

Analysis of gene expression patterns in various topographic sites of gastritis patients infected with *Helicobacter pylori*

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ABSTRACT

Background and Objectives: *Helicobacter pylori* infection primarily contributes to gastric carcinogenesis. This study examined the expression of *cyclin E1*, β -catenin, *PPAR- δ* , and miR-9 in different gastric regions of gastritis patients with and without *H. pylori* infection.

Materials and Methods: Gastric biopsies were obtained from 108 participants divided into three groups, including healthy ($n = 15$), gastritis ($n = 53$), and *H. pylori*-positive gastritis ($n = 40$). Bacterial culture and molecular characterization were performed to confirm *H. pylori* presence. Gene expression was assessed using SYBR Green-based RT-qPCR. Statistical analyses revealed correlations between the expression of *cyclin E1*, β -catenin, *PPAR- δ* , miR-9, and the presence of *H. pylori* in patients with gastritis compared to healthy controls.

Results: The *cagA*-positive strains were significantly associated with the expression of *cyclin E1*, β -catenin, *PPAR- δ* ($P < 0.001$), and miR-9 ($P < 0.01$). *Cyclin E1* and β -catenin expression were upregulated in gastritis and *H. pylori*-positive gastritis patients. The *PPAR- δ* expression was highest in *H. pylori*-positive gastritis patients, specifically in the antrum tissues. miR-9 showed significant downregulation in *H. pylori*-positive gastritis samples.

Conclusion: *cagA*-positive *H. pylori* infection was linked to distinct gene expression changes that varied by gastric region. Further studies are needed to explore the mechanisms involved.

Keywords: *Helicobacter pylori*; Gastritis; Stomach neoplasms; Cyclin E1; β -catenin; Peroxisome proliferator-activated receptor delta; MicroRNAs

INTRODUCTION

Helicobacter pylori is a microaerophilic bacterium that can subtly colonize the gastric mucosa of billions of individuals worldwide (1). This infection is a notorious precursor to several gastroduodenal conditions,

including gastric and duodenal peptic ulcers, gastric cancer, and mucosa-associated lymphoid tissue lymphoma (2, 3). The spectrum of clinical outcomes attributed to *H. pylori* infection can be related to a dynamic interaction of bacterial virulence factors, host genetic predispositions, and environmental agents

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(4). This interplay triggers chronic gastritis in different locations and with various severities of inflammation within the stomach, which would result in distinct disease phenotypes classified as antral-predominant gastritis, corpus-predominant gastritis, and pangastritis (2, 5). This pathogen is considered a Group 1 carcinogen that is present in over 80% of gastric cancer cases, with infection rates reaching up to 90% in developing countries by the age of 10 years (6). Thus, timely diagnosis and proper treatment of *H. pylori* infection can result in early detection and reduction of the incidence of gastric cancer (7).

Chronic gastric inflammation caused by *H. pylori* is accompanied by the secretion of proinflammatory cytokines such as interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α), which can reshape the gastric microenvironment (8). As a result, *H. pylori* activates a cascade of oncogenic pathways, disrupts cellular signaling networks, and induces hallmarks of cancer, such as sustained proliferation, invasion, and metastasis (9). Alterations in the Wnt/ β -catenin signaling axis, epidermal growth factor receptor (EGFR), and focal adhesion kinase (FAK) pathways play vital roles in mediating *H. pylori*-induced cancer progression (10). Importantly, *H. pylori* *cagA* oncoprotein promotes tumorigenesis through phosphorylation-dependent and independent mechanisms and directly affects epithelial-mesenchymal transition (EMT), cell migration, and proliferation (11).

One of the key regulators influenced by *H. pylori* is peroxisome proliferator-activated receptor delta (PPAR- δ), which influences the tumor microenvironment by modulating chemokine signaling, such as the CCL20/CCR6 axis, and supports immune evasion and cancer progression (12). Dysregulation of PPAR- δ is strongly associated with gastric cancer and correlates with poor clinical outcomes. Similarly, microRNA 9 (miR-9), with its dual oncogenic and tumor-suppressive roles, is a crucial modulator of gastric carcinogenesis (13). In gastric cancer, miR-9 downregulates tumor suppressor genes such as *E-cadherin* and ultimately triggers EMT and metastasis (13). Furthermore, its upregulation influences the expression of vascular endothelial growth factor (VEGF), angiogenesis, and cell proliferation, which would exacerbate oncogenic events (14). Cyclin E1 serves as a key regulator of the G1/S transition that can participate in gastric cancer progression by disrupting cell cycle checkpoints and resulting in uncontrolled cell division (15). Additionally, stabilized

β -catenin can activate genes involved in proliferation, cell survival, and invasion to induce an aggressive tumor phenotype (16). Through these multifaceted pathways, *H. pylori* intensifies inflammatory signaling and alters the expression of oncogenic and tumor-suppressor genes (17, 18). Given the molecular impact of *H. pylori* on cancer progression, this study aimed to evaluate the influence of this infection on the expression levels of *cyclin E1*, *β -catenin*, *PPAR- δ* , and miR-9 in antrum and body biopsy samples obtained from *H. pylori*-positive and -negative patients with gastritis to identify potential topography-based biomarkers for early intervention in gastric cancer.

MATERIALS AND METHODS

Study population. This study was conducted on gastric biopsy samples collected from 108 adult patients (aged 20-80 years) undergoing upper gastrointestinal endoscopy at the Research Institute for Gastroenterology and Liver Diseases, Tehran, Iran, between 2019 and 2021. Exclusion criteria included previous antibiotic treatment, use of non-steroidal anti-inflammatory drugs, proton pump inhibitors, or H₂ receptor blockers within one month before endoscopic examination, current or previous smoking, alcohol consumption, and a positive first-degree family history of gastric cancer. Participant eligibility was assessed using a standardized questionnaire. To minimize potential confounding effects on gene expression analyses, individuals with comparable lifestyle characteristics and clinical backgrounds were enrolled whenever possible. Written informed consent was obtained from all participants before sample collection. The study protocol was approved by the Ethics Review Committee of the Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran (ethics approval code: IR.SBMU.RIGLD.REC.1399.046).

Sample collection. A total of four gastric biopsies, two from the antrum and two from the gastric body, were collected from each participant, including healthy controls, using an identical endoscopic sampling protocol. This approach enabled region-matched comparisons between study groups while minimizing potential topographic bias. Two biopsy samples, one antrum and one body, for culture from each patient were transferred to transport medium contain-

ing thioglycolate, 1.3 g/L agar (Merck Co., Hamburg, Germany), and 3% yeast extract (Oxoid Ltd., Basingstoke, UK). The remaining two biopsies from each gastric region were established in RNA FIX solution (Qiagen, Hilden, Germany) and initially stored at 4°C for 24 h before being transferred to -20°C for preservation until RNA extraction.

***H. pylori* culture and identification.** Gastric biopsies were transferred to brain heart infusion broth (Merck Co., Hamburg, Germany) supplemented with 10% bovine serum in an aseptic condition. Then, the tissue was ground and homogenized, and inoculated onto Brucella agar supplemented with 7% sheep blood, 10% fetal calf serum (FCS), and the selective campylobacter antibiotics (vancomycin 2 mg/L, polymyxin B 0.05 mg/L, trimethoprim 1 mg/L, and amphotericin B 2.5 mg/L). Plates were incubated under microaerophilic conditions (10% CO₂, 5% O₂, and 85% N₂) at 37°C for 3-5 days in the Innova® CO-170 system (New Brunswick Scientific, USA). Colonies suggestive of *H. pylori* were further characterized by biochemical tests, including catalase and urease activity assays. Gram staining was performed for morphological confirmation. Molecular detection was performed by amplifying 16S rRNA and *cagA* genes using specific primers listed in Table 1.

RNA isolation and cDNA synthesis. Total RNA was extracted using the RNA Total RNeasy Plus Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. The quality and concentration of the RNA samples were assessed spectrophotometrically at 260/280 nm by a NanoDrop spectrophotometer (ND-1000, Thermo Scientific, Waltham, MA, USA). The purified RNA was reverse-transcribed to cDNA using the BioFACT RT-kit (BIOFACT Co., Ltd., Daejeon, South Korea) according to the manufacturer's guidelines. Synthesized cDNA samples were stored at -20°C for further analysis.

Quantitative real-time PCR. The expression levels of *PPAR-δ*, *cyclin E1*, and *β-catenin* were quantified using SYBR Green-based RT-qPCR. The reactions were performed in a Rotor-Gene 3000 thermocycler (Qiagen GmbH, Hilden, Germany) using gene-specific primers (Table 1). Primer specificity was confirmed by melt curve analysis. Amplification efficiencies were determined using standard curves generated from serial cDNA dilutions. All primer pairs demonstrated amplification efficiencies within the standard range of. *β-actin* was used as the house-keeping gene and internal control. Each reaction (20 μL total volume) included 10 μL SYBR Green master mix, 0.5 μL forward and reverse primers (10 μM

Table 1. The oligonucleotide sequences used in this study

Target gene	Oligonucleotide sequence	Amplicon (bp)	Annealing temperature (°C)	Reference
16S rRNA	F: GGCTATGACGGGTATCCGGC R: GCCGTGCAGCACCTGTTTC	764	58	(19)
<i>cagA</i>	F: AATACACCAACGCCTCCAAG R: TTGTTGCCGCTTTTGCTCTC	400	57	(20)
<i>cyclin E1</i>	F: CCCGGTCATCATCTTCTTTG R: AGAAATGGCCAAAATCGACA	125	55	(21)
<i>β-catenin</i>	F: AGCTTCCAGACACGCTATCAT R: CGGTACAACGAGCTGTTTCTAC	98	55	(21)
<i>PPAR-δ</i>	F: AACTGCAGATGGGCTGTGAC R: CAGCTTCATGCGGATCGTAC	149	55	(22)
miR-9	F: AACTCCAGCTGGGTCTTTGGTTATCTAG R: TGGTGTCTGAGTCGGCAATTCAGTTG RT: CTCAACTGGTGTCTGAGTCGGCAATTCAGTTGA	~90	58	This study
<i>RNU6</i>	F: AACTCCATCTGGGTCTGTAAGCGTTC R: TGGTGTCTGAGTCGGCAATTCAGTTG	~90	58	(23)
<i>β-actin</i>	F: ATGTGGCCGAGGACTTTGATT R: AGTGGGGTGGCTTTTAGGATG	107	57	(21)

RT, reverse transcriptase

each), 2 μ L cDNA, and deionized water. The thermocycling conditions included initial denaturation at 95°C for 15 sec, 40 cycles of denaturation at 95°C for 20 sec, and annealing at 55°C for 40 sec.

To analyze the expression of miR-9, a stem-loop primer was employed as the forward primer, while a universal reverse primer stabilized the cDNA structure. *RNU6* served as the internal control for this gene. The RT-qPCR protocol for miR-9 included initial denaturation at 95°C for 4 min, 30 cycles of denaturation at 95°C for 1 min, annealing at 58°C for 45 sec, and extension at 72°C for 1 min. A final extension step at 72°C for 15 sec was performed to ensure complete amplification. All RT-qPCR reactions were conducted in duplicate to enhance reliability. Relative gene expression levels were assessed by the $2^{-\Delta\Delta C_t}$ method. Expression levels of *PPAR- δ* , *cyclin E1*, and *β -catenin* were normalized to *β -actin*, while miR-9 expression was normalized to *RNU6*.

Statistical analysis. Statistical analyses were conducted via SPSS software (v20.0, IBM Corp.) and R (4.4.2). Data were expressed as mean $\pm$ standard deviation (SD). Between-group comparisons were conducted by employing independent t-tests, LSD multiple comparison tests, or one-way ANOVA, as appropriate. To compare gene expression levels between paired antrum and body samples obtained from the same patients, the Wilcoxon signed-rank test was performed. Tukey's post hoc test (for multiple comparisons between more than two groups) was further used to calculate statistical significance between groups. Statistical significance was set at $P < 0.05$.

RESULTS

Characteristics of participants. As presented in Table 2, a total of 108 participants were included in this study and divided into healthy ($n = 15$), gastritis ($n = 53$), and gastritis with *H. pylori* infection ($n = 40$) groups. Gender distribution did not show any significant differences, with females representing 32 (60.4%) and males 21 (39.6%) among the gastritis group, while in the gastritis patients with concomitant *H. pylori* infection, 22 (55%) were female and 18 (45%) were male. Age distribution showed significant differences among all participants ($P < 0.05$), with the majority in the gastritis group aged 40-59

years (64.2%). In patients with gastritis and concomitant *H. pylori*, a significant proportion were aged 40-59 years (42.5%) and ≥ 60 years (30%), which demonstrated a trend toward increased *H. pylori* prevalence in older cases. Blood group analysis revealed a significant association between blood type and group distribution ($P < 0.001$). The A⁺ blood group was most common in the gastritis group (26.4%), while the A⁺ and O⁺ blood groups were equally prevalent among the gastritis patients with concomitant *H. pylori* (20% each). AB⁻ and O⁻ blood groups were absent in the *H. pylori*-positive gastritis patients. No statistically significant differences were observed across ethnic groups, although the Fars individuals represented the largest proportion.

Expression of cyclin E1 was significantly upregulated in *H. pylori*-infected gastritis patients. The expression levels of each gene exhibited considerable variation across different groups (Table 3). As illustrated in Fig. 1, *cyclin E1* expression showed an increasing pattern in patients compared to healthy controls, with the most significant elevation observed in individuals with gastritis and *H. pylori*. In the antrum, the expression of *cyclin E1* was significantly elevated in gastritis patients with *H. pylori* ($P < 0.001$) compared to healthy controls. A similar pattern was observed in the body, where *cyclin E1* expression remained significantly higher among individuals with gastritis and *H. pylori* ($P < 0.01$). *cyclin E1* expression exhibited a statistically significant increase ($P < 0.01$) from healthy individuals to gastritis patients with concomitant *H. pylori*.

Topographic comparison of gene expression between antrum and body sites. Direct paired comparisons between antrum and body specimens revealed significant topography-dependent differences in gene expression patterns (Table 4). In healthy individuals, *cyclin E1*, *β -catenin*, and *PPAR- δ* expressions were significantly higher in the antrum than in the body, while miR-9 expression was significantly reduced in antrum samples. *cyclin E1*, *β -catenin*, and *PPAR- δ* expressions were significantly elevated in the antrum compared with the body ($P < 0.001$) among the gastritis and *H. pylori*-positive gastritis groups. The expression of miR-9 was significantly lower in the antrum of gastritis patients ($P < 0.001$) and gastritis patients infected with *H. pylori* ($P < 0.01$).

Table 2. Baseline characteristics of study participants

Characteristics	Healthy (n = 15)	Gastritis (n = 53)	Gastritis +HP (n = 40)	P value
Gender				
F	8	32	22	NS
M	7	21	18	
Age group, years				
0-19	0	3	0	P < 0.05
20-39	7	9	11	
40-59	8	34	17	
60 ≥	0	7	12	
Blood group				
A-	4	1	0	P < 0.001
A+	0	14	8	
AB-	2	0	0	
AB+	2	5	1	
B-	2	1	1	
B+	0	6	8	
O-	1	3	0	
O+	2	12	8	
Unknown	2	11	14	
Ethnic group				
Fars	5	31	21	NS
Kurd	2	6	3	
Lur	3	1	4	
Turk	3	12	10	
Unknown	2	3	2	
CG				
Negative	15	0	0	P < 0.001
Positive	0	53	40	
IM				
Negative	15	48	35	NS
Positive	0	5	5	
Dysplasia				
Negative	15	50	38	NS
Positive	0	3	2	

CG, chronic gastritis; F, female; HP, *Helicobacter pylori*; IM, intestinal metaplasia; M, male; NS, not significant

Expression of β -catenin was significantly elevated in *H. pylori*-positive gastritis patients. The β -catenin expression displayed a marked upregulation across different gastric regions, as shown in Fig. 2. In the antrum, β -catenin expression was significantly higher in gastritis ($P < 0.01$) and *H. pylori*-positive gastritis patients ($P < 0.001$) groups compared to healthy controls. Similarly, β -catenin levels were significantly elevated in gastritis ($P < 0.05$) and in

patients with *H. pylori* ($P < 0.001$) in the body. β -catenin expression demonstrated a progressive increase in *H. pylori*-positive gastritis patients compared to healthy controls ($P < 0.05$), regardless of the gastric topography.

***H. pylori* infection upregulated the expression of PPAR- δ among gastritis patients.** As shown in Fig. 3, PPAR- δ expression was consistently elevated in all disease groups, with the highest levels detected in *H. pylori*-positive gastritis patients. In the antrum, PPAR- δ expression was significantly increased in gastritis ($P < 0.01$) and *H. pylori*-positive gastritis ($P < 0.001$) patients compared to healthy controls. A similar pattern was observed in the body, with PPAR- δ levels significantly upregulated in gastritis ($P < 0.05$) and *H. pylori*-positive gastritis ($P < 0.01$) patients. PPAR- δ expression was significantly elevated ($P < 0.01$) in *H. pylori*-positive gastritis patients.

Expression of miR-9 was downregulated in *H. pylori*-infected gastritis patients. As shown in Fig. 4, miR-9 expression was downregulated in the patient groups. In the antrum, miR-9 expression was significantly reduced in gastritis ($P < 0.01$) and *H. pylori*-positive gastritis ($P < 0.05$) patients compared to healthy controls. However, no significant changes in miR-9 levels were observed in the body. When all specimens were considered collectively, miR-9 expression was significantly downregulated ($P < 0.05$) in the gastritis patients with concomitant *H. pylori*.

***cagA*-positive *H. pylori* strains significantly alter gene expression in gastric tissue.** As indicated in Table 5, the presence of *H. pylori cagA* virulence factor was significantly associated with the expression of cyclin E1, β -catenin, PPAR- δ ($P < 0.001$), and miR-9 ($P < 0.01$). Among the 40 *H. pylori*-positive gastric samples, 21 (52.5%) were classified as *cagA*-positive. *cagA*-positive samples exhibited significantly elevated expression levels of cyclin E1 (2.691 ± 0.251) and β -catenin (2.709 ± 0.216) compared to *cagA*-negative samples (cyclin E1: 1.507 ± 0.682 , β -catenin: 1.677 ± 0.623). PPAR- δ expression was notably higher in *cagA*-positive samples (1.975 ± 0.190) compared to the negative ones (1.468 ± 0.355). Conversely, miR-9 expression levels were markedly lower in the *cagA*-positive samples (0.540 ± 0.171) than in the *cagA*-negative samples (0.750 ± 0.181).

Table 3. Gene expression level of *cyclin E1*, β -catenin, *PPAR- δ* , and miR-9 in gastric tissue samples of study groups. Mean expression levels $\pm$ standard deviation (SD) are presented for each group, along with statistical significance (P values) comparing patients to healthy controls

Target gene	Groups				
	Healthy (n=15)	Gastritis (n=53)		Gastritis + HP (n=40)	
	Mean $\pm$ SD	Mean $\pm$ SD	P value	Mean $\pm$ SD	P value
<i>cyclin E1</i>	1.000 $\pm$ 0.051	1.200 $\pm$ 0.061	0.63398	2.725 $\pm$ 0.242	0.00794
β -catenin	1.000 $\pm$ 0.069	1.476 $\pm$ 0.133	0.2521	2.738 $\pm$ 0.209	0.0109
<i>PPAR-δ</i>	1.000 $\pm$ 0.065	1.400 $\pm$ 0.123	0.3006	2.001 $\pm$ 0.184	0.0414
miR-9	1.000 $\pm$ 0.070	0.738 $\pm$ 0.131	0.163	0.563 $\pm$ 0.166	0.018

HP, *Helicobacter pylori*; miR-9, microRNA-9; *PPAR- δ* , peroxisome proliferator-activated receptor delta; SD, standard deviation

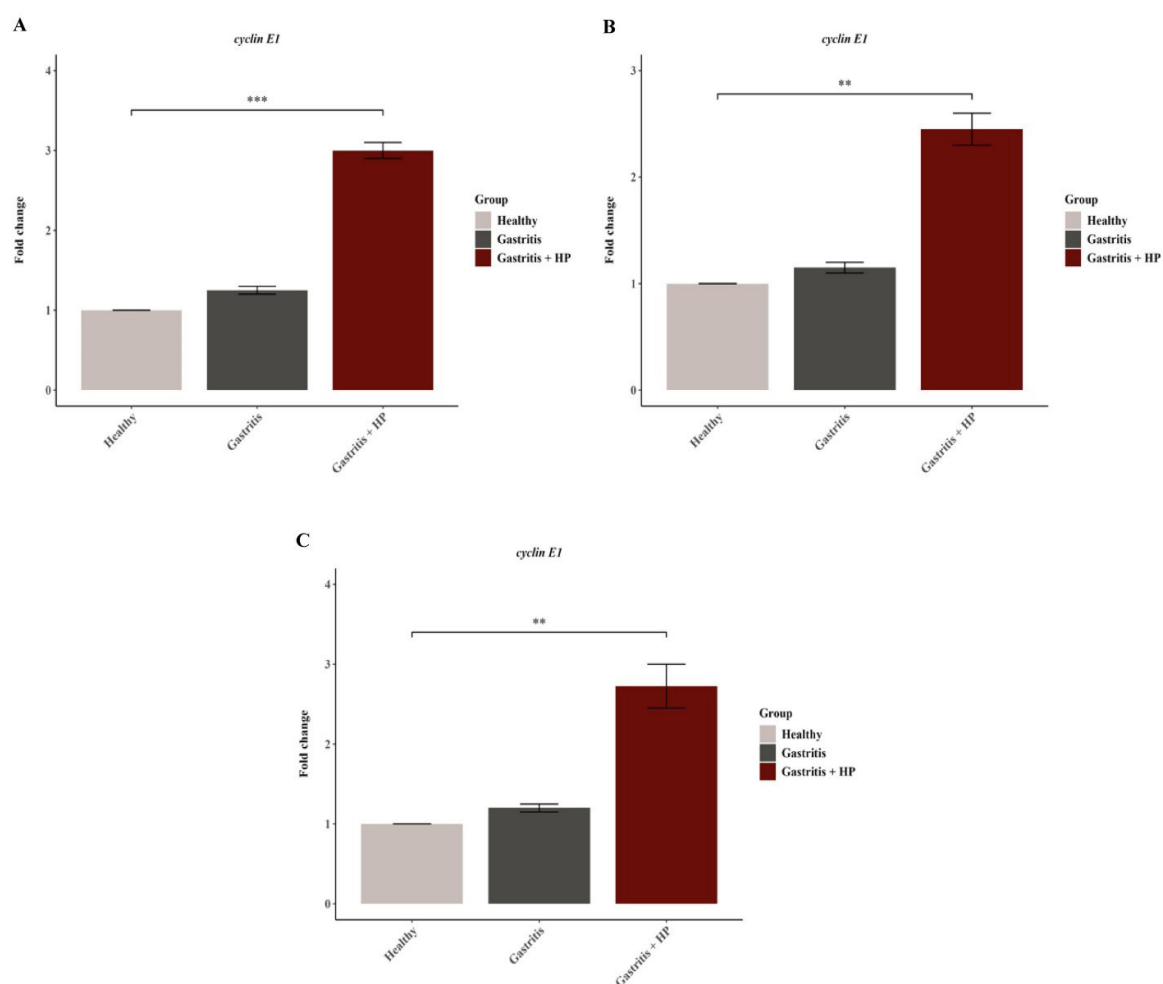


Fig. 1. Differential gene expression of *cyclin E1* in the antrum (A), body (B), and total (C) samples across study groups. Fold change was used to compare gene expression in each study group relative to healthy controls. P values reflect the significant differences in the mean expression levels of each study group compared to healthy controls. Statistical significance is represented as follows: $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***). HP, *Helicobacter pylori*.

Table 4. Topographic comparison of gene expressions between the antrum and body samples among study groups

Target gene	Healthy Mean $\pm$ SD (n = 15)		P value	Gastritis Mean $\pm$ SD (n = 53)		P value	Gastritis + HP Mean $\pm$ SD (n = 40)		P value
	Antrum	Body		Antrum	Body		Antrum	Body	
<i>cyclin E1</i>	1.03 $\pm$ 0.03	0.99 $\pm$ 0.05	P < 0.05	1.28 $\pm$ 0.07	1.12 $\pm$ 0.05	P < 0.001	2.95 $\pm$ 0.26	2.50 $\pm$ 0.22	P < 0.001
<i>β-catenin</i>	1.02 $\pm$ 0.07	0.97 $\pm$ 0.06	P < 0.05	1.60 $\pm$ 0.14	1.35 $\pm$ 0.12	P < 0.001	2.94 $\pm$ 0.22	2.53 $\pm$ 0.20	P < 0.001
<i>PPAR-δ</i>	1.04 $\pm$ 0.07	0.98 $\pm$ 0.06	P > 0.05	1.51 $\pm$ 0.13	1.29 $\pm$ 0.12	P < 0.001	2.30 $\pm$ 0.19	1.70 $\pm$ 0.17	P < 0.001
miR-9	0.97 $\pm$ 0.07	1.00 $\pm$ 0.05	P > 0.05	0.58 $\pm$ 0.12	0.90 $\pm$ 0.14	P < 0.001	0.49 $\pm$ 0.15	0.64 $\pm$ 0.18	P < 0.01

HP, *Helicobacter pylori*; PPAR- δ , peroxisome proliferator-activated receptor delta; miR-9, microRNA-9; SD, standard deviation

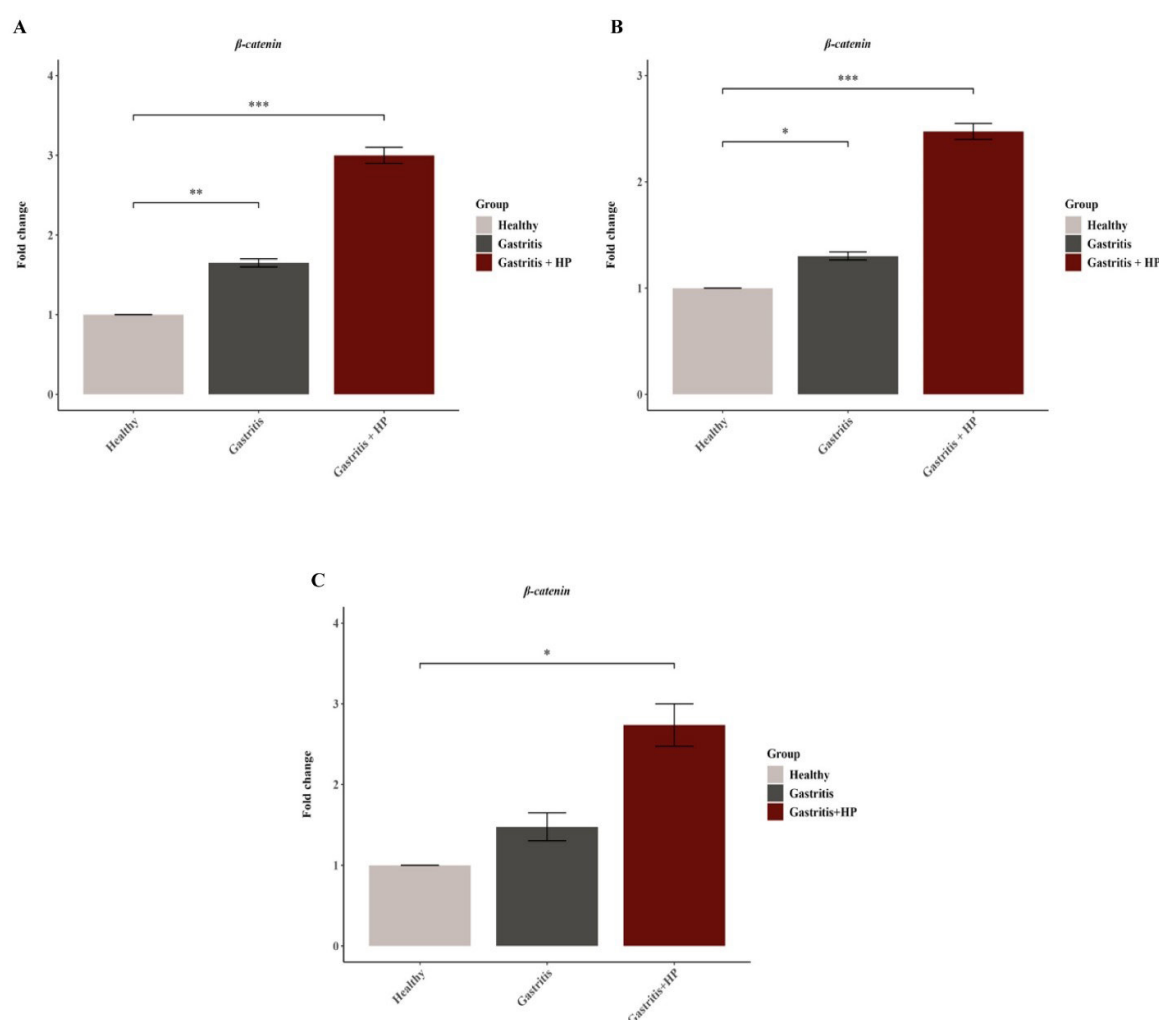


Fig. 2. Differential gene expression of β -catenin in the antrum (A), body (B), and total (C) samples across study groups. Fold change was used to compare gene expression in each study group relative to healthy controls. P values reflect the significant differences in the mean expression levels of each study group compared to healthy controls. Statistical significance is represented as follows: P < 0.05 (*), P < 0.01 (**), P < 0.001 (***). HP, *Helicobacter pylori*.

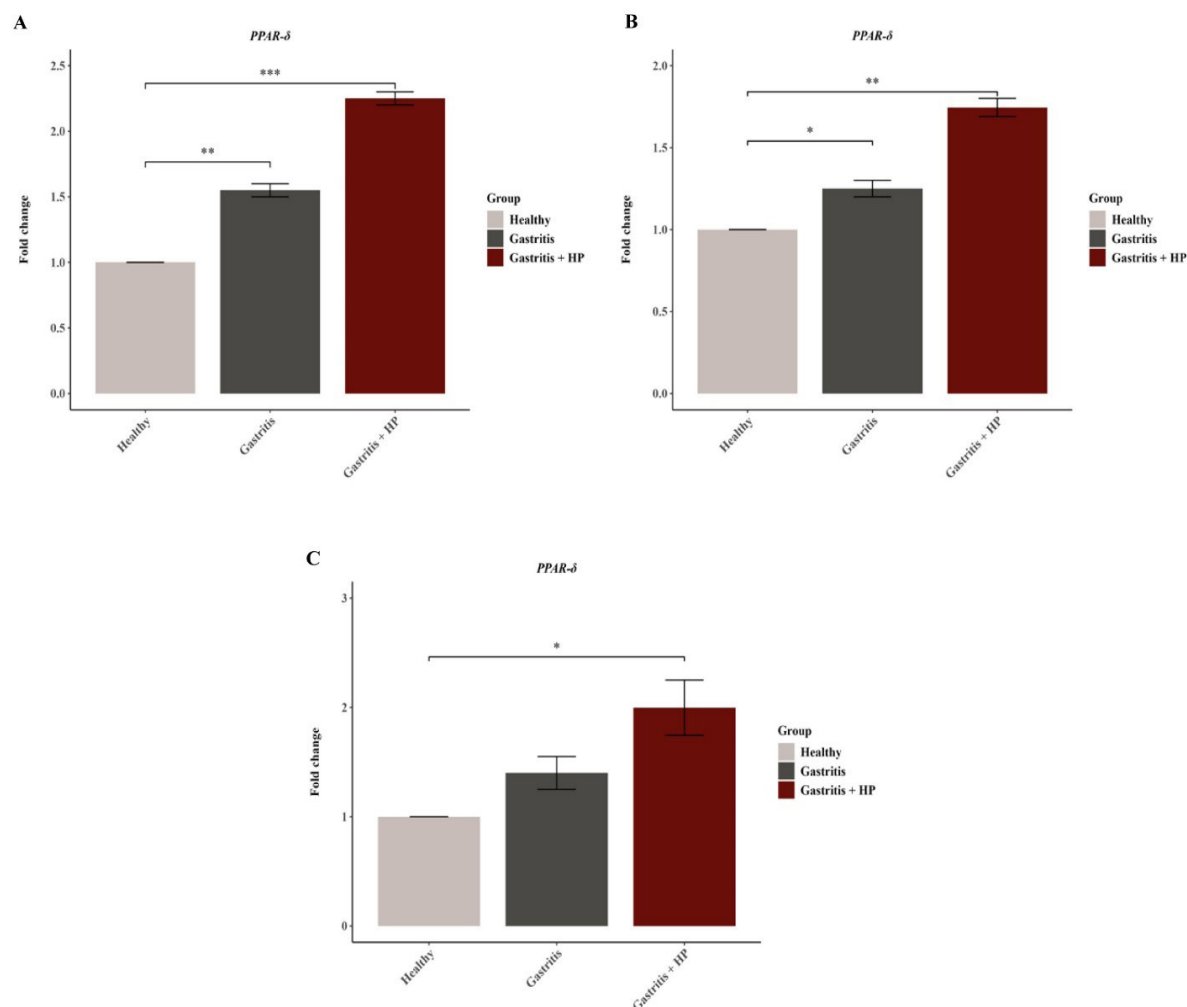


Fig. 3. Differential gene expression of *PPAR-δ* in the antrum (A), body (B), and total (C) samples across study groups. Fold change was used to compare gene expression in each study group relative to healthy controls. P values reflect the significant differences in the mean expression levels of each study group compared to healthy controls. Statistical significance is represented as follows: $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***). HP, *Helicobacter pylori*.

Gastritis severity and histopathology were associated with gene expression levels. Gene expression values were summarized as the mean of the antrum and body measurements for each patient to match the patient-level histopathologic assessment. Spearman correlation was used for gastritis severity, and Mann–Whitney U test was used for intestinal metaplasia and dysplasia. Analyses in the healthy group were not applicable because no histopathologic variation was present. As shown in Table 6, higher gastritis severity was associated with increased *β-catenin* expression among the gastritis patients ($\rho = 0.288$, $P = 0.037$), while similar trends for *cyclin E1* did not reach statistical significance. In the gastritis + HP group, severity-related correlations were weaker and

non-significant. Intestinal metaplasia was associated with altered *PPAR-δ* expression in both groups, while dysplasia did not show significant associations with any of the examined genes.

DISCUSSION

The ability of *H. pylori* to manipulate host molecular pathways and disrupt gastric pathophysiology has remained a compelling area of investigation (2, 17). Chronic infection with *H. pylori cagA*-positive strains has been introduced as the strongest risk factor for gastric cancer (2). The assessment of *cagA* using in vitro cultured gastric epithelial cells has in-

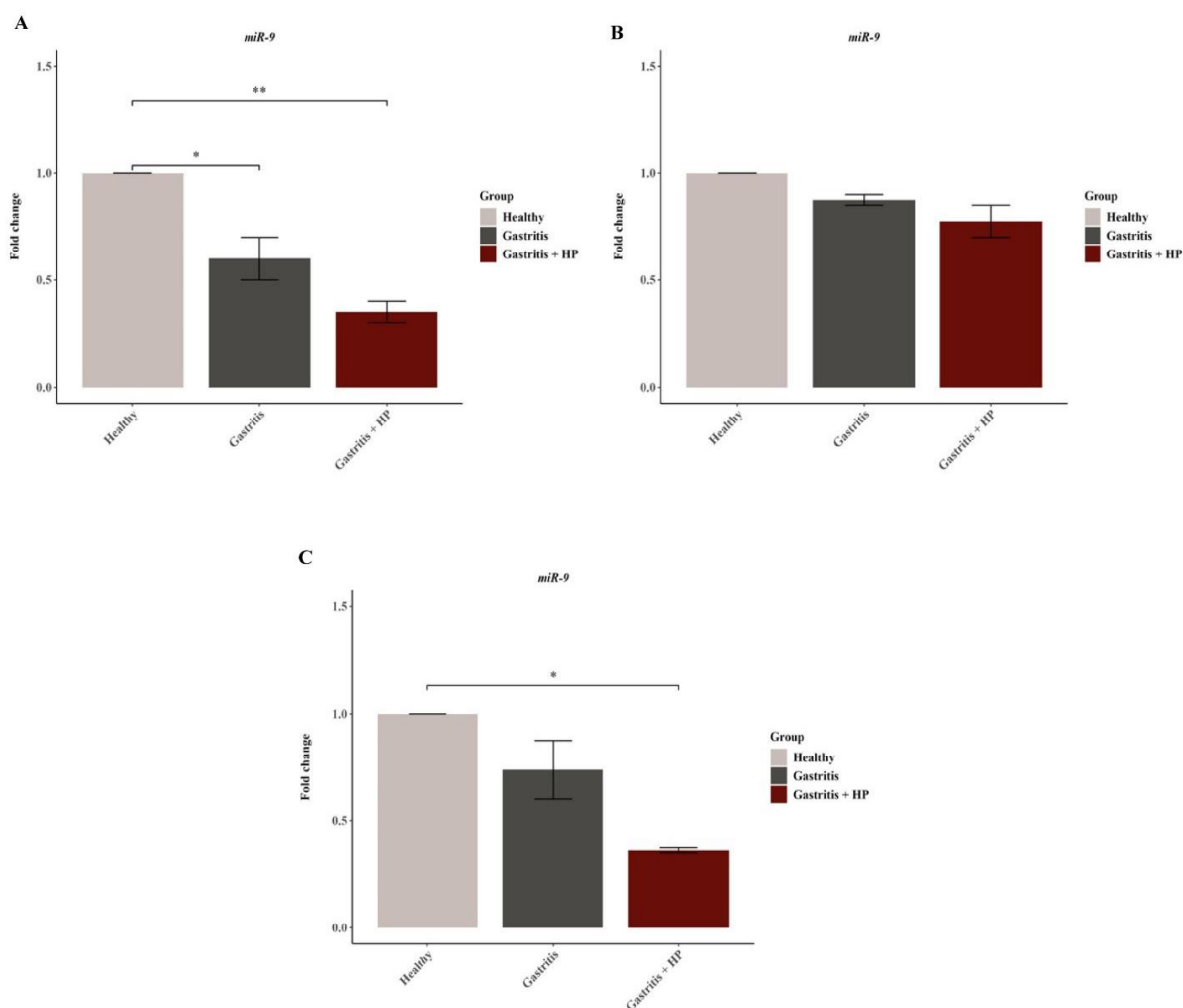


Fig. 4. Differential gene expression of miR-9 in the antrum (A), body (B), and total (C) samples across study groups. Fold change was used to compare gene expression in each study group relative to healthy controls. P values reflect the significant differences in the mean expression levels of each study group compared to healthy controls. Statistical significance is represented as follows: P < 0.05 (*), P < 0.01 (**), P < 0.001 (***). HP, *Helicobacter pylori*.

Table 5. Association of *cagA* status with the expression of cyclin E1, β -catenin, PPAR- δ , and miR-9 in *H. pylori*-positive gastric samples

Target gene	Gastritis + HP		P value
	<i>cagA</i> positive (n=21)	<i>cagA</i> negative (n=19)	
	Mean $\pm$ SD	Mean $\pm$ SD	
<i>cyclin E1</i>	2.691 $\pm$ 0.251	1.507 $\pm$ 0.682	3.33e-07
β -catenin	2.709 $\pm$ 0.216	1.677 $\pm$ 0.623	7.08e-07
PPAR- δ	1.975 $\pm$ 0.190	1.468 $\pm$ 0.355	7.10e-06
miR-9	0.540 $\pm$ 0.171	0.75 $\pm$ 0.181	0.00058

CagA, cytotoxin-associated gene A; miR-9, microRNA-9; PPAR- δ , peroxisome proliferator-activated receptor delta; SD, standard deviation

indicated that the nonphysiological scaffolding actions of *cagA* can promote the malignant transformation of the cells by activating multiple phenotypic cancer hallmarks, including sustained proliferation, evasion of growth suppressors, invasiveness, resistance to cell death, and genomic instability (24). It has been indicated that the in vivo oncogenic activity of *cagA* is further potentiated in the presence of chronic inflammation (25). In this study, *cagA*-positive *H. pylori* infection of gastritis patients has been associated with elevated expression of *cyclin E1*, β -catenin, and PPAR- δ , alongside suppressed miR-9 levels. These molecular changes can suggest a coordinated strategy by *H. pylori* to manipulate host cellular mechanisms and potentially promote carcinogenesis. It should be noted that *cagA* is a key bacterial oncopro-

Table 6. Association between histopathology and gene expression by study group

Histopathologic variable	Gene	Gastritis (n=53) P value	Gastritis + HP (n=40) P value
Gastritis severity (Spearman ρ)	<i>cyclin E1</i>	0.252 (P = 0.069)	0.182 (P = 0.260)
	<i>β-catenin</i>	0.288 (P = 0.037)	0.031 (P = 0.848)
	<i>PPAR-δ</i>	0.008 (P = 0.956)	-0.121 (P = 0.456)
	miR-9	-0.124 (P = 0.376)	-0.080 (P = 0.622)
IM (mean $\pm$ SD)	<i>cyclin E1</i>	P: 1.19 $\pm$ 0.05 N: 1.20 $\pm$ 0.04 (P = 0.647)	P: 2.67 $\pm$ 0.15 N: 2.73 $\pm$ 0.18 (P = 0.380)
	<i>β-catenin</i>	P: 1.44 $\pm$ 0.03 N: 1.48 $\pm$ 0.10 (P = 0.237)	P: 2.84 $\pm$ 0.11 N: 2.72 $\pm$ 0.15 (P = 0.113)
	<i>PPAR-δ</i>	P: 1.31 $\pm$ 0.10 N: 1.41 $\pm$ 0.09 (P = 0.037)	P: 2.19 $\pm$ 0.09 N: 2.03 $\pm$ 0.13 (P = 0.007)
	miR-9	P: 0.72 $\pm$ 0.02 N: 0.74 $\pm$ 0.10 (P = 0.584)	P: 0.56 $\pm$ 0.08 N: 0.57 $\pm$ 0.11 (P = 0.937)
Dysplasia (mean $\pm$ SD)	<i>cyclin E1</i>	P: 1.18 $\pm$ 0.05 N: 1.20 $\pm$ 0.04 (P = 0.427)	P: 2.59 $\pm$ 0.35 N: 2.73 $\pm$ 0.17 (P = 0.577)
	<i>β-catenin</i>	1.43 $\pm$ 0.07 N: 1.48 $\pm$ 0.09 (P = 0.427)	2.72 $\pm$ 0.24 N: 2.74 $\pm$ 0.14 (P = 0.877)
	<i>PPAR-δ</i>	P: 1.37 $\pm$ 0.12 N: 1.40 $\pm$ 0.09 (P = 0.758)	P: 1.96 $\pm$ 0.25 N: 2.06 $\pm$ 0.13 (P = 0.577)
	miR-9	P: 0.67 $\pm$ 0.03 N: 0.74 $\pm$ 0.10 (P = 0.125)	P: 0.47 $\pm$ 0.14 N: 0.57 $\pm$ 0.10 (P = 0.310)

HP, *Helicobacter pylori*; IM, intestinal metaplasia; miR-9, microRNA-9; N, negative; P, positive; PPAR- δ , peroxisome proliferator-activated receptor delta; SD, standard deviation

tein that can be delivered into gastric epithelial cells via the type IV secretion system of *H. pylori* (11). Once entered the host cell, *cagA* undergoes tyrosine phosphorylation at Glu-Pro-Ile-Tyr-Ala motifs in its C-terminal region and acts as a nonphysiological scaffold that disrupts multiple signaling proteins in both tyrosine phosphorylation-dependent and -independent manners (24). Furthermore, it has been elucidated that *cagA* can mediate the disruption of

tight junctions and cellular polarity, which would exacerbate tumor progression by creating inflammatory conditions favorable for the aberrant expression of these oncogenic factors (26).

Cyclin E1 is a critical regulator of the G1-S phase cell cycle transition, which is essential for maintaining normal cellular proliferation, and its overexpression is frequently observed in gastric cancer (27). Under normal conditions, *cyclin E1* levels are tightly

controlled through ubiquitin-mediated degradation by Glycogen synthase kinase-3 β (GSK-3 β) (28). However, unphosphorylated *cagA* interacts with the hepatocyte growth factor receptor and disrupts this regulatory mechanism by activating the PI3K/Akt pathway, which can inhibit GSK-3 β and result in the stabilization of *cyclin E1* (29). Dysregulated activity of *cyclin E1*-CDK2 can result in retinoblastoma protein phosphorylation, which can relieve E2F inhibition and facilitate uncontrolled cell cycle progression (30). Further reinforcing *cyclin E1* overexpression, *cagA* enhances β -catenin signaling, which directly induces *cyclin E1* transcription (31). Recent findings suggest that β -catenin also forms a complex with transcription factor EB (TFEB), which is a master regulator of lysosomal biogenesis, in order to regulate a distinct subset of Wnt target genes (32). Wnt activation promotes TFEB nuclear localization and Tankyrase-mediated PARsylation, which enables TFEB to cooperate with β -catenin-TCF/LEF1 in Wnt target gene regulation. The lower miR-9 expression among *cagA*-positive samples in the present study shows that miR-9 potentially suppresses gastric cancer cell proliferation and invasion by targeting Cyclin D1 and Ets1 (33).

The concurrent upregulation of β -catenin in our study under *H. pylori* influence could have potentially activated transcriptional programs and the Wnt/ β -catenin pathway that enhance cell survival, proliferation, invasion, and EMT (21, 34). In uninfected cells, β -catenin is maintained at low levels through APC/GSK-3 β -dependent degradation (35). However, *cagA* directly interacts with β -catenin to prevent its degradation and facilitate its nuclear translocation (36). Once in the nucleus, β -catenin cooperates with TCF/LEF transcription factors to upregulate oncogenes, including *cyclin E1* and *CD44* (37). *CagA*-related disruption of cellular adhesion can destabilize the β -catenin-E-cadherin complex and facilitate tumor progression (38). While *CagA* promotes cancer stem cell-like properties in gastric cancer cells, including increased Nanog and Oct4 expression, via Wnt/ β -catenin signaling, β -catenin can bind to the Nanog and Oct4 promoters during *H. pylori* infection. *CagA* may trigger EMT in gastric cancer cells, characterized by increased TWIST1 and vimentin and reduced E-cadherin expression (39, 40). However, *H. pylori*-induced suppression of miR-9 in our study has potentially boosted β -catenin signaling and resulted in oncogenic activation. Our findings

suggest a potential model in which *H. pylori*, mostly *cagA*-positive strains, promotes activation of the Wnt/ β -catenin signaling pathway, which can lead to increased *cyclin E1* expression and enhanced cellular proliferation. Concurrent upregulation of *PPAR- δ* may support inflammatory and tumor-promoting signaling, while suppression of miR-9 may remove inhibitory control over multiple oncogenic pathways. The existence of these interconnected molecular networks involved in early gastric carcinogenesis interactions needs to be investigated in future studies.

Another significant finding of the present study is the upregulation of *PPAR- δ* in *H. pylori*-positive samples. *PPAR- δ* is a nuclear receptor that governs fatty acid oxidation and tumor metabolism to facilitate cancer cell survival under hypoxic conditions (41, 42). Mechanistically, *PPAR- δ* interacts with transcriptional regulators, like Yap1 and Sox9, to cause gastric epithelial cell proliferation and survival (43). Additionally, this receptor participates in modulating the CCL20/CCR6 axis chemokine signaling that would result in immune evasion and metastatic progression via recruiting immunosuppressive cells like Tregs and MDSCs and reducing CD8⁺ cytotoxic T cells (12). Notably, the concomitant elevated expression of *PPAR- δ* , as well as β -catenin and *cyclin E1* in our study, may represent an immunosuppressive environment, which would likely lead to sustained chronic inflammation and cause gastric adenocarcinoma (44). Thus, it can be suggested that in this study, *cagA*-induced NF- κ B activation upregulated *PPAR- δ* , which in turn could promote β -catenin nuclear translocation and lead to strengthening Wnt-driven tumorigenesis. Of note, miR-9 exhibits context-dependent roles in gastric carcinogenesis. In some gastric cancer models, increased miR-9 expression has been associated with reduced E-cadherin expression and enhanced invasive properties, while other studies have demonstrated a tumor-suppressive role for miR-9 through inhibition of Cyclin D1 and Ets1. (13, 33). Accordingly, it can be suggested that *H. pylori*-related cell cycle dysregulation upregulates *cyclin E1*, as well as the suppression of tumor-suppressor markers such as miR-9, to create a microenvironment that is conducive to uncontrolled growth and transformation. The observed association between increasing gastritis severity and elevated β -catenin expression can support the involvement of Wnt/ β -catenin signaling in the progression of gastric mucosal damage and early oncogenic transfor-

mation. Although *PPAR-δ* expression was elevated in diseased tissues, no significant association with gastritis severity was observed in the present study. The results are consistent with the Correa cascade that describes a progression from chronic gastritis to gastric cancer (45). Moreover, the differential expression of genes between the antrum and body samples in our study highlights the influence of topographical physiology and localized and systemic effects of *H. pylori* infection. The antrum primarily secretes gastrin and mucus, while the body is responsible for acid and pepsinogen production (46, 47). The preference may contribute to the upregulation of inflammatory and oncogenic pathways in the antrum compared to the body in *H. pylori*-positive cases, as *cagA* targets Wnt/β-catenin and NF-κB differently by topography due to differences in cellular receptors and local microenvironments (17). In contrast, the body, despite lower bacterial density, experiences distinct molecular alterations potentially driven by systemic cytokine diffusion or *H. pylori*-mediated alterations in acid production. For instance, the reduced miR-9 expression observed in the body compared to the antrum can illustrate a regulatory response to chronic inflammation throughout these regions.

CONCLUSION

The present study investigated the relationship between *H. pylori* infection and the extent of oncogenic gene expression in gastric antrum and body specimens of patients with gastritis. Gene expression analysis of biopsy samples revealed that *H. pylori* infection can cause upregulation of *cyclin E1*, *β-catenin*, and *PPAR-δ* and downregulation of miR-9, with these effects being more significant in patients infected with *cagA*-positive strains compared to those harboring *cagA*-negative strains. Furthermore, these alterations were more remarkable in the antrum than in the body of the stomach, which highlights the role of topological factors in tumor initiation and development. The cross-sectional design of the current study does not permit evaluation of temporal relationships between gene expression alterations and disease progression. To better understand the molecular nature of *H. pylori*-induced gastric cancer, future research should involve longitudinal patient sampling and follow-up, as well as in vivo experiments spanning all stages of cancer progression. Additionally, the func-

tional consequences of the observed transcriptional changes require further validation of the identified biomarkers, as well as mechanistic investigations to determine how the molecular alterations contribute to the progression from gastritis to gastric cancer. High-throughput analyses, including genomic, epigenetic, and transcriptomic profiling, could reveal the driving pathways of the gastric malignancy. Additionally, identifying predictive biomarkers for risk stratification would enable early intervention in patients at high risk of developing gastric cancer due to *H. pylori* infection. Taken together, understanding the temporal dynamics of molecular alterations, along with the interplay between host genetic predisposition, dietary influences, and co-infection, is important for achieving a comprehensive understanding of *H. pylori*-induced gastric carcinogenesis.

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