACCGCTC	55	185	This study
alpA R	TTCCCGTCCAACTTACCGAA			
alpB F	TCAACTTGCGAGCAGACCTA	57	218	This study
alpB R	AGCCATAGACCCCATACACG			
oipA F	CTCCACGCTGAAAGGAATGG	55	233	This study
oipA R	CCATTTCCTGCGAATCGGTT			

*The primers yield a fragment of 229 or 334 bp depending on the presence of a repetitive sequence of 105 nucleotides in some iceA2 alleles [40].

TABLE 2: Demographic data of patients in this study.

	Gastritis	Gastric cancer	Total	*P* value
H. pylori infection (positive/negative)	23/27	11/19	34/46	0.487
Sex (male/female)	24/26	24/6	48/32	0.005
Age range (18–30/31–45/46–60/61–85)	10/18/17/5	0/0/7/23	10/18/24/28	0.000

TABLE 3: Demographic data of the prevalence of *H. pylori* infection among males and females with various age groups.

H. pylori infection	Sex (*n*: 80)		Age group (*n*: 80)			
	Male	Female	18–30	31–45	46–60	61–85
H. pylori infection (positive, *n*: 34)	19	15	2	10	11	11
H. pylori infection (negative, *n*: 47)	29	17	8	8	13	17
Total	48	32	10	18	24	28

TABLE 4: Frequency of *H. pylori* virulence genes' cDNA in biopsy samples of gastric and gastric carcinoma patients with *H. pylori* infection.

Gene/Disease	Gastritis, *H. pylori* positive (N = 23)	Gastric cancer, *H. pylori* positive (N = 11)	*H. pylori*-positive samples (N = 34) (%)	P value
cagA	1 (4.3%)	5 (45.5%)	17.6	0.008
CagA-EPIYAC	1 (4.3%)	4 (36.4%)	14.7	0.029
cagT	17 (73.9%)	11 (100%)	82.4	0.145
cagY	5 (21.7%)	8 (72.7%)	38.2	0.008
cagE	0 (0.0%)	5 (45.5%)	14.7	0.002
vacA s1m1	7 (30.4%)	5 (45.5%)	35.3	0.391
vacA s1m2	16 (69.6%)	6 (54.5%)	64.7	0.391
SabA/B	15 (65.2%)	9 (81.8%)	70.6	0.074
BabA2/B	8 (24.8%)	7 (63.6%)	44.1	0.469
oipA	20 (87%)	11 (100%)	91.2	0.535
AlpA/B	17 (73.9%)	10 (90.9%)	79.4	0.552
HopQI/II	9 (39.1%)	9 (81.8%)	42.9	0.074
IceA1/2	23 (100%)	11 (100%)	100	1.000

findings show that simultaneous gene expression in some *H. pylori* virulence genes could be related to clinical outcomes.

3.4. Pathological Characters of Gastric Carcinoma Biopsy Samples. Table 5 shows the frequency of tumor regions in gastric carcinoma patients. The cardia region's frequency was 46.66% and the highest frequency compared to other tumor regions in gastric carcinoma, including the body, lesser curvature, and antrum regions. Pathology findings showed that out of 30 biopsy samples with gastric carcinoma, 11 cases (37.5%) had *H. pylori* infection. These findings were similar to the urea breath test results and *H. pylori* 16s rRNA molecular detection. The present study results showed no significant difference statistically between gastric carcinoma tumor regions and *H. pylori* infection ($P = 0.276$). The absence of the *alpA/B* gene's cDNA in the body tumor region significantly differed from other gastric carcinoma tumor regions ($P = 0.024$). The frequency of G1, G2, G3, and G4 tumor grades in gastric carcinoma samples were 7 (23.33%), 14 (46.66%), 8 (26.66%), and 1 (3.33%), respectively. All cases of *H. pylori* infection were detected in patients with the G1 and G2 tumor stages. 63.63% of gastric carcinoma tumors with *H. pylori* infection were in the G2 tumor stage ($P = 0.047$). The frequency of *oipA*, *cagT,* and *iceA* genes' cDNAs was similar in gastric carcinoma samples with G1 and G2 tumor grades ($P = 0.047$) (Table 6). This study showed a significant correlation statistically between the frequency of the *cagY* gene's cDNA and G2 tumor grade in gastric carcinoma samples ($P = 0.007$).

4. Discussion

Our study results showed some positive correlations and relationships among transcripts of *H. pylori* virulence genes detected in gastric biopsy samples of gastritis and gastric carcinoma patients with *H. pylori* infection. We surveyed statistical aspects of *H. pylori* virulence genes' cDNA's frequency in gastric biopsy samples and their relationship

with clinical outcomes, including gastritis, gastric carcinoma, gastric carcinoma tumor region, and tumor grade. The study was conducted to detect *H. pylori* virulence genes' cDNA frequency, each alone and in combination (Tables 4–6). We surveyed frequency of *H. pylori* adhesin's genes' cDNA that include combination transcripts' cDNA as alp^+/oip^+, $alp^+/oip^+/hopQ^+$, sab^+/bab^+, and $alp^+/oip^+/hopQ^+/sab^+/bab^+$ and their statistic's correlations with clinical outcomes (Table 7).

Numerous studies have shown the correlation of *H. pylori vacA s1* and *cagA* genotypes with clinical outcomes [27, 40–46]. On the other hand, some studies have shown the opposite effects of *cagA* and *vacA* on gastric epithelial cells [46]. Vacuolating cytotoxin A inhibits the production of hummingbird phenotypes in gastric epithelial cells that are created by CagA toxin [46]. The EPEYC motif of CagA protein via stimulating SHP-2 (Src homolog2 domain-containing tyrosine phosphatase) phosphatase creates needle-like protrusions on the surface of epithelial cells that are called hummingbird phenotypes with a high rate of growth and proliferation rather than normal cells [46]. However, the results of some molecular epidemiology studies show that $cagA^+$ strains likely are $vacAs1^+$ genotype simultaneously [46]. Our study results show an inverse correlation coefficient −0.465 (Spearman's static test) between the frequency of vacAs1m2 and cagA genes' cDNA in gastritis gastric carcinoma samples with *H. pylori* infection ($P = 0.01$).

Some studies show that *H. pylori* $cagA^+/vacAs1^+/babA2^+$ genotypes significantly correlate with peptic ulcers and gastric cancer [47]. *babA* gene expression in European strains of *H. pylori* is 40–70%, but in East Asian and American strains, it is 70–100% [7, 21, 43, 44]. There is a high homology between the nucleic acid sequences of 5 and 3 ends of the *babA*, *babB*, and *babC* genes, which lead to recombination among these three genes and consequently turn on or off gene expression [43, 48]. The result of our study showed that the frequency of the *bab* gene's cDNA is 63.6% and 24.8% in gastric cancer and gastritis biopsy samples with *H. pylori* infection, respectively (Table 4). There

TABLE 5: Frequency of *H. pylori* virulence genes' cDNA in gastric antral biopsy samples of gastric carcinoma patients with stomach tumors in different areas.

Variable/Tumor area	N (GC)	Cardia (N 14)	Body (N 4)	Lesser curvature (N 6)	Antrum (N 6)	P value
H. pylori infection	11	3	1	3	4	0.276
cagA	5	1	0	2	2	0.662
cagA-EPIYAC	4	0	0	2	2	0.284
cagT	11	3	1	3	4	0.276
cagY	8	2	1	2	3	0.92
cagE	5	1	0	2	2	0.662
vacA s1m1	5	0	0	2	3	0.260
vacA s1m2	6	3	1	1	1	0.260
SabA/B	9	3	0	3	3	0.494
BabA2/B	7	2	0	2	3	0.810
oipA	11	3	1	3	4	0.276
AlpA/B	10	3	0	3	4	0.024
HopQI/II	9	2	0	3	4	0.156
IceA1/2	11	3	1	3	4	0.458

TABLE 6: Frequency of *H. pylori* virulence genes' cDNA in gastric carcinoma biopsy samples with G1 and G2 tumor grades. All samples with G3 and G4 tumor grades were without *H. pylori* infection.

Variable/Tumor grade	N	G1 (N 7)	G2 (N 14)	P value
H. pylori infection	11	4 (36.36%)	7 (63.64%)	0.047
cagA	5	1 (20%)	4 (80%)	0.303
cagA-EPIYAC	4	1 (25%)	3 (75%)	0.554
cagT	11	4 (36.36%)	7 (63.64%)	0.047
cagY	8	1 (12.5%)	7 (87.5%)	0.007
cagE	5	1 (20%)	4 (80%)	0.303
vacA s1m1	5	1 (20%)	4 (80%)	0.303
vacA s1m2	6	3 (50%)	3 (50%)	0.545
SabA/B	9	3 (33.33%)	6 (66.67%)	0.856
BabA2/B	7	2 (28.57%)	5 (81.43%)	0.735
oipA	11	4 (36.36%)	7 (63.64%)	0.047
AlpA/B	10	4 (40%)	6 (60%)	0.378
HopQI/II	9	4 (44.44%)	5 (53.64%)	0.308
IceA1/2	11	4 (36.36%)	7 (63.64%)	0.047

TABLE 7: The combined cDNA detected in gastric antral biopsy samples. Some samples had single cDNA that we did not mention. These findings were collected of 23 and 11 gastric antral biopsies in gastritis and gastric cancer samples with *H. pylori* infection, respectively. There were no significant correlations between *H. pylori* virulence genes' cDNA combinations and clinical outcomes ($P > 0.05$).

alp/oip (Gastritis/GC)	*alpA + alpB + oipA* (6/3)	*alpA + oipA* (10/7)			
alp/oip/hopQ (Gastritis/GC)	*alpA + alpB + oipA + hopQ1* (2/3)	*alpA + oipA + hopQ2* (5/6)	*alpA + oipA + hopQ1* (1/0)	*oipA + hopQ1* (1/0)	
cagA (Gastritis/GC)	*cagA + cagT + cagY + cagA-EPIYAC + cagE* (0/4)	*cagA + cagT + cagY* (1/5)	*cagA + cagT + cagY + cagE* (0/5)	*cagA + cagT* (1/5)	*cagT + cagY* (5/8)
sab/bab (Gastritis/GC)	*sabA + babA2 + babB* (1/0)	*sabA + sabB + babB* (1/0)	*sabA + sabB + babA2* (0/1)	*sabB + babA2 + babB* (0/1)	*sabA + babB* (2/2)
	sabB + babA2 (0/2)	*sabA + babA2* (4/1)			

were positive correlation coefficients between the frequency of the *bab* gene's cDNA and *cagA*, *cagA-EPIYAC*, *sab*, *hopQ*, and *alp* genes' cDNA ($P < 0.05$).

hopQI[+] genotypes are predominant in East Asia and are associated with *cagA*[+] genotypes, whereas *hopQII*[+] genotypes are predominant in West and Europa and have no association with *cagA*[+] genotypes [27, 42]. Some studies have demonstrated HopQ adhesin's effects on the injection and entry of cagA protein into gastric epithelial cells in the company with T4SS [42]. The progress of gastric mucosa

tissue inflammation increases sialic acid antigens' expression on the gastric epithelial cells [8]. *H. pylori* is attached to sialic acid antigens by SabA adhesin and lead to a chronic and persistent *H. pylori* infection [8]. *H. pylori* is attached to gastric epithelial cells by OipA membrane protein in most *H. pylori* clinical strains [7, 25, 26]. The results of some studies show that *oipA* gene expression is directly related to the expression of *cagA* and *vacA* genes [25, 26]. Our study results show that the oipA gene's cDNA's frequency had a positive correlation coefficient of 0.406 with the frequency of the *sab* gene's cDNA ($P = 0.017$). Studies show that AlpAB lipoprotein adhesin in *H. pylori* Western strains leads to different cell signaling in gastric epithelial cells than Eastern strains [23]. The study results showed a positive correlation of 0.349 between the frequency of $alp^+/oip^+/hopQ^+$ cDNA and gastritis ($P = 0.049$).

As a remarkable result, there was a low frequency of *cagA* gene's cDNA in gastric biopsy samples with *H. pylori* infection versus gastric carcinoma biopsy samples ($P = 0.008$). Four samples of the *cagA* gene's cDNA in gastric carcinoma biopsy samples had an EPIYAC sequence ($P = 0.029$). The cagY and cagE genes' cDNA frequency had a significant difference statistically between gastric biopsy samples with *H. pylori* infection and gastric carcinoma biopsy samples with *H. pylori* infection ($P = 0.008$ and 0.002, respectively). We compared all frequency of *cag* pathogenicity island genes' cDNA between two groups of patients with gastritis and gastric cancer in this study (Table 7). The results showed there are positive correlations between cag genotypes and clinical outcomes. The genotypes with transcripts of *cagA-EPIYAC$^+$/cagT$^+$/cagY$^+$/cagE$^+$* had a positive strong correlation of 0.692 with gastric carcinoma ($P = 0.005$). On the other hand, the strains of *H. pylori* without any transcript of cag pathogenicity island genes and *cagT$^+$* had a low positive correlation of 0.320 with gastritis ($P = 0.065$). Although *H. pylori* did not correlate with clinical outcomes, transcripts of *cagY*, *cagE*, *oipA,* and *IceA* genes' cDNA had a significant correlation statistically with G2 tumor grade in gastric carcinoma biopsy samples (Table 6). The gastric biopsy samples without any *alp* gene's cDNA correlated with the body tumor area in gastric carcinoma ($P = 0.024$) (Table 5). The results showed a remarkable difference in *H. pylori* virulence genes' expression between body area tumor and other areas tumor of the stomach. It can be due to differences between the origin and pathophysiology of *H. pylori* infection. In this regard, based on previous studies, most of the gastric cancers in patients with *H. pylori* infection are in the antral and cardia areas [49–51].

To explain the low frequency of some *H. pylori* virulence genes' cDNA in this study, we would say that multiplying control mechanisms reduce gene expression [52–54]. The two-components ArsRS system, which is affected by acidic pH, regulates *H. pylori* genes' expression, including *sabA* and *cagA* [52]. Changing the ORFs direction of cag pathogenicity islands' promotors leads to an unequal gene expression [31, 32]. On the other hand, the molecular mechanisms such as single strand mispairing and the activity of nine types of methyltransferases regulate and limit *H. pylori* genes' expression.

5. Conclusion

The results' statistics analysis shows that some separate and combinatorial transcripts of *H. pylori* virulence genes are related to clinical outcomes.

Data Availability

The data used to support the findings of this study are included within the article.

Additional Points

(i) Transcript's profile of *H. pylori* virulence genes, a bacterium with a high level of genetic diversity, is remarkable in clinical strains of gastritis and gastric cancer patients (ii) This study shows a high *H. pylori* strain frequency in gastric antral biopsy samples of gastritis patients without the *cagA* gene's cDNA to assess *cagA* gene expression (iii) Evaluation of pathological features of gastric biopsy specimens with *Helicobacter pylori* infection shows positive correlation coefficients between the frequency of *H. pylori* virulence genes' cDNA and clinical outcomes, including gastritis, gastric adenocarcinoma, tumor grades, and gastric tumor area

Ethical Approval

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

Disclosure

This study results from Doctor Manouchehr Ahmadi Hedayati' (Ph.D. of Medical Bacteriology) thesis.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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