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B-cell Lymphoma 9 (*BCL9*) Gene Methylation as a Biomarker for Gastrointestinal Diseases and Gastric Cancer Risk

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Abstract

In this study, the link between bacterial infection and *BCL9* gene methylation was examined, which is crucial to cancer formation. Also, it examined patients' gene methylation levels for stomach ulcers, gastritis [acute and chronic], and duodenal ulcers and cancer risk. Using MS-RT-PCR, BCL-9 methylation was associated more strongly with clinical variables. Substantial differences in BCL-9 methylation between patients and controls (median 1.368, Interquartile Range [0.912-1.468] vs. 1.001, [0.861-1.211], $p<0.05$). Interestingly, males had substantially greater BCL-9 methylation levels than females (median 1.186, Interquartile Range [1.139-1.308] vs. 0.986, Interquartile Range [0.875-1.150], $p<0.05$). A significant difference was observed in BCL-9 methylation between acute and chronic gastritis patients (median 1.213, Interquartile Range [1.083-1.378] vs. 1.013, Interquartile Range [0.855-1.130], $p<0.05$), suggesting that chronic inflammation may impact BCL-9 methylation patterns. BCL-9 methylation was strongly linked with treatment status (r= 0.293, $p<0.01$). BCL-9 methylation was linked to gastrointestinal diseases such as chronic gastritis. This strongly suggests that *BCL9* gene methylation can be detected early. This evidence can be utilized to create cancer treatment and prevention medicine.

Key Words: Gastric cancer; *Helicobacter pylori*; DNA methylation; MS-RT- PCR technology.

مثيلة جين ليمفوما الخلايا البائية 9 (*BCL9*) كعلامة حيوية لأمراض الجهاز الهضمي وخطر الإصابة بسرطان المعدة

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الخلاصة

في هذه الدراسة قمنا بفحص العلاقة بين العدوى البكتيرية ومثيلة الجين *BCL9*، وهو أمر بالغ الأهمية لتكوين السرطان. فحصنا مستويات المثيلة الجينية للمرضى فيما يتعلق بقرحة المعدة والتهاب المعدة [الحاد والمزمن] وقرحة الاثني عشر ومخاطر الإصابة بالسرطان. باستخدام MS-RT-PCR، ارتبط مثيلة BCL-9 بالمتغيرات السريرية بقوة أكبر. اختلافات كبيرة في مثيلة BCL-9 بين المرضى والضوابط (المتوسط 1.368، النطاق الربعي [1.468-0.912] مقابل 1.001، [1.211-0.861]، P<0.05). ومن المثير للاهتمام أن الذكور لديهم مستويات مثيلة BCL-9 أعلى بكثير من الإناث (المتوسط 1.186، النطاق الربعي

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[1.308-1.139] مقابل 0.986، النطاق الربعي [1.150-0.875]، p<0.05). لاحظنا اختلافًا كبيرًا في مثيلة BCL-9 بين مرضى التهاب المعدة الحاد والمزمن (المتوسط 1.213، النطاق الربعي [1.083- 1.378 مقابل 1.013، النطاق الربعي[0.855-1.130] ، P <0.05)، مما يشير إلى أن الالتهاب المزمن قد يؤثر على BCL- 9 أنماط المثيلة. ترتبط مثيلة BCL-9 بقوة بحالة العلاج (r = 0.293، p <0.01). يرتبط مثيلة BCL-9 بأمراض الجهاز الهضمي مثل التهاب المعدة المزمن. يشير هذا بقوة إلى أنه يمكن اكتشاف مثيلة الجين *BCL9* مبكرًا. يمكن استخدام هذا الدليل في إنشاء دواء لعلاج السرطان والوقاية منه.

1. Introduction

DNA methylation (epigenetic mark) is a heritable change where DNA methyl-transferases (DNMTs) covalently transfer a methyl group (CH_4) to the fifth carbon atom (C-5) position of the cytosine ring of DNA [1]. Covalent methylation of cytosine nucleotides, or DNA methylation, usually takes place in the presence of CpG. Via de-novomethylation (DNMT3A and DNMT3B) or methyl group removal (TET1, TET2, and TET3), a complex protein system in the cell writes the pattern of DNA methylation. A number of factors faithfully duplicate methylation patterns during DNA replication (UHRF1D and NMT1) [2, 3]. Through their interacting microenvironment, *Helicobacter pylori* infections also cause genetic or epigenetic alterations in a variety of cell types, including fibroblasts, epithelial cells, immune cells, and stem cells. About two out of every five individuals will have cancer at some point in their lives, and the prevalence is still rising globally [4]. They also disrupt DNA repair pathways, leading to neoplastic transformation, and induce dysregulation of critical cellular processes like cell division, apoptosis, migration, and motility [5, 6]. The exact mechanism and reason for the development of precancerous diseases and stomach cancer as a result of long-term *H. pylori* infection remain unknown despite extensive research [7]. Despite new findings linking *H. pylori* infection, DNA methylation, and stomach cancer, there are still a number of unanswered questions. These include how bacterial virulence factors like Cytotoxin-associated gene A (CagA), Vacuolating cytotoxin A (VacA), and cag-Pathogenicity Island (cagPAI) affect DNA methylation, how they interact with other epigenetic modifications, and whether or not they can be used as biomarkers for gastric cancer [8, 9]. Growing data points to B-cell CLL/lymphoma 9 protein (BCL9) involvement in a number of illnesses, most notably human malignancies [10]. Many types of cancer, including myeloma, have overexpressed *BCL9* [11]. As an oncoprotein, *BCL9* primarily facilitates the growth of cancerous cells by maintaining their division, encouraging their proliferation and migration, preventing apoptosis, modifying the immune system and tumor microenvironment (TME), and controlling chromosomal instability and karyotype for tumor evolution [12 - 14]. Although *BCL9* was long thought to be a part of the canonical Wnt signaling system, more recent research has shown that *BCL9* has a variety of roles outside of the Wnt signaling pathway [15].

Non-coding RNAs have a significant role in controlling *BCL9*. Two mature, single-stranded miRNAs, miR-30a-3p and miR-30a-5p, are produced by the double-stranded miR-30a precursor in gastric cancer. They fulfill important biological roles in two different ways. Through *BCL9*, miR-30a-3p controls the nuclear translocation of b-catenin and suppresses the production of Cyclooxygenase-2 (COX-2). *BCL9*'s untranslated region (3` UTR) is bound by miR-30a-5p, which thus suppresses *BCL9* expression [16]. Furthermore, it is discovered that the long non-coding RNA (lncRNA) nuclear paraspeckle assembly transcript 1 (NEAT1) has the ability to sponge miR-30a. NEAT1 and miR-30a (miR-30a-3p and miR-30a-5p) have a negative correlation. NEAT1 indirectly increases *BCL9* expression because miR-30a-5p negatively affects *BCL9* expression [17]. According to Elsarraj *et al.*, [18], *BCL9* controls both the direct targets of STAT3, which are integrin b3, COX-2, forkhead box O1 (FOXO1), p-c-Jun, and EGFR, as well as the upstream regulators of STAT3, which are IGF, PDGF, HER2, ERK/MAPK, HGF, ILK, and IL-6. Moreover, a number of tumor suppressors are upregulated in response to downregulation of *BCL9*, such as phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase, dual-specificity protein phosphatase (PTEN), cyclin-dependent kinase inhibitor 1B (CDKN1B), and BCL2-associated agonist of cell death (BAD) [19]. In this research, the relationship of *BCL9* to the development of stomach cancer was investigated, and many interactions were studied, including clinical and demographic ones. In addition, the importance and the possibility of exploiting methylation of *BCL9* as a biomarker for stomach cancer with possibility of exploiting it as a diagnostic and therapeutic target was demonstrated.

2. Materials and Methods

Clinical specimens

The 100 specimens in this study are split into 40 samples (control group), consisting of roughly healthy individuals who have not been diagnosed with digestive system diseases. Both males and females, aged 18 to 76, were represented. Additionally, 60 samples of male and female patients with a range of ages (19–71) who displayed digestive system symptoms and signs (diarrhea, vomiting, weight loss, and indigestion) were included in this investigation. Face-to-face procedures were used for the patient interviews. A number of questions about each patient's clinical picture, past medical history, demographics, previous therapies, and family history were included in the questionnaire. Through the specialist physicians, an endoscopy of oesophago-gastroduodenoscopy (OGD) was performed in the digestive system unit/Fallujah Hospital/ Anbar/ Fallujah in the period from 14- March- 2023 to 15- September- 2023. They were accurately diagnosed by a physician through histological examination as suffering from a stomach disorder. A biopsy was also taken to detect, isolate, and molecularly diagnose *H. pylori*. The specimens were immersed in the medium and allowed to incubate at room temperature (RT) aerobically. After incubation for 10 minutes, 1, 2, and 24 hours, the test result is visible. According to Uotani and Graham, the development of a pink-red or red-violet color denotes positive results [19].

The Methylation Specific-Real-Time amplification (MS-RT-PCR) for BCL9

Using the Monarch® Genomic DNA Purification Kit Reagent procedure from New England Biolabs/ USA, genomic DNA was extracted from the 100 biopsy samples that were collected. Using a DNA MethylEdge™ Bisulfite Conversion System (Promega Corporation, USA), through which all extracted DNA was treated with this substance, in order to detect CpG islands.

Then, using MS-RT-PCR technology and using primers that were specifically designed to determine the methylation promoter in *BCL9* gene to detect methylation pattern; these primers were arranged as follows:

BCL9 Left M	5'- GCGTAGTTAGGTTAAGTTTGCGA - 3'
BCL9 Right M	5'- TTCTAACTCCCCCTCTTACTACGAT - 3'
BCL9 Left U	5'- GTGTGTAGTTAGGTTAAGTTTGTGA - 3'
BCL9 Right U	5-' TTCTAACTCCCCCTCTTACTACAAT - 3'

The MS-RT-PCR technology was accomplished using a SimpliAmp Thermal Cycler Applied Biosystem/ USA, where the active ingredients were mixed to obtain the final volume (20 µL), and the technique was completed carefully and with high accuracy. Using SYBR Green PCR SuperMix, Synthol / Russia, components were SYBR Green PCR Master Mix (10 µL), MgCl (1 µL), forward primer (1 µL), reverse primer (1 µL), nuclease- free- water (5 µL), and DNA (2 µL).

MS-RT-PCR protocol

Three major steps that make up MS-RT-PCR were performed in each cycle for a real-time PCR reaction. Reactions were normally run for 45 cycles, except for the first step, which is only done in one cycle. The procedure was performed in sterile conditions and included the following stages: initial denaturation at 95°C for 5 min one cycle; denaturation 95°C for 20 sec., annealing at 59 °C for 20 sec., and extension at 72 °C for 20 sec.

Statistical analysis

Statistical analysis was carried out using GraphPad Prism 8 (GraphPad Software, CA, USA). Continuous variables, such as age, were presented as the median and interquartile range (IQR), while categorical variables, including sex, smoking status, comorbidities, residence, *H. pylori* infection, and medical treatment, were presented as frequencies and percentages. Additionally, Pearson correlation coefficients were calculated to assess relationships between various clinical, molecular, and epigenetic factors, and the results were visualized using a correlation matrix and heatmap. Promoter DNA methylation levels of CpG island *BCL-9* were compared between different groups using Mann-Whitney U tests. A *p*-value<0.05 was regarded as statistically significant for all statistical tests.

3. Results and discussion

Demographic and clinical characteristics

Out of 100 participants in the current study, sixty patients had various gastric diseases (Table 1). There was no discernible difference in the groups' median ages (p=0.813), with the control group's median age being 42 years (interquartile range, IQR=25.3) and the patient group's median age being 38 years (IQR=21.8). Furthermore, there was a comparable gender distribution in both groups: in the control group, there were 37.5% males and 41.7% females, while in the sick group, there were 46.7% males and 53.3% females (p=0.414). The proportion of smokers was also relatively similar in both groups, with 30% of participants being smokers and 70% being non-smokers (*p*=0.999). The proportion of patients with comorbidities was higher in the patient group (40%) than in the control group (22.5%), although the difference was not statistically significant (p=0.084). The distribution of residence was also similar between the groups, with 57.5% of the control group and 55% of the patient group residing in urban areas, while 42.5% and 45% resided in rural areas, respectively (*p*=0.840). A notable difference was observed in the prevalence of *H. pylori* infection between the two groups. While none of the participants in the control group tested positive for *H. pylori* infection, a significant proportion (88.3%) of the patient group was found to be infected (*p*=0.001). Lastly, the proportion of participants receiving medical treatment was comparable between the control group (10%) and the patient group (63.3%), with no significant difference observed (*p*=0.834).

The frequency and percentage of patients with various gastric conditions in the patient group, are presented in Figure 1A. Gastritis was the most prevalent condition, affecting 51.7% of patients, followed by gastric cancer (23.3% of patients), duodenal ulcers (13.3% of patients), and gastric ulcers (11.7% of patients). Furthermore, the distribution of these conditions by sex is depicted in Figure 1B. Notably, gastritis was equally prevalent among males (16 patients) and females (15 patients), while duodenal ulcers were more common in females (6 patients) compared to males (2 patients), but without any significant differences. Gastric cancer affected an equal number of males and females (7 patients each), and gastric ulcers showed a similar distribution, with 3 male and 4 female patients. Moreover, the age-wise distribution of these gastric conditions is illustrated in Figure 1C. Interestingly, gastritis was more prevalent in patients aged 40 years or younger (21 patients) compared to those over 40 years (10 patients). In contrast, gastric cancer was more common in patients over 40 years (10 patients) than in those aged 40 years or younger (4 patients). Gastric ulcers and duodenal ulcers showed a similar distribution across both age groups, with 3 and 4 patients aged 40 years or younger and 4 patients each in the over 40 years age group, respectively.

Table 1: Demographic and clinical characteristics of the study participants.

	Controls (n= 40)	Patients (n= 60)	*P*
Age, years	42 (25.3)	38 (21.8)	0.813
Sex			
Male	15 (37.5)	28 (46.7)	0.414
Female	25 (41.7)	32 (53.3)	
Smoking status			
Smoker	12 (30)	18 (30)	0.999
Non-smoker	28 (70)	42 (70)	
Comorbidities			
Present	9 (22.5)	24 (40)	0.084
Absent	31 (77.5)	36 (60)	
Residence			
Urban	23 (57.5)	33 (55)	0.840
Rural	17 (42.5)	27 (45)	
***H. pylori* infection**			
Positive	0 (0)	53 (88.3)	0.001
Negative	40 (100)	7 (11.7)	
Medical treatment			
Yes	24 (10)	38 (63.3)	0.834
No	16 (90)	22 (36.7)	

The age is presented as median (and interquartile range, IQR), and categorical data (sex, residence, comorbidities, and smoking status) are presented as frequencies (and percentages). P-values were calculated using a Mann Whitney's U test for age and Fisher's exact test for categorical data.

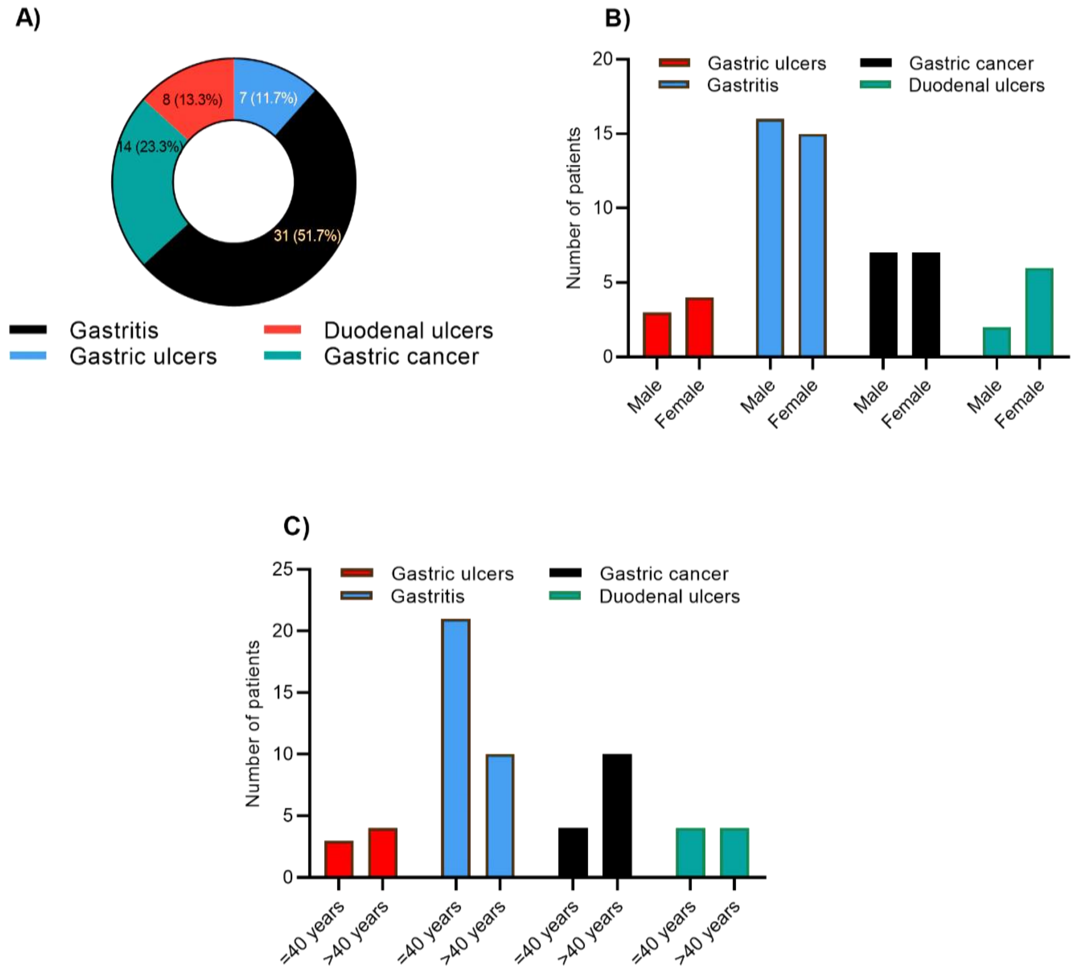


Figure 1: Distribution of gastric conditions among patients: A) Frequency and percentage of patients with gastric ulcers, gastritis, gastric cancer, and duodenal ulcers, B) Distribution of gastric conditions by sex, and C) Age-wise distribution of gastric conditions, categorized into patients aged 40 years or younger and those over 40 years.

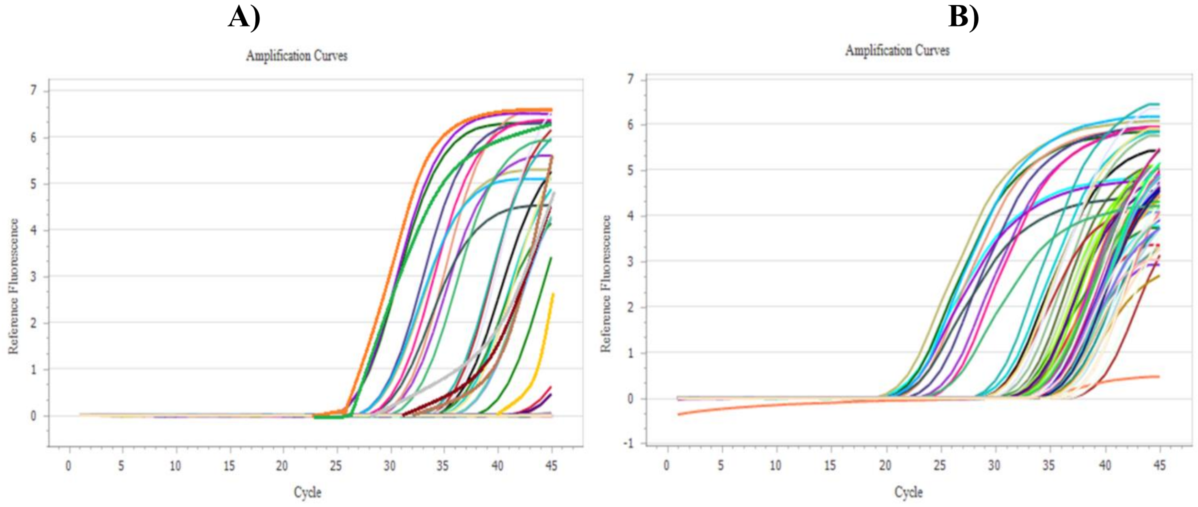


Figure 2: Each curve in this graph represents an instantaneous state measured at each RTq-PCR cycle, indicating amplification of the target DNA sequence.

The number of cycles (from 0 to 45) is shown on the x-axis for each RTq-PCR cycle performed as the target DNA is amplified exponentially. The y-axis indicates the levels and amounts of fluorescence, which increase in direct proportion as DNA is amplified. The Ct value represents the point where the curve intersects with the threshold line. **A)** Amplification curve for *BCL9* Methylated samples. **B)** Amplification curve for *BCL9* Unmethylated samples.

DNA methylation

The amplification curve for the hundred *BCL9* Methylated samples is displayed in Figure 2 A. The Ct value ranged from (22.899– 44.321). The amplification curve for the hundred *BCL9* Unmethylated samples is shown in Figure 2 B, with a Ct value ranging from (26.881–40.132). The current study investigated DNA methylation patterns of CpG island *BCL-9* in relation to clinical and pathological factors in gastric disease. According to Muhammad *et al*., [21], DNA methylation is the primary epigenetic change in cancer cells. Epigenetic dysregulation is a hallmark of cancer.

According to Wu *et al*., [22], *BCL9* is regarded as a crucial developmental regulator and a proven oncogenic driver in a variety of cancer types, mostly by enhancing Wnt/b-catenin signaling. The results are summarized in Table 2 and visualized in Figure 3 for *BCL-9*. *BCL-9* methylation showed more pronounced associations with certain clinical factors (Table 2 and Figure 3). There was a significant difference in *BCL-9* methylation between patients and controls (median 1.368, IQR [0.912-1.468] *vs*. 1.001, [0.861-1.211], $p<0.05$), several noteworthy associations was observed. Most strikingly, *BCL-9* methylation levels were significantly higher in males compared to females (median 1.186, IQR [1.139-1.308] *vs*. 0.986, IQR [0.875-1.150], $p<0.05$). Additionally, a significant difference was found in *BCL-9* methylation between acute and chronic gastritis cases (median 1.213, IQR [1.083-1.378] *vs*. 1.013, IQR [0.855-1.130], $p<0.05$), which suggests that the chronicity of inflammation may influence *BCL-9* methylation patterns. *BCL9* acts as a co-activator for β-catenin, a central component of the Wnt signaling pathway. When Wnt ligands bind to their receptors, β-catenin is stabilized and translocates to the nucleus, where it partners with TCF/LEF transcription factors to activate target genes that promote cell proliferation and survival [23].

Other factors such as age, smoking status, residence, chronic disease status, and treatment status did not significantly correlate with *BCL-9* methylation (all $p>0.05$). Infection with *H. pylori* showed a difference in the methylation level of the gene, but statistically, it is small and does not reach a significant difference ($p=0.760$). This is often explained by the fact that infection with bacteria slightly affects the methylation level of this gene. The risk of gastric cancer (GC) is associated with DNA methylation in the stomach mucosa caused by *H. pylori*. On the other hand, the CpG island methylator phenotype (CIMP) is characterized by elevated levels of methylation specific to cancer and offers unique molecular and clinicopathological aspects of GC [24]. Also, in this study, it became clear that there is no significant difference between cancerous and non-cancerous cases, even though there is a small difference between them (median 1.149, IQR [0.959-1.237] *vs*. 1.140, IQR [0.885-1.323], $p=0.839$). The methylation status of *BCL9* could serve as a potential biomarker for gastric cancer. Detecting *BCL9* methylation may help to assess the aggressiveness of the tumor and could aid in prognostic evaluations.

Table 2: DNA methylation levels of *BCL-9* in relation to clinical and pathological factors in gastric disease.

Clinical and Pathological factors	BCL-9 methylation ($2^{-\Delta\Delta Ct}$)	
	Median (IQR)	*p*-value
DNA methylation		
Control	1.001 (0.861-1.211)	0.041
Patients (All)	1.368 (0.912-1.468)	
Sex		
Male	1.186 (1.139-1.308)	0.001
Female	0.986 (0.875-1.150)	
Age		
≤40 years	1.149(0.886-1.296)	0.969
>40 years	1.139(1.013-1.216)	
Smoking status		
Smoker	1.218(1.171-1.291)	0.070
Non-smoker	1.105(0.904-1.208)	
Residence		
Urban	1.130(0.891-1.213)	0.445
Rural	1.149(1.013-1.292)	
Chronic disease		
Yes	1.153(0.886-1.240)	0.866
No	1.149(0.959-1.237)	
Treatment		
Yes	1.128(0.901-1.283)	0.703
No	1.176(1.020-1.217)	
Diseases		
Cancers	1.140(1.036-1.221)	0.839
Non-cancers	1.149(0.902-1.274)	
Gastritis		
Acute	1.213(1.083-1.378)	0.026
Chronic	1.013(0.855-1.130)	
***H. pylori* status**		
+ve	1.149(0.959-1.237)	0.760
-ve	1.140 (0.885-1.323)	

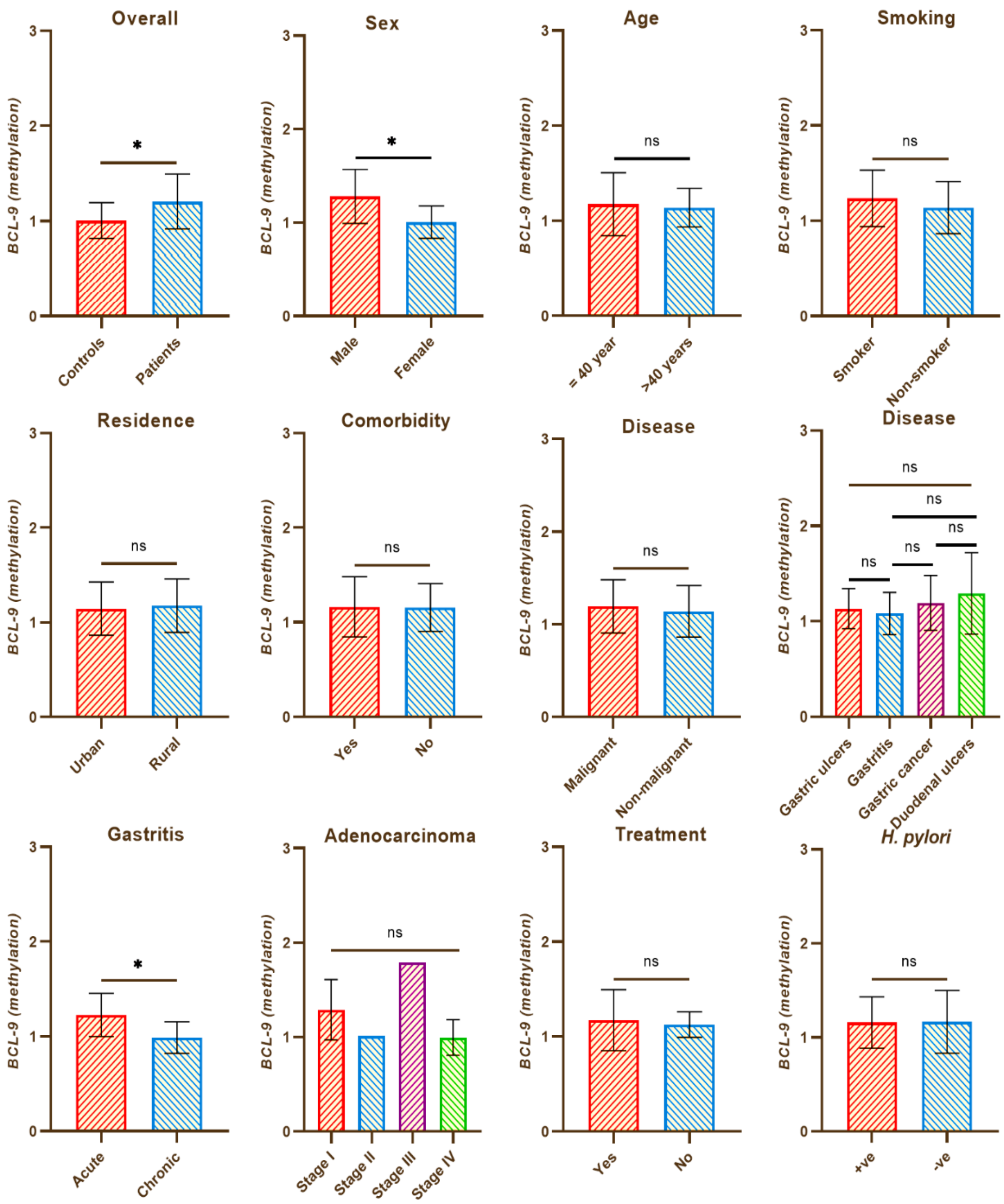


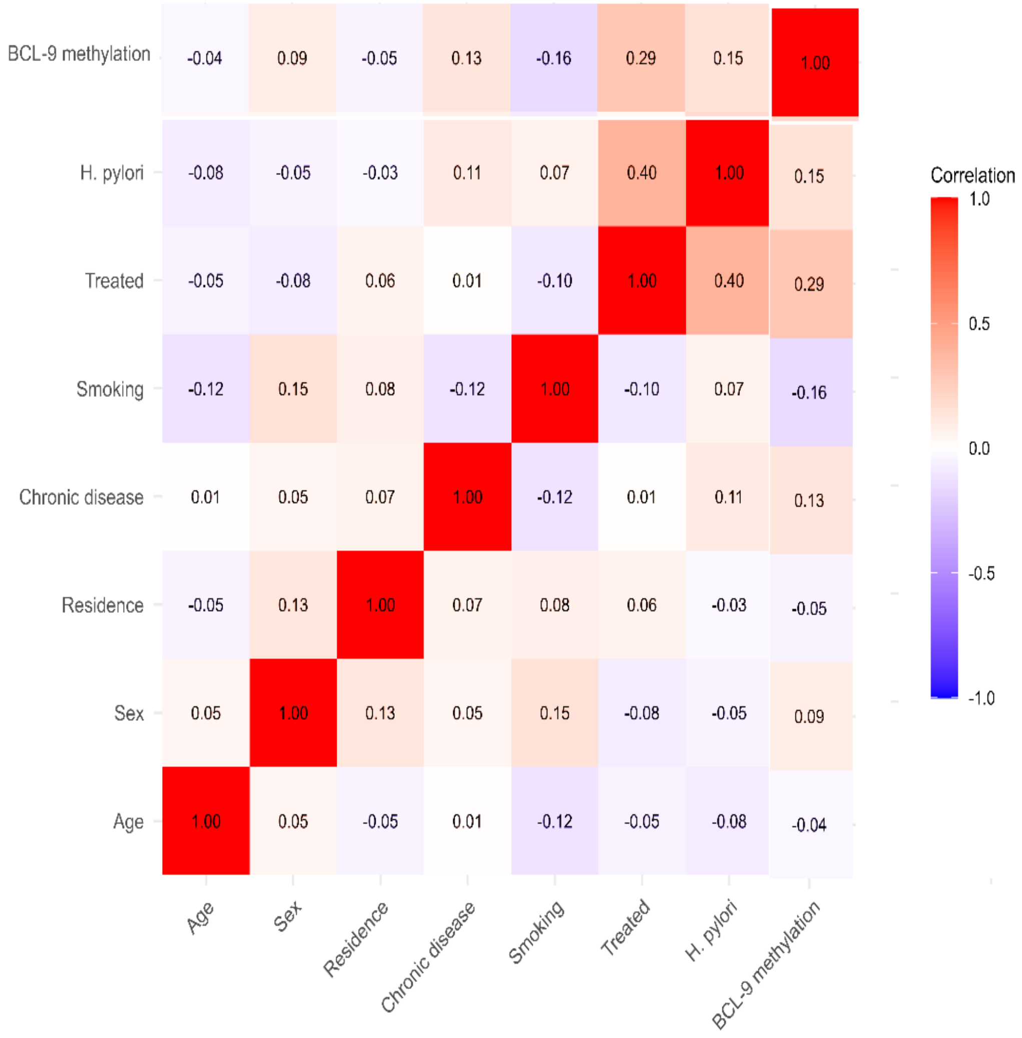
Figure 3: *BCL-9* methylation patterns in gastric disease. The figures depict the DNA methylation levels of *BCL-9* ($2^{-\Delta\Delta Ct}$) in relation to various clinical and pathological factors in gastric disease. Box plots represent the median, interquartile range, and outliers for each category. Statistically significant differences ($p<0.05$) are observed for overall specimen, sex, and gastritis type as indicated by asterisks (*).

Correlation analysis

This study also studied the relationships between various clinical, molecular, and epigenetic factors in gastric disease. The correlation matrix is presented in Table 3 and Figure 4. *H. pylori* infection showed some of the strongest correlations in the analysis. The *H. pylori* positivity also correlated positively with treatment status (r= 0.395, $p<0.01$). The epigenetic markers, *BCL-9* promoter CpG island methylation, exhibited notable correlations with several factors. *BCL-9* methylation correlated positively with treatment status (r= 0.293, $p<0.01$). Only *BCL-9* methylation was negatively correlated with age, residence and smoking (r= -

0.035, r= -0.049, and r= -0.159, respectively). One kind of lymphoid tissue that influences both local and systemic immune responses is gut-associated lymphoid tissue [25].

BCL9 amplification was found in a notably larger percentage of basal-like invasive breast cancers than in luminal breast cancers [18]. However, other demographic factors such as age, sex, residence, and smoking status did not correlate significantly with epigenetic markers. However, there is still a lack of extensive clinical validation for DNA methylation acting as tumor biomarkers, which prevents their application in clinical practice [26]. A major contributing factor to the development of GC is aberrant DNA methylation caused by *H. pylori*; DNA methylation serves as a link between *H. pylori* and GC. Cytotoxin-associated gene A (*CagA*), which causes aberrant methylation of the gene promoter and causes carcinogenesis in normal gastric cells, is the basis for *H. pylori*'s oncogenicity [27]. According to recent research, macrophages can interact with the Wnt signaling system, and activation of the Wnt signaling pathway can cause initial resistance to immunotherapy [28].



	Age	Sex	Residence	Chronic disease	Smoking	Treated	H. pylori	BCL-9 methylation
BCL-9 methylation	-0.04	0.09	-0.05	0.13	-0.16	0.29	0.15	1.00
H. pylori	-0.08	-0.05	-0.03	0.11	0.07	0.40	1.00	0.15
Treated	-0.05	-0.08	0.06	0.01	-0.10	1.00	0.40	0.29
Smoking	-0.12	0.15	0.08	-0.12	1.00	-0.10	0.07	-0.16
Chronic disease	0.01	0.05	0.07	1.00	-0.12	0.01	0.11	0.13
Residence	-0.05	0.13	1.00	0.07	0.08	0.06	-0.03	-0.05
Sex	0.05	1.00	0.13	0.05	0.15	-0.08	-0.05	0.09
Age	1.00	0.05	-0.05	0.01	-0.12	-0.05	-0.08	-0.04

Figure 4: Heatmap displaying the study's variable correlation coefficients. On the color scale, red denotes a positive correlation, white indicates no association, and blue represents a negative correlation. The degree of association is shown by the color's intensity. Numbers in each cell represent the correlation coefficient.

Table 3: Correlation matrix of variables in the study.

Variable	Age	Sex	Residence	Chronic disease	Smoking	Treated	*H. pylori*	*BCL-9* methylation
Age	1.000	0.051	-0.051	0.008	-0.119	-0.055	-0.075	-0.035
Sex	0.051	1.000	0.125	0.051	0.154	-0.079	-0.049	0.088
Residence	-0.051	0.125	1.000	0.065	0.078	0.060	-0.027	-0.049
Chronic disease	0.008	0.051	0.065	1.000	-0.120	0.006	0.107	0.130
Smoking	-0.119	0.154	0.078	-0.120	1.000	-0.097	0.072	-0.159
Treated	-0.055	-0.079	0.060	0.006	-0.097	1.000	0.395**	0.293**
H. pylori	-0.075	-0.049	-0.027	0.107	0.072	0.395**	1.000	0.145
***BCL-9* methylation**	-0.035	0.088	-0.049	0.130	-0.159	0.293**	0.145	1.000

*Pearson correlation coefficients are shown as values. ** denotes p<0.01, and * denotes p<0.05.*

The coding for binary variables was as follows: Residence (0= Rural, 1= Urban), Sex (0= Female, 1= Male), H. pylori, (0= Negative, 1= Positive), treated (0= No, 1= Yes), smoking status (0= No, 1= Yes), and chronic disease (0= No, 1= Yes).

Although the potential genetic and epigenetic variations are not fully understood, recent evidence suggests that *H. pylori* induces changes in the methylation of important genes after infection and enhances their function by releasing reactive oxide and nitric oxide. Some have even shown that it has the ability to activate DNA methyl transferases [31, 32]. These enzymes are responsible for the acquisition of genomic methylation patterns. It is important because it strengthens the hypothesis that chronic *H. pylori* inflammation induces epigenetic disruption of some "tumor-progenitor genes" such as CDH1 and APC, in non-neoplastic stem cells of the "healthy" stomach, possibly increasing their replicative potential and reducing their differentiation. The current finding by Chehelgerdi *et al*., [33] turned out that relevant epigenetic events occur in chronic *H. pylori* gastritis and may represent the beginning of gastric carcinogenesis. This is significant because it strengthens the hypothesis. Based on this discovery, it is feasible to find diagnostic solutions appropriate for untying the knot of stomach cancer and finding more accurate and efficient solutions.

According to earlier research, *BCL9* and *BCL9L* are expressed at distinct phases of the development of the mouse mammary gland, with *BCL9L* expression decreasing during post-lactational involution [20]. Recent research by Wei *et al.* has shown that *BCL9* suppression alters macrophage polarization into the M1 and M2 subtypes. It is becoming more widely recognized that macrophage polarization has a crucial pathogenetic role in inflammatory and malignant disorders [30]. Targeting the epigenetic regulation of genes like *BCL9* may represent a therapeutic strategy, potentially using drugs that modify DNA methylation to restore normal expression patterns of important cancer-related genes.

In the current study, several limitations and challenges were encountered in terms of application and analysis. Although RT-PCR is widely used for gene methylation analysis, the precise amount of methylation is limited. RT-PCR requires carefully designed primers, which can cause nonspecific interactions with unmethylated regions, leading to incomprehensible and unanalyzable results. Identifying genes of interest in a particular study can be difficult when there is gene similarity. Experimental conditions, such as temperature or concentration of different materials, can be challenging, as they can produce erroneous Ct values due to differences in fluorescence intensity or cycle. Modern techniques also require highly efficient, modern, and sophisticated equipment to perform correctly. Interpreting methylation results can be complicated by the interaction of numerous environmental and genetic factors that influence statistical analysis. Individual variation in methylation levels among the population requires a large number of participants and a more comprehensive study.

4. Conclusion

The current results indicate that the methylation level of the gene changed after infection with *H pylori* and gender. Infection with *H. pylori* showed a difference in the methylation level of *BCL-9* gene. The methylation level is closely related to various stomach conditions, most notably acute gastritis. It is possible to use *BCL9* as a therapeutic target to prevent GC and as a biomarker of high-risk gastritis. This leads to strong evidence that detecting the molecular level of methylation of *BCL9* gene has an effective early diagnostic role. Research in this area may lead to the development of novel diagnostic and treatment strategies targeting the epigenetic landscape of gastric cancer. This evidence can be exploited in the future to develop a suitable drug to treat the condition and prevent cancer.

5. Novelty and Impact

One potential treatment approach could be to target the *BCL9* gene's epigenetic regulation, potentially using drugs that modify DNA methylation to restore normal expression patterns of important cancer-related genes.

Ethics Approval

The University of Baghdad College of Science Research Ethics Committee approved this study (CSEC/0723/0055).

Conflict of Interest

I confirm that neither I nor any other researcher involved in this research have any conflict of interest or potential for personal gain in this research.

References

[1] F. M. Lafta, R. M. AL-Jumaily, and L. M. Rasoul, "Global DNA Methylation Levels in Epstein-Barr-Virus-Positive Iraqi Patients with Acute Lymphoblastic Leukaemia," *Iraqi Journal of Science*, vol. 64, no. 3, pp. 1109–1118, Feb. 2023, doi: 10.24996/ijs.2023.64.3.7.

[2] T. Davletgildeeva and N. A. Kuznetsov, "The Role of DNMT Methyltransferases and TET Dioxygenases in the Maintenance of the DNA Methylation Level," *Biomolecules*, vol. 14, no. 9, pp. 1117, 2024, doi: 10.3390/biom14091117.

[3] R. H. Hamid and R. M. AL-Jumaily, "Evaluation of Oxidative Stress activity, DNA Damage and Global DNA Methylation among Patients with Chronic Kidney Disease," *Iraqi Journal of Science*, vol. 65, no. 9, pp. 4963–4972, 2024, doi: 10.24996/ijs.2024.65.9.13.

[4] R. K. Mwafaq, A. K. Abbas, and L. A. H. Abdullah, "Evaluation of PARP-1 by immunohistochemistry in a sample of Iraqi patients with gastric cancer," *Iraqi Journal of Science*, vol. 63, no. 2, pp. 467-479, 2022, doi: 10.24996/ijs.2022.63.2.5.

[5] R. S. Aboud, "Evaluation of anti- Helicobacter pylori IgG level in the serum of patients with autoimmune thyroid disease," *Iraqi Journal of Science*, vol. 52, no. 4, pp. 440–444, 2024, doi: 10.24996/ijs.2011.52.4.%g.

[6] Y. B. Qi, R. B. Hu, S. Z. Ding, M. N. Khan, L. Lei, and P. R. Wei, "Helicobacter pylori Infection, DNA Methylation, and Gastric Carcinogenesis," *Journal of Carcinogenesis and Mutagenesis*, vol. 11, p. 352, 2020, doi: 10.35248/2157-2518.20.11.352.

[7] M. M. Ibraheem, "Evaluation of CD14 expression in Helicobacter pylori positive and Helicobacter pylori negative gastritis," *Iraqi Journal of Science*, vol. 58, no. 4, pp. 2070-2075, 2021, doi: 10.24996/ijs.2017.58.4B.9.

[8] N. A. Aburahma and N. A. Kadhim, "Changes of Serum Electrolytes and Serum Vitamin C Levels in a Sample of Iraqi patients Infected with Helicobacter pylori (H. pylori)," *Iraqi Journal of Science*, vol. 64, no. 8, pp. 3793-3798, 2023, doi: 10.24996/ijs.2023.64.8.6.

[9] S. Salvatori, I. Marafini, F. Laudisi, G. Monteleone, and C. Stolfi, "Helicobacter pylori and Gastric Cancer: Pathogenetic Mechanisms," *International Journal of Molecular Sciences*, vol. 24, no. 3, p. 2895, 2023, doi: 10.3390/ijms24032895.

[10] M. Mani, D. E. Carrasco, Y. Zhang, K. Takada, M. E. Gatt, J. Dutta-Simmons, H. Ikeda, F. Diaz-Griffero, V. Pena-Cruz, M. Bertagnolli, L. L. Myeroff, S. D. Markowitz, K. C. Anderson, and D. R. Carrasco, "*BCL9* promotes tumor progression by conferring enhanced proliferative, metastatic, and angiogenic properties to cancer cells," *Cancer Research*, vol. 69, no. 19, pp. 7577–7586, 2009, doi: 10.1158/0008-5472.CAN-09-0773.

[11] Z. Wen and J. E. Jr Darnell, "Mapping of Stat3 serine phosphorylation to a single residue (727) and evidence that serine phosphorylation has no influence on DNA binding of Stat1 and Stat3," *Nucleic Acids Research*, vol. 25, no. 11, pp. 2062–2067, 1997, doi: 10.1093/nar/25.11.2062.

[12] K. Suzuki, Y. Okuno, N. Kawashima, H. Muramatsu, T. Okuno, X. Wang, S. Kataoka, Y. Sekiya, M. Hamada, N. Murakami, D. Kojima, K. Narita, A. Narita, H. Sakaguchi, K. Sakaguchi, N. Yoshida, N. Nishio, A. Hama, Y. Takahashi, K. Kudo, and S. Kojima, "MEF2D-*BCL9* Fusion Gene Is Associated With High-Risk Acute B-Cell Precursor Lymphoblastic Leukemia in Adolescents," *Journal of Clinical Oncology*, vol. 34, no. 28, pp. 3451–3459, 2016, doi: 10.1200/JCO.2016.66.5547.

[13] T. B. K. Watkins, E. L. Lim, M. Petkovic, S. Elizalde, N. J. Birkbak, G. A. Wilson, D. A. Moore, E. Grönroos, A. Rowan, S. M. Dewhurst, J. Demeulemeester, S. C. Dentro, S. Horswell, L. Au, K. Haase, M. Escudero, R. Rosenthal, M. A. Bakir, H. Xu, K. Litchfield, and C. Swanton, "Pervasive chromosomal instability and karyotype order in tumour evolution," *Nature*, vol. 587, no. 7832, pp. 126–132, 2020, doi: 10.1038/s41586-020-2698-6.

[14] J. Li, X. Chen, X. Ding, Y. Cheng, B. Zhao, Z. C. Lai, K. Al Hezaimi, R. Hakem, K. L. Guan, and C. Y. Wang, "LATS2 suppresses oncogenic Wnt signaling by disrupting β-catenin/*BCL9* interaction," *Cell Reports*, vol. 5, no. 6, pp. 1650–1663, 2013, doi: 10.1016/j.celrep.2013.11.037.

[15] M. Jiang, Y. Kang, T. Sewastianik, J. Wang, H. Tanton, K. Alder, P. Dennis, Y. Xin, Z. Wang, R. Liu, M. Zhang, Y. Huang, M. Loda, A. Srivastava, R. Chen, M. Liu, and R. D. Carrasco, "*BCL9* provides multi-cellular communication properties in colorectal cancer by interacting with paraspeckle proteins," *Nature Communications*, vol. 11, no. 1, p. 19, 2020, doi: 10.1038/s41467-019-13842-7.

[16] X. Liu, Q. Ji, C. Zhang, X. Liu, Y. Liu, N. Liu, H. Sui, L. Zhou, S. Wang, and Q. Li, "miR-30a acts as a tumor suppressor by double-targeting COX-2 and *BCL9* in H. pylori gastric cancer models," *Scientific Reports*, vol. 7, no. 1, p. 7113, 2017, doi: 10.1038/s41598-017-07193-w.

[17] X. Rao, X. Liu, N. Liu, Y. Zhang, Z. Zhang, L. Zhou, G. Han, R. Cen, N. Shi, H. Zhu, C. Gong, Q. Huang, Q. Ji, and Q. Li, "Long noncoding RNA NEAT1 promotes tumorigenesis in H. pylori gastric cancer by sponging miR-30a to regulate COX-2/*BCL9* pathway," *Helicobacter*, vol. 26, no. 6, p. e12847, 2021, doi: 10.1111/hel.12847.

[18] H. S. Elsarraj, Y. Hong, D. Limback, R. Zhao, J. Berger, S. C. Bishop, A. Sabbagh, L. Oppenheimer, H. E. Harper, A. Tsimelzon, S. Huang, S. G. Hilsenbeck, D. P. Edwards, J. Fontes, F. Fan, R. Madan, B. Fangman, A. Ellis, O. Tawfik, D. L. Persons F., and Behbod, "*BCL9*/STAT3 regulation of transcriptional enhancer networks promote DCIS progression," *NPJ Breast Cancer*, vol. 6, no. 12, 2020, doi: 10.1038/s41523-020-0157-z.

[19] N. Datta, S. Chakraborty, M. Basu, and M. K. Ghosh, "Tumor Suppressors Having Oncogenic Functions: The Double Agents," *Cells*, vol. 10, no. 1, p. 46, 2020, doi: 10.3390/cells10010046.

[20] N. A. Urgessa, P. Geethakumari, P. Kampa, R. Parchuri, R. Bhandari, A. R. Alnasser, A. Akram, S. Kar, F. Osman, G. D. Mashat, H. H. Tran, and A. P. Arcia Franchini, "A Comparison Between Histology and Rapid Urease Test in the Diagnosis of Helicobacter Pylori in Gastric Biopsies: A Systematic Review," *Cureus*, vol. 15, no. 5, p. e39360, 2023, doi: 10.7759/cureus.39360.

[21] D. Liu, Y. Liu, W. Zhu, Y. Lu, J. Zhu, X. Ma, Y. Xing, M. Yuan, B. Ning, Y. Wang, and Y. Jia, "Helicobacter pylori-induced aberrant demethylation and expression of GNB4 promotes gastric carcinogenesis via the Hippo-YAP1 pathway," *BMC Medicine*, vol. 21, no. 1, p. 134, 2023, doi: 10.1186/s12916-023-02842-6.

[22] M. Wu, H. Dong, C. Xu, M. Sun, H. Gao, F. Bu, and J. Chen, "The Wnt-dependent and Wnt-independent functions of *BCL9* in development, tumorigenesis, and immunity: Implications in therapeutic opportunities," *Genes and Diseases*, vol. 11, no. 2, pp. 701–710, 2023, doi: 10.1016/j.gendis.2023.03.012.

[23] K. He, W. J. Gan, "Wnt/β-Catenin Signaling Pathway in the Development and Progression of Colorectal Cancer," *Cancer Management and Research*, vol. 15, pp. 435–448, 2023, doi: 10.2147/CMAR.S411168.

[24] S. Tahara, T. Tahara, J. Yamazaki, T. Shijimaya, N. Horiguchi, K. Funasaka, T. Fukui, Y. Nakagawa, T. Shibata, M. Naganuma, T. Tsukamoto, and N. Ohmiya, "Helicobacter pylori infection associated DNA methylation in primary gastric cancer significantly correlates with specific molecular and clinicopathological features," *Molecular Carcinogenesis*, vol. 63, no. 2, pp. 266–274, 2024, doi: 10.1002/mc.23650.

[25] X. Wang, Y. Dong, H. Zhang, Y. Zhao, T. Miao, G. Mohseni, L. Du, and C. Wang, "DNA methylation drives a new path in gastric cancer early detection: Current impact and prospects," *Genes and Diseases*, vol. 11, no. 2, pp. 847–860, 2023, doi: 10.1016/j.gendis.2023.02.038.

[26] M. Tilahun, A. Gedefie, C. Belayhun, Z. Sahle, and A. Abera, "Helicobacter pylori Pathogenicity Islands and Giardia lamblia Cysteine Proteases in Role of Coinfection and Pathogenesis," *Infection and Drug Resistance*, vol. 15, pp. 21–34, 2022, doi: 10.2147/IDR.S346705.

[27] M. L. Tigue, M. A. Loberg, J. A. Goettel, W. A. Weiss, E. Lee, and V. L. Weiss, "Wnt Signaling in the Phenotype and Function of Tumor-Associated Macrophages," *Cancer Research*, vol. 83, no. 1, pp. 3–11, 2023, doi: 10.1158/0008-5472.CAN-22-1403.

[28] N. Zatula, M. Wiese, J. Bunzendahl, W. Birchmeier, C. Perske, A. Bleckmann, F. H. Brembeck, "The *BCL9*-2 proto-oncogene governs estrogen receptor alpha expression in breast tumorigenesis," *Oncotarget*, vol. 5, no. 16, pp. 6770–6787, 2014, doi: 10.18632/oncotarget.2252.

[29] V. Vafaizadeh, D. Buechel, N. Rubinstein, R. K. R. Kalathur, L. Bazzani, M. Saxena, T. Valenta, G. Hausmann, C. Cantù, K. Basler, and G. Christofori, "The interactions of *Bcl9*/*Bcl9*L with β-catenin and Pygopus promote breast cancer growth, invasion, and metastasis," *Oncogene*, vol. 40, no. 43, pp. 6195–6209, 2021, doi: 10.1038/s41388-021-02016-9.

[30] Z. Wei, M. Yang, M. Feng, Z. Wu, R. Rosin-Arbesfeld, J. Dong, and D. Zhu, "Inhibition of *BCL9* Modulates the Cellular Landscape of Tumor-Associated Macrophages in the Tumor Immune Microenvironment of Colorectal Cancer," *Frontiers in Pharmacology*, vol. 12, p. 713331, 2021, doi: 10.3389/fphar.2021.713331.

[31] M. A. Valenzuela, J. Canales, A. H. Corvalán, and A. F. Quest, "Helicobacter pylori-induced inflammation and epigenetic changes during gastric carcinogenesis," *World Journal of Gastroenterology*, vol. 21, no. 45, pp. 12742–12756, 2015, doi: 10.3748/wjg.v21.i45.12742.

[32] R. Capparelli and D. Iannelli, "Epigenetics and Helicobacter pylori," *International Journal of Molecular Sciences*, vol. 23, no. 3, p. 1759, 2022, doi: 10.3390/ijms23031759.

[33] M. Chehelgerdi, F. Behdarvand Dehkordi, M. Chehelgerdi, H. Kabiri, H. Salehian-Dehkordi, M. Abdolvand, S. Salmanizadeh, M. Rashidi, A. Niazmand, S. Ahmadi, S. Feizbakhshan, S. Kabiri, N. Vatandoost, and T. Ranjbarnejad, "Exploring the promising potential of induced pluripotent stem cells in cancer research and therapy," *Molecular Cancer*, vol. 22, no. 1, p. 189, 2023, doi: 10.1186/s12943-023-01873-0.