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Article

Correlation Between Helicobacter Pylori Infection, Gene Methylation, and Host Genetic Polymorphisms in Gastric Cancer

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Abstract: This study investigated how Helicobacter pylori (Hp) infection and host genetic variations contribute to gene-specific DNA methylation patterns in the development of gastric cancer in Iraqi patients. Seven genes (CYP2E1, HIN-1, MT2A, CASP3, CDKN1A, MSH6, and EpCAM) known to play roles in inflammation, DNA repair, and apoptosis were selected for methylation analysis. A total of 67 gastric tissue samples were analyzed using Methylation-Sensitive High-Resolution Melting (MS-HRM), including samples from 30 gastric cancer patients and 37 gastritis patients (subdivided by Hp status and post-eradication treatment). Results revealed significantly higher methylation frequencies in gastric cancer, particularly for CYP2E1 (80%), CASP3 (86.7%), and EpCAM (20%), compared to gastritis. Notably, CDKN1A exhibited 100% methylation across all cases, regardless of Hp status or disease stage. Certain polymorphic genotypes, including CYP2E1 T/T and CASP3 A/A, were strongly associated with increased methylation. Post-eradication analysis showed only limited reversal of methylation in some genes, such as MSH6, EpCAM, and MT2A. These findings suggest that the combined impact of Hp infection, host genetic variation, and epigenetic silencing may contribute significantly to gastric carcinogenesis. Moreover, gene methylation patterns, particularly in CYP2E1, CASP3, and EpCAM, may serve as valuable biomarkers for early detection and risk stratification of gastric cancer in clinical practice.

Keywords: DNA methylation, gastric cancer, Helicobacter pylori, gene polymorphism, MS-HRM, epigenetics.

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Introduction

Gastric cancer (GC) remains among the most serious health problems in several countries [1]. Worldwide, gastric cancer is the third most common cancer. Gastric carcinogenesis is a complex, multi-step, and multifactorial outcome. Many factors play a role in its etiology, with environmental factors being primary among them. One of the most intriguing of these is H. pylori (Hp), a gram-negative microorganism. The etiological factors involved in the genesis of gastric cancer are not entirely known. Epidemiological studies demonstrate the importance of environmental factors, notably dietary ones, in

the pathogenesis of gastric cancer. A new factor has been investigated in gastric oncogenesis: it is postulated that infection with Helicobacter pylori (Hp) leading to chronic gastritis, mucosal atrophy, and intestinal metaplasia participates in the chain of events in the genesis of gastric cancer [2]. In recent studies we observed that patients with Helicobacter pylori infection presented DNA damage in the epithelial cells of the gastric mucosa, which correlated with the intensity of the inflammatory response in the mucosa and with the pathogenic cagA and vacA genotypes of H. pylori [3]. These results open the perspective of investigating the possible correlation between the different genotypes of the bacteria and the clinicopathological characteristics of gastric cancer. The association between H. pylori and chronic gastritis has been widely demonstrated; so much so that in countries with a high incidence of H. pylori, the incidence of gastric cancer is also high. The different studies that exist on this matter demonstrate a significant association and there is no room for discussion [4].

H. pylori employ a series of mechanisms that allow it to colonize the gastric mucosa, such as: good motility, strong adhesion to epithelial cells, and an appropriate microenvironment to perpetuate the infection. On the other hand, there are H. pylori genotypes that encode various virulence factors such as the cagA, vacA, and babA2 proteins. These genes largely determine the pathogenicity and aggressiveness of the bacterium, which has been evidenced in several studies demonstrating their association with gastric cancer and premalignant lesions [4]. Genetic susceptibility factors include polymorphisms in genes involved in critical processes of gastric carcinogenesis, such as: the inflammatory and immune response to infection, including genes responsible for recognizing H. pylori in gastric epithelial cells and activating signaling pathways that trigger pro-inflammatory and apoptotic responses; protection of the gastric mucosa; metabolism and detoxification of carcinogenic compounds; repair of oxidative damage; cell proliferation and adhesion; and DNA repair. The genes of these mechanisms, and very especially those related to the immunoinflammatory response, have thus been the most analyzed in candidate gene analysis strategies [5].

Individual genetic susceptibility has been associated with specific genetic polymorphisms, mostly single nucleotide polymorphisms (SNPs), which could modify the effect of environmental exposures, partly explaining the great variability in the worldwide incidence of gastric cancer. Specific allelic variants of different genes are associated with an increased risk of developing gastric cancer. Among the single nucleotide polymorphisms related to gastric carcinogenesis is the enzyme methylenetetrahydrofolate reductase (MTHFR) gene [6]. This SNP is linked to increased susceptibility to GC because the mutation decreases MTHFR enzyme activity, leading to DNA hypomethylation and oncogene expression. Much of the improvement in the knowledge of genetic variability and its application in association studies is due to the international [7-8].

Various epidemiological studies have also focused on genetic variability in genes involved in DNA repair and its association with gastric carcinogenesis [5,7]. In recent years, several discrepant association studies have been reported between the potentially functional polymorphisms of XRCC1, Arg194Trp, Arg280His and Arg399Gln, and the risk of gastric cancer. [9] Another group of polymorphisms that have been analyzed are those located in genes responsible for recognizing H. pylori and mediators of the innate immune response, TLRs and NODs. These polymorphisms can modify the ability of receptors to recognize H. pylori or its derivatives and, consequently, cause defects in the cellular signaling they transmit in response. [10]. Genetic variability in genes for gastric mucosal protection may also be an influential factor in the risk of gastric cancer. These genes encode the main proteins for the formation and maintenance of the gastric mucosa, mucins and trefoil peptides, which play an important role in protecting the epithelium against pathogens and other external aggressions [11].

The association between Hp and gastric carcinoma has been established more with the intestinal type of gastric cancer. This is because the intestinal type of gastric cancer has precancerous lesions such as atrophic gastritis and intestinal metaplasia. However, the specific role Hp plays in carcinogenesis has not been determined. This study seeks to address that deficiency by examining the methylation status of essential genes and their correlation with H. pylori infection, cancer advancement, and genetic polymorphisms. The objective is to find possible early biomarkers and learn how genetics and infection work together to raise the risk of gastric cancer.

Materials and Methods

The objective of this study was to compare hypermethylated genes in tissue samples from patients with gastric cancer and gastritis, both prior to and following Helicobacter pylori eradication therapy, to investigate potential correlations. We used the MS-HRM technique to look at tissue samples and compare the methylation levels between groups. Standard lab tools were micropipettes, centrifuge tubes, PCR accessories, a microcentrifuge, a vortex mixer, and a thermal cycler. The Applied Biosystems™ Veriti™ 96-Well Thermal Cycler was used to make PCR copies. We used the Bio-Rad CFX96 Touch™ Real-Time PCR Detection System to look at methylation. The PureLink™ Genomic DNA Kit (Thermo Fisher Scientific) was used to get the DNA out, and the Zymo EZ DNA Methylation-Gold™ Kit (Zymo Research, USA) was used to change the DNA into bisulfite. We got both methylated and unmethylated control DNA samples from MilliporeSigma's CpGenome™ Universal Methylated and Unmethylated DNA Kit. The MS-HRM test was done with Bio-Rad's Precision Melt Supermix and validated primers that only worked with the CYP2E1, HIN-1, CASP3, MT2A, MSH6, EpCAM, and CDKN1A genes. To keep the samples safe and reliable, all reagents were made with distilled water that did not contain any nucleases.

Patient Selection and Tissue Collection: This study was conducted as a team effort led by the Department of Medical Genetics at Baghdad Medical College, Iraq, between February 2023 and April 2024, in collaboration with the Departments of Gastroenterology, General Surgery, and Pathology. Information about the research was provided to the patients included in the study, and consent forms were obtained. Tissue samples taken from 30 cases who were operated on with a diagnosis of gastric cancer and reported to have intestinal-type gastric cancer constituted the cancer patient group of our study. Biopsy samples taken from 37 cases who underwent endoscopy with a diagnosis of gastritis. Cases diagnosed as Hp(+) by the Gastroenterology Department were given medical treatment for *Hp* eradication and called for post-treatment follow-up. Only 15 cases completed their treatment within the study period and came for follow-up 3–8 months later; samples were taken by control endoscopy and analysed. For the evaluation of *Hp* status, microscopic examination, culture growth, and urease test were performed on the tissues. Cases where Hp was detected in any examination were evaluated as Hp(+), and cases where Hp could not be detected in any examination were evaluated as Hp(-) and grouped accordingly. In the cancer group consisting of 30 cases, 5 cases were diagnosed as Hp(-) and 25 cases as Hp(+). In the gastritis group consisting of 37 cases, 14 cases were diagnosed as Hp(-) and 23 cases as Hp(+). The age distribution of the gastritis patient group (n=37) was between 21-75 years, with a group mean age of 46.60 ± 2.39. The age distribution of the gastric cancer patient group (n=30) was between 36-79 years, with a group mean age of 62.27 ± 2.05.

Tissue Homogenization: Fresh tissues stored in the refrigerator at -20°C until analysis, were processed in groups for the study. Tissue fragments, approximately 1-10 mg in size, were placed in petri dishes and mechanically minced and crushed using a scalpel blade. The minced and crushed tissues were transferred to 1.5 ml tubes with the addition of 500 µl of PBS solution and centrifuged at 14,000 rpm for 10 minutes. The supernatant above the pellet in the tubes was removed. 500 µl of PBS solution was added, and the tubes were vortexed to resuspend the pellet and then centrifuged again at 14,000 rpm for 10 minutes. This washing and removal of unwanted tissue fragments step was repeated two or three times. After the final centrifugation step, the supernatant was removed. To the tubes, 200 µl of tissue lysis buffer (Roche, MagNa Pure DNA Tissue Lysis Buffer) and 20 µl of Proteinase K (Roche, Proteinase K, Recombinant, PCR grade) were added. The tubes were then incubated for three hours in a shaking water bath preheated to 60°C. To ensure better homogenization, the tubes were periodically vortexed at specific intervals. At the end of this stage, the tissues had been converted into a homogenized liquid.

DNA Extraction from Homogenized Tissues: DNA isolation was performed using a nucleic acid isolation robot. The MagNa Pure Compact Nucleic Acid Isolation Robot protocol was set to "Total Nucleic Acid Plasma 500," "sample volume 500 µl," and "elution volume 100 µl." The names of the loaded samples were entered, and the cartridge and tubes were placed. 280 µl of PBS was added to the homogenized tissue liquids to achieve a total volume of 500 µl, and they were loaded into the robot.

After DNA isolation was complete, the DNA products obtained from the robot were stored in a -20°C refrigerator for subsequent bisulfite modification. The EpiTect® Bisulfite Kit (Qiagen) was used for the bisulfite modification of the obtained DNA products. The positive/methylated control sample to be used in the HRM stage, CpGenome™ Universal Methylated DNA (Millipore-Chemicon® International), and the negative/unmethylated control sample, CpGenome™ Universal Unmethylated DNA (Millipore-Chemicon® International), were modified at this stage. The primer sequences for the genes whose methylation patterns were evaluated in our study are provided in Table 1.

Table 1. Primer sequences of analyzed genes

Gene	Primer Type	Primer Sequence (5' → 3')	Product Size (bp)	Reference(s)
CASP3	Methylated	F: TCGTATTTCGGGATTCGGTC R: ACCTAAACGCAAACCCCGC	133	[12]
	Unmethylated	F: TTGTATTTTGGGATTTGGTT R: AACTAAACACAAACCCCAC	129	
MLH1	Methylated	F:TATATCGTTCGTAGTATTCGTGT R: TCCGACCCGAATAAACAACGTA	143	[13]
	Unmethylated	F:TTGATGTAGATGTTTTATTAGTTGT R:ACCACCTCATCATAACTACCCACA	161	
MSH2	Methylated	F: TCGTGGTCGGACGTCGTTC R: CAACGTCTCCTTCGACTACACCG	142	[14-15]
	Unmethylated	F: GGTTGTTGTGGTTGGATGTTGTTT R:CAACTACAACATCTCACTACACCA	148	
MT2A	Methylated	F: TGCGGTGTGCGTTTAGTT R: AAACCCAACAACCAACGA	118	[16]
	Unmethylated	F: TGTGGTGTGTGTTTAGTTGTG R: CCAACAACCAACAACTATTTTTA	126	
CDKN1A	Methylated	F:TTATTAGAGGTGGGGCGGATCGC R: GACCCCGAACCGCGACCGTAA	151	[17]
	Unmethylated	F:TATTAGAGGGTGGGGTGGATTGT R: CAACCCCAAACCACAACCATAA	153	
HIN-1	Methylated	F:GTTTCTGGTTTTGTTCGGGTAGTC R: GCAAACCCCAAAAAAACGACG	140	[18]
	Unmethylated	F: AAGTTTTGGTTTGTTTGGTAGTT	146	

		R: CACACAACCCCAAAAAACAACA		
CYP2E1	Methylated	F: TCCGGGTCAAAGCGGGC R: CGTCAATTCCCATGCCCTTGC	139	[19]
	Unmethylated	F: GATGGGAGTTTGGGTTAAAG R:ACAACTCTCACAACTAAAATCAC	134	

2.1 Performing Methylation Analysis on Modified DNA Products Using the MS-HRM (Methylation-Sensitive High-Resolution Melting) Method

The methodology at this stage were performed using the LightCycler® 480 real-time PCR (Roche) instrument. PCR amplification and high-resolution melting analysis were carried out using the compatible 'LightCycler® 480 High-Resolution Melting Master' kit. The 50-cycle amplification stages applied according to the kit's user manual are specified below as bullet points. The HRM analysis stage was configured according to the recommended standards. For each gene, a separate plate was used. A total of 82 samples: 30 cancer, 14 Hp(-) gastritis, 23 Hp(+) gastritis, 15 post-treatment Hp(+) gastritis loaded into separate wells at 10 µl each. Positive/methylated control, negative/unmethylated control samples, and their %75, %50, %25 intermediate value samples. HRM mix and distilled water samples as negative controls for the reaction. Additionally, 10 µl of HRM master mix, 2 µl of primer F (forward) and 2 µl of primer R (reverse) (at a concentration of 10 µM), 2 µl of Mg^{2+} (at a concentration of 2.5 µM), and 1 µl of 'RNase-free' water were added to each well, reaching a total volume of 27 µl per well. The LightCycler® 480 real-time PCR (Roche) instrument was prepared and run within the framework of this protocol. After the process was completed, evaluations were performed using the instrument's own analysis software.

2.2 Assessing Host Genetic Polymorphisms Involved in Gastric Cancer

To understand how inherited genetic variations (called polymorphisms) in certain genes may influence the risk of developing gastric cancer, especially in relation to the genes already studied for DNA methylation [20], such as *CYP2E1, HIN-1, MT2A, CASP3, CDKN1A, MSH6,* and *EpCAM* - a detailed molecular approach would be used. Initially, DNA would be taken from blood samples using standard method. After making sure the DNA was suitable at particular parts of each gene, typically the parts where significant genetic changes are found. These SNPs would then be identified using reliable techniques such as PCR-RFLP, allele-specific PCR, or real-time PCR with TaqMan probes. For more detailed analysis, methods like Sanger sequencing or modern tools like microarrays and next-generation sequencing (NGS) may also be used. Once the genotypes are determined, the data would be analysed statistically to look for links between certain genetic variations and the presence of disease (gastritis or gastric cancer), as well as their relationship with *H. pylori* infection and methylation patterns. This combined approach could help pinpoint genetic factors that may increase the risk of gastric cancer in the Iraqi population.

2.3 Statistical analysis

Statistical analysis of the data was evaluated in SPSS 15 package program. Normal distribution analyses of the quantitatively evaluated data were performed. Mann-Whitney you and Wilcoxon *t*-tests were used to evaluate the data that did not show normal distribution. In addition, Spearman's correlation analysis and Chi^2 analysis were applied.

Result

Study groups were formed with 30 patients in the gastric cancer (intestinal type) group (5 patients with Hp - / 25 patients with Hp +) and 37 patients in the gastritis group (14 patients with Hp - / 23 patients with Hp +). The age distribution of the gastritis group (n=37) was between 21 and 75 years, with a mean age of 46.60 ± 2.39; the age distribution of the gastric cancer group (n=30) was between 36

and 79 years, with a mean age of 62.27 ± 2.05. Based on these data, a statistically significant difference was found between the gastritis and stomach cancer patient groups in terms of age (t= -4.85; SD= 65; p< 0.001). In the group of 23 Hp(+) gastritis patients who received medical treatment for Hp eradication, 15 patients completed their treatment and returned for follow-up during the study period. Of these 15 patients who underwent follow-up endoscopies, only 4 were found to have eradicated Hp, while the remaining 11 patients were found to have persistent Hp infection.

3.1. Analysis Of PCR Product Methylation Patterns

The genomic DNA sequences derived from tissue samples of the study participants undergo bisulfite modification. The goal of this process was to change unmethylated cytosine nucleotides into uracil nucleotides in the DNA sequence, which would set methylated cytosines apart. PCR amplification and high-resolution melting analysis were then performed using the LightCycler® 480 Real-Time PCR. The analysis data were evaluated against two databases: "Gene Scanning" and "Tm Calling," and similar results were obtained. Additionally, the device's "Difference Plot" database was used to analyze the graphical distribution of the samples based on methylation differences.

The analysis of the high-resolution melting data was performed using the LightCycler® 480 "Gene Scanning" software program. This evaluation is based on the different fluorescence levels between the samples. Normalized melting profiles are directly compared, and the DNA methylation level in the case samples is evaluated based on their similarity to the melting profiles of standards with known methylated/unmethylated ratios. In this algorithm, the melting temperature curves of the PCR products from the cases are compared with the melting temperature curves of control PCR products for which the ratio of unmethylated to methylated is known. The software graphs for the control and patient groups used in this evaluation are shown below (Figures 1–2). Comparisons were made between the individual patients and the groups collectively with the control curves to determine the methylation status of the relevant genes.

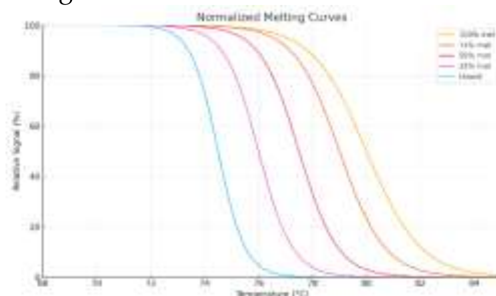

Figure 1. Melting curves for positive/methylated and negative/unmethylated control samples, and their 75%, 50%, and 25% intermediate samples. The temperature increases as the methylation rate increases.

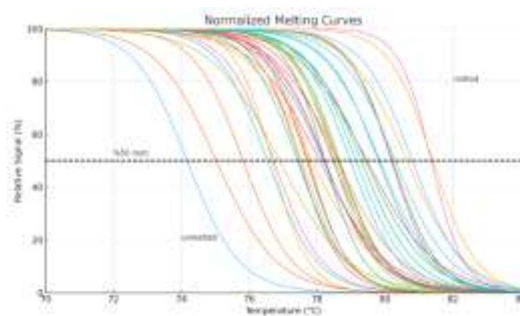

Figure 2. Overall view of the melting curves of the CASP3 gene for the studied sample groups.

Melting temperature data were also evaluated using the "Tm calling" software program. In this algorithm, the melting profiles of the samples differ depending on whether they are methylated or unmethylated. Products amplified from methylated DNA have higher Tm values due to the presence of CpG sequences in the amplicon. Unmethylated cytosine nucleotides are converted to uracil

nucleotides through bisulfite modification, resulting in lower Tm values. Due to the mixture of methylated and unmethylated DNA in the samples, peaks were obtained in the regions corresponding to two different temperature values in the "melting peak" graphs (Figures 3–4).

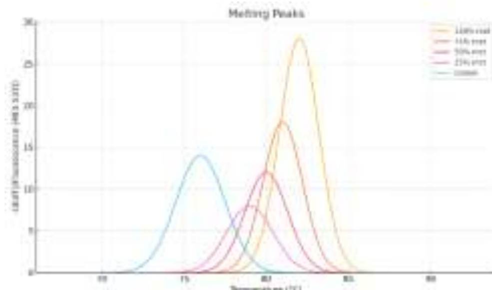

Figure 3. Melting curves of positive/methylated and negative/unmethylated control samples, and their 75%, 50%, and 25% intermediate samples, based on their fluorescence peaks.

Due to the mixture of methylated and unmethylated DNA, two peaks resulting from different melting temperatures are observed. The lower melting temperature indicates samples containing unmethylated cytosine nucleotides that have been converted to uracil nucleotides, while the higher melting temperature indicates methylated cytosine nucleotides. Relative differences in temperatures are also observed based on methylation rates.

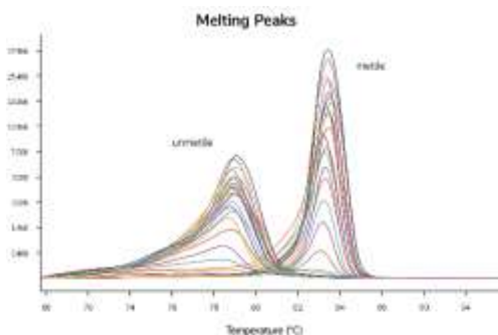

Figure 4. Melting curves of the studied sample groups based on their fluorescence peaks for the CASP3 gene.

The "difference plot" analysis program of the LightCycler® 480 Real Time PCR device groups the samples loaded onto the plate by marking them according to their sequence differences. As observed in Figures 5 and 6, the sequence differences between unmethylated and methylated samples are shown as graphical distributions. The data obtained from this graphical grouping of the two databases used in the study and the device were observed to be consistent.

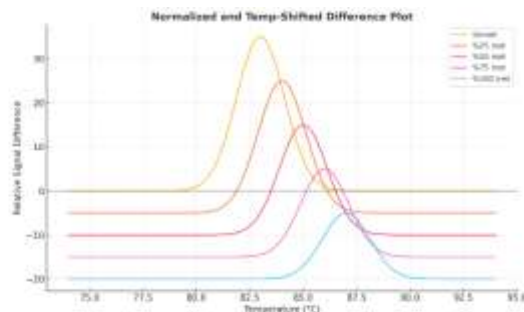

Figure 5. Melting curves of positive/methylated and negative/unmethylated control samples, and their 75%, 50%, and 25% intermediate samples, as grouped using the LightCycler® 480 Real Time PCR instrument's "difference plot" analysis program.

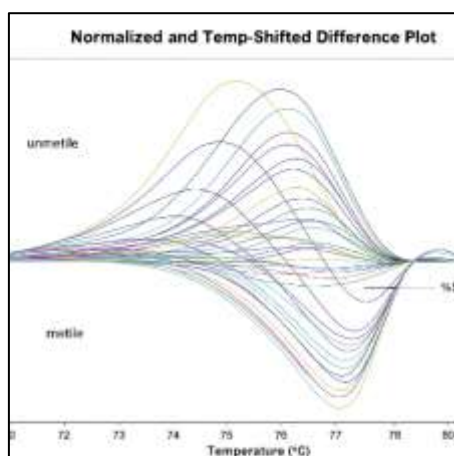

Figure 6. Melting curves of CASP3 gene samples from the studied sample groups, as grouped using the LightCycler® 480 Real Time PCR instrument's "difference plot" analysis program.

3.2 Aberrant methylation rates according to patient groups

In current study, tissue samples obtained from 37 gastritis and 30 gastric cancer patients were evaluated for the CYP2E1, HIN-1, MT2A, CASP3, CDKN1A, MSH6, and EpCAM genes.

When all cases were evaluated together, an abnormal methylation pattern was observed in at least one gene in all (100%) gastritis and gastric cancer cases. The number of cases with aberrant methylation in three or more genes was 34/37 in the gastritis patient group, while all 30 patients (100%) in the gastric cancer patient group had aberrant methylation in three or more genes. The average number of hypermethylated genes was 3.46 in the gastritis patient group and 4.87 in the gastric cancer patient group. Pre- and post-treatment methylation patterns were evaluated in only 15 patients in the gastritis patient group. When aberrantly methylated genes were compared in only 15 cases, a decrease in hypermethylation rates was observed in some genes. Conversely, although no aberrant methylation patterns were detected in some genes before treatment, hypermethylated patterns were observed in post-treatment samples. The same findings were obtained in repeat analyses of pre- and post-treatment samples (Figure 7) and (Table 2).

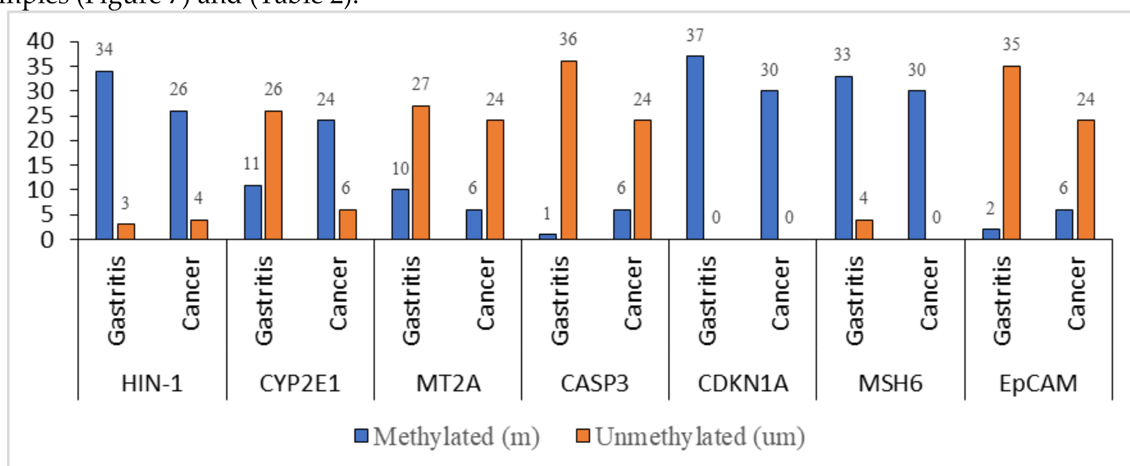

Figure 7: Column chart evaluation of aberrant methylation frequencies detected for the CYP2E1, HIN-1, MT2A, CASP3, CDKN1A, MSH6, and EpCAM genes in gastritis and gastric cancer cases. Table 4.1 Aberrant methylation frequencies detected for the CYP2E1, HIN-1, MT2A, CASP3, CDKN1A, MSH6, and EpCAM genes in gastritis and gastric cancer cases.

Table 2. Gene Methylation Frequencies of CYP2E1, HIN-1, MT2A, CASP3, CDKN1A, MSH6, and EpCAM in Gastritis and Gastric Cancer Patients With and Without *Helicobacter pylori* Infection

Group	CYP2E1	HIN-1	MT2A	CASP3	CDKN1A	MSH6	EpCAM
Gastritis (n = 37)							
Hp(+) Pre-treatment (n = 23)	3	22	7	1	23	22	1
Hp(+) Post-treatment (n = 15)	5	15	2	1	15	13	0
Hp(−) (n = 14)	8	12	3	0	14	11	1
Cancer (n=30)							
Hp(+) (n = 25)	19	21	6	6	21	25	4
Hp(−) (n = 5)	5	5	0	0	5	5	2

3.3. Methylation rates of the analysed genes in patient groups

CYP2E1 Gene Findings: Regarding the CYP2E1 gene, 29.7% of gastritis cases were hypermethylated, while hypermethylation was observed at a higher frequency in Hp(-) cases compared to Hp(+) cases (57.14% and 13.04%). Methylation rates were also observed to decrease in two cases examined after medical eradication therapy in Hp(+) cases. However, in the other three cases examined after treatment, no aberrant methylation was observed before treatment, while post-treatment analyses revealed gene hypermethylation at 100%, 50%, and 75%. Similar data were obtained in repeated analyses. Due to the high methylation rate observed after treatment in these three cases, the methylation level increased from 13.04% before treatment to 33.3%. In the remaining cases, no change was detected in their pre-treatment unmethylated status. CYPB1 gene hypermethylation was observed in 80% of gastric cancer cases. Hypermethylation of the gene was observed in all Hp(-) gastric cancer cases and in 76% of Hp(-) cases.

HIN-1 Gene Findings: HIN-1 was observed as a gene with high rates of hypermethylation in gastritis and gastric cancer patient groups. Aberrant methylation was observed in 91.9% of gastritis cases. This gene was hypermethylated in 22 of 23 Hp(-) cases (95.7%), while hypermethylation was detected in 85.7% of Hp(-) cases. Hypermethylation was detected at a rate of "100%" in all Hp(+) cases, while hypermethylation was detected at a rate of "100%" in nine Hp(-) cases, at a rate of "75%" in two cases, and at a rate of "50%" in one case. No change was observed in HIN-1 gene hypermethylation rates before and after treatment, and all control cases also showed a rate of "100%" methylation. HIN-1 gene hypermethylation was detected in 86.7% of gastric cancer cases. Hypermethylation was observed in all samples from Hp(-) cases, with methylation rates at rates of "50% or above." The average hypermethylation rate for the HIN-1 gene in Hp(+) gastric cancer cases was calculated as 84%.

MT2A Gene Findings: Aberrant methylation was observed in 27.02% of gastritis cases in the MT2A gene. Hypermethylation was detected in 30.4% of Hp(+) patient group samples, while hypermethylation was detected in 21.4% of Hp(-) patient group samples. In control analyses of Hp(+) patients after medical eradication treatment, no evidence of hypermethylation was found in samples where aberrant methylation was observed except for one case. In this case (G44), MT2A hypermethylation, observed at a rate of 25% before treatment, was hypermethylated at a rate of 50% in the post-treatment analysis. In another case (G42), where no aberrant methylation was observed before treatment, the gene was found to be hypermethylated at a rate of 25% after treatment. The same data were obtained after repeating the analyses. When comparing the methylation levels of Hp(+) gastritis patients before and after treatment, it was observed that the methylation level, which was 30.4% before treatment, decreased to 13.3%. In the gastric cancer patient group, MT2A aberrant methylation was detected in 20% of the cases, and all cases were from the Hp(+) gastric cancer group. No methylation changes were observed in this gene in the Hp(-) patients.

CASP3 Gene Findings: CASP3 gene hypermethylation was rarely observed in the gastritis patient group. CASP3 methylation was not detected in any of the Hp(-) gastritis patient group samples, while a "100% hypermethylated" CASP3 gene methylation pattern was observed in only one of the Hp(+) gastritis patient group samples. Because this patient did not return for follow-up, no assessment of his status after Hp medical eradication treatment could be made. In contrast, because 100% hypermethylated CASP3 was observed in the post-treatment samples of one patient (G35), whose CASP3 methylation was not detected before treatment, repeat analyses were performed on pre- and post-treatment samples, and similar findings were obtained.

CASP3 gene methylation alterations were positive in 86.7% of gastric cancer cases. CASP3 gene hypermethylation was observed in all Hp(-) gastric cancer cases (100%) and in 84% of Hp(+) gastric cancer cases. In the gastric cancer patient group, hypermethylation rates of 50% or more were detected in all cases. These were 50% methylation in 3/30 patients (10.0%), 75% methylation in 1/30 patients (3.3%), and 100% methylation in 22/30 patients (73.4%). In the Hp(-) gastric cancer group, the CASP3 gene was 100% hypermethylated in all five cases. In conclusion, from a general assessment, aberrant methylation of the CASP3 gene is particularly significant in the gastric cancer patient group.

CDKN1A Gene Findings: One of the most interesting findings in current study was aberrant hypermethylation of the CDKN1A gene. CDKN1A hypermethylation was observed at varying rates in all samples from both the gastritis and gastric cancer patient groups. Therefore, hypermethylation was observed at a 100% rate in these groups. In the majority of the Hp(+) patient group who came for follow-up after medical eradication treatment, no change in CDKN1A hypermethylation rates was observed. However, methylation rates were found to increase in three cases: one from 50 to 100 and two from 75 to 100. Aberrant methylation patterns related to the CDKN1A gene are observed at high rates in gastritis and stomach cancer cases. Hp eradication treatment was not found to have any effect on CDKN1A methylation rates.

MSH6 Gene Findings: The MSH6 gene is among the genes we examined with high rates of methylation abnormalities. 89.2% of the gastritis patients and 93.3% of the gastric cancer patients were methylated. The methylation rate among Hp(-) samples in the gastritis patient group was 78.6%, while this rate was 95.7% in the pre-treatment Hp(+) samples. After Hp eradication treatment, 86.7% methylation was observed. The MSH6 hypermethylation rate in gastric cancer patients was 93.3%, and it was 92.0% and 100% in the Hp(+) and Hp(-) gastric cancer patient groups, respectively. Conclusively, the MSH6 gene is a gene that is hypermethylated at a high frequency in both gastritis and gastric cancer patients. However, when compared with other genes with high aberrant methylation, the MSH6 gene shows lower rates of methylation.

EpCAM Gene Findings: Among the DNA repair genes, EpCAM stood out as the gene with the least aberrant methylation among the genes we examined. Hypermethylation was detected at a rate of 25% in only one patient in the Hp(+) gastritis patient group. A normal methylation pattern was observed in the control sample after medical treatment given due to Hp(+). Among Hp(-) gastritis cases, hypermethylation was observed at a rate of 25% in only one case (G14).

Aberrant methylation of the EpCAM gene was observed at a higher rate in the gastric cancer patient group than in the gastritis patient group. While an aberrant methylation pattern was observed in 20% of all gastric cancer cases, varying degrees of hypermethylation were detected in 4/25 Hp(-) gastric cancer cases and 2/5 Hp(-) gastric cancer cases.

In conclusion, EpCAM gene methylation abnormalities are observed at a higher frequency in gastric cancer cases. When all data are evaluated together, the methylation rates of genes across disease groups are summarized in Table 3.

Table 3. Methylation Rates (%) of Genes in Disease Groups

Disease Group	CYP2E1	HIN-1	MT2A	CASP3	CDKN1A	MSH6	EpCAM
Gastric Cancer (n = 30)	80.0	86.7	20.0	86.7	100.0	93.3	20.0
Hp(+) Gastric Cancer (n = 25)	76.0	84.0	24.0	84.0	100.0	92.0	16.0

Hp(−) Gastric Cancer (n = 5)	100.0	100.0	0.0	100.0	100.0	100.0	40.0
Gastritis (n = 37)	29.7	91.9	27.0	2.7	100.0	89.2	5.4
Hp(+) Gastritis (n = 23)	13.0	95.7	30.4	4.3	100.0	95.7	4.3
Hp(−) Gastritis (n = 14)	57.1	85.7	21.4	0.0	100.0	78.6	7.1
Post-treatment Hp(+) Gastritis (n = 15)	33.3	100.0	13.3	6.7	100.0	86.7	0.0

When the Hp(+) and Hp(-) patient groups were compared in the gastritis patient group consisting of a total of 37 cases, it was determined that CYP2E1 gene hypermethylation increased significantly in Hp(-) gastritis cases, whereas the increases in HIN-1 and MSH6 gene methylations in Hp(+) gastritis cases were significant (**Figure** 8. The mean rank, Z, and P values indicate significant methylation differences in CYP2E1 ($Z = -2.61$, $p < 0.05$), HIN-1 ($Z = -4.79$, $p < 0.001$), and MSH6 ($Z = -2.60$, $p < 0.05$) between groups. Other genes—MT2A, CASP3, CDKN1A, and EpCAM—showed no statistically significant variation between Hp(+) and Hp(−) groups.

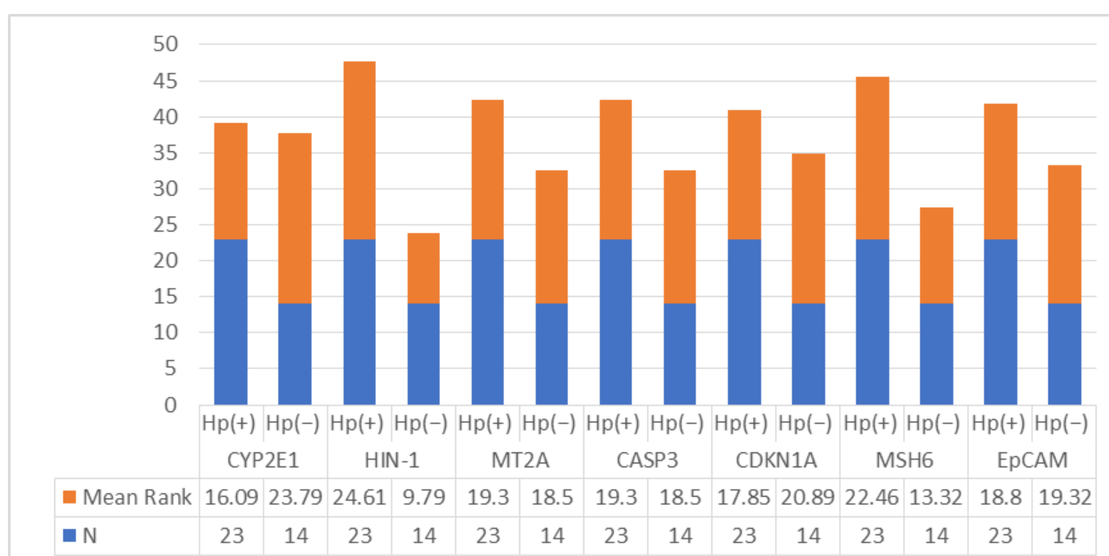

Figure 8. Statistical Analysis of Comparison Between Hp(+) and Hp(−) Gastritis Patient Groups

In Hp(+) gastritis cases, no significant difference was observed in gene methylation rates before and after Hp medical eradication therapy. The fact that Hp was eradicated in only four of the treated cases supports our data (Figure 9).

When we compared the methylation status of these four cases before and after Hp eradication therapy, no methylation was detected in the CYP2E1, CASP3, and EpCAM genes before or after treatment, while no change was detected in the methylation status of the HIN-1 and CDKN1A genes after treatment. In the MSH6 gene, methylation rates decreased in two cases after treatment, while methylation rates increased in the other two. In the MT2A gene, methylation, which was detected at 50% before treatment in three cases, was completely recovered to 0% after treatment, while no methylation was detected in the remaining case before or after. The comparison of mean ranks before and after *H. pylori* eradication in Hp(+) gastritis patients revealed no statistically significant changes in gene methylation. Although CDKN1A showed a noticeable increase ($Z = -1.90$, $p = 0.09$), all p-values exceeded 0.05, indicating that eradication therapy had limited effect on methylation profiles.

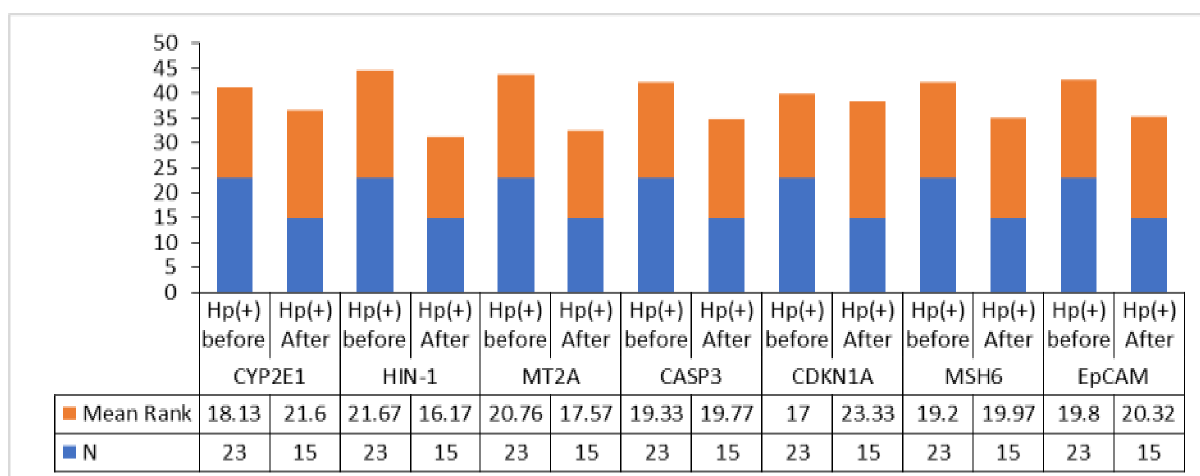

Figure 9. Statistical analysis of the comparison of Hp(+) gastritis cases before and after Hp medical eradication treatment

When the Hp(+) and Hp(-) patient groups were compared in a total of 30 gastric cancer patient group, no significant differences were found in terms of gene methylation between the Hp(+) and Hp(-) gastric cancer patient groups (Figure 10). In gastric cancer patients, no statistically significant methylation differences were observed between Hp(+) and Hp(−) groups across all genes studied. Although EpCAM showed a higher mean rank in Hp(−) cases ($Z = -2.31$, $p = 0.11$), the result was not significant ($p > 0.05$), suggesting minimal influence of Hp status.

When the gastric cancer and gastritis patient groups were compared with each other, it was determined that the CYP2E1, MT2A, CASP3, MSH6, and EpCAM gene methylation rates were significantly increased in the gastric cancer patient group (Figure 11). Statistical comparison between gastric cancer and gastritis groups revealed significantly higher methylation in gastric cancer for CYP2E1 ($p < 0.001$), MT2A ($p < 0.001$), CASP3 ($p < 0.05$), MSH6 ($p < 0.001$), and EpCAM ($p < 0.001$). No significant differences were observed for HIN-1 ($p = 1.000$) and CDKN1A ($p = 0.080$).

When the Hp(-) gastric cancer and Hp(-) gastritis patient groups were compared in terms of methylation rates of the analyzed genes, it was determined that the hypermethylation rates of the HIN-1, MT2A, and EpCAM genes were significantly increased in the Hp(-) cancer group (Figure 12). In Hp(−) groups, significant methylation differences were found for HIN-1 ($p < 0.05$), MT2A ($p < 0.001$), and EpCAM ($p < 0.001$), with higher levels in gastric cancer patients. No significant differences were noted for CYP2E1, CASP3, CDKN1A, or MSH6, though trends suggest elevated methylation in cancer cases.

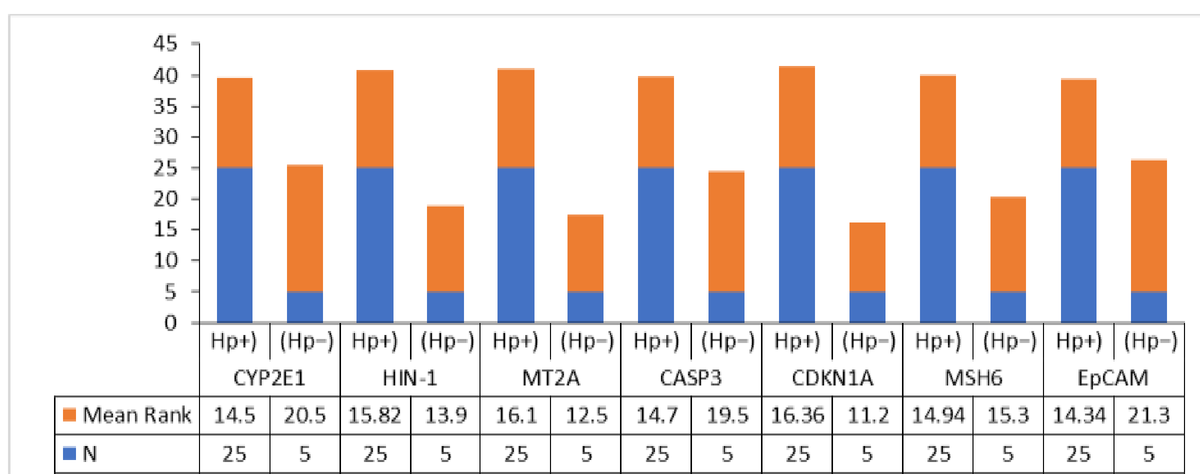

Figure 10. Statistical analysis of the comparison of the Hp(+) and Hp(-) patient groups (groups 1 and 2) in the gastric cancer patient group.

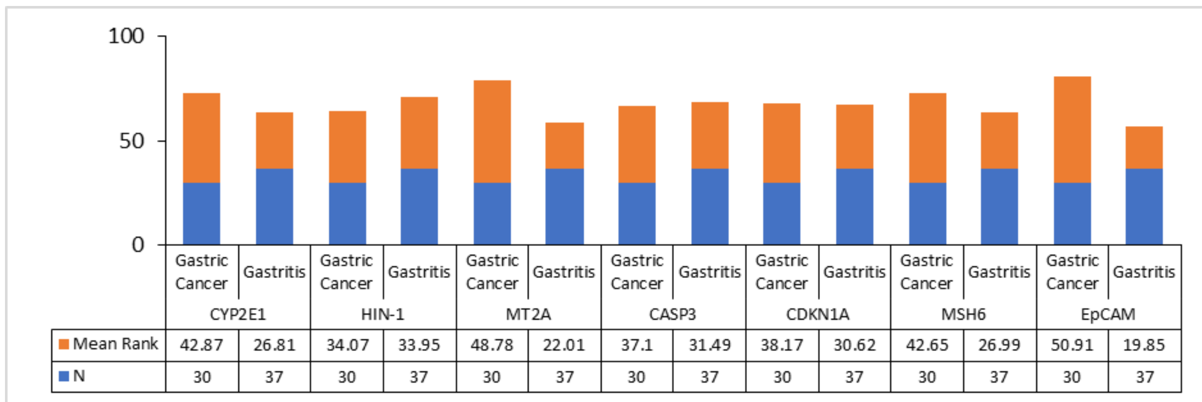

Figure 11. Statistical analysis of the comparison of the gastric cancer and gastritis patient groups

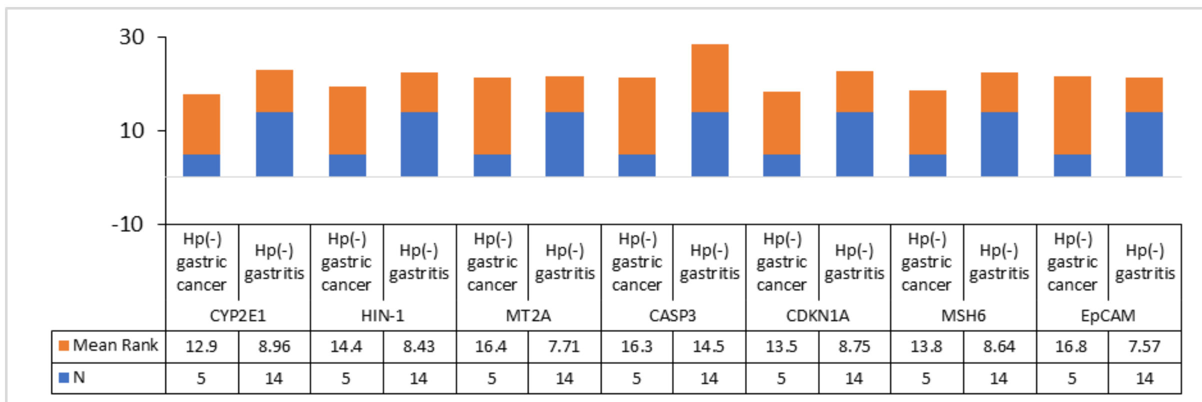

Figure 12. Statistical analysis comparing the Hp(-) gastric cancer and Hp(-) gastritis patient groups

When the Hp(+) gastric cancer and Hp(+) gastritis patient groups were compared in terms of methylation rates of the analyzed genes, it was determined that the hypermethylation rates of the CYP2E1, MT2A, MSH6, and EpCAM genes were significantly increased in the Hp(+) gastritis group. Conversely, hypermethylation of the HIN-1 gene ($p<0.05$) was found to be significant in the Hp(+) gastritis samples (Figure 13). In Hp(+) patients, gastric cancer cases showed significantly higher methylation for CYP2E1, MT2A, and EpCAM ($p < 0.001$), and MSH6 ($p < 0.05$). HIN-1 methylation was higher in gastritis ($p < 0.05$). CASP3 and CDKN1A showed no significant differences. These findings highlight distinct epigenetic alterations between cancer and gastritis in Hp(+) individuals.

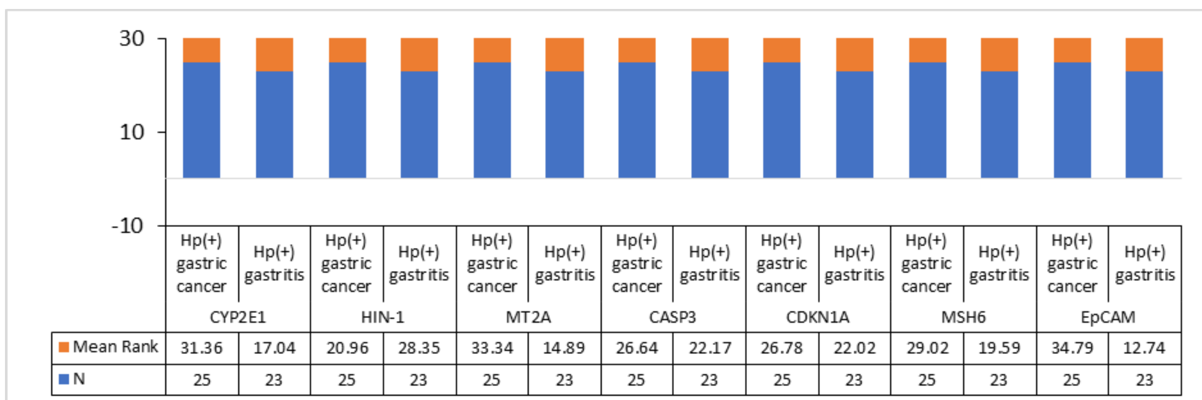

Figure 13. Statistical analysis comparing the Hp(+) gastric cancer and Hp(+) gastritis patient groups

The effects of smoking, chronic medication use, and the presence of Hp on methylation patterns in the CYP2E1, HIN-1, MT2A, CASP3, CDKN1A, MSH6, and EpCAM genes were investigated in

gastric cancer and gastritis patient groups. No statistically significant differences were found in the effects of these parameters on gene methylation patterns.

3.4. Comparison of aberrant Gene Number

In current study, we compared the aberrant methylation rates of seven genes in which methylation patterns were analyzed between the gastritis and stomach cancer patient groups (Figure 14). When all cases were evaluated together, aberrant methylation patterns were observed in 1-3 genes in 20 cases. Of these cases, 90% (18/20) were gastritis patients, while only two cancer patients had hypermethylation in one to three genes. Conversely, of the 47 cases with hypermethylation in four or more genes, 59.6% were gastric cancer patients, while 40.4% were gastritis patients. Hypermethylation was observed at a high frequency in five to six genes in the gastric cancer patient group. Statistically significant differences were found between the groups in terms of the number of genes with aberrant methylation patterns.

When the sample rates with methylation abnormalities in the genes evaluated in current study were compared between the gastritis patient group and the gastric cancer patient group, it was observed that the frequency of gastric cancer cases with aberrant methylation of the CYP2E1, CASP3, and EpCAM genes was significantly higher than that of gastritis patients.

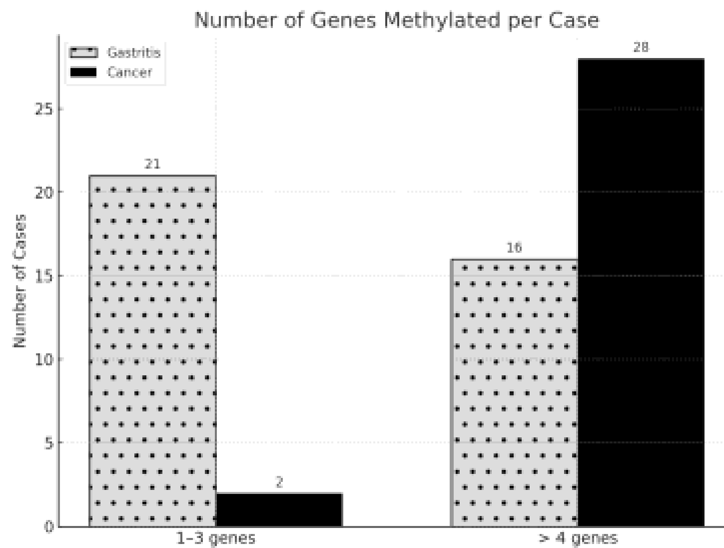

Figure 14. Column chart comparison of gastritis and stomach cancer cases based on the number of hypermethylated genes ($\chi^2 = 13.94$, $P < 0.001$)

3.5 Gene Methylation Quantification

When using the MS-HRM method, the positive/methylated control sample CpGenome™Universal Methylated DNA (Millipore-Chemicon ® International) and the negative/unmethylated control sample CpGenome™Universal Unmethylated DNA (Millipore-Chemicon ® International), whose quantitative methylation statuses were known, were also loaded into each plate well along with the patient group samples. The 25%, 50%, and 75% intermediate dilutions obtained by diluting these were used. The average of the quantitative methylation values of all study groups relative to the control samples was calculated using the software programs of the LightCycler ® 480 Real Time PCR (Roche) device (Table 4). Groups were evaluated in column charts based on these averages.

Table 4. Average Quantitative Methylation Rates of Genes According to Disease Groups (%)

Disease Group	CYP2E1	HIN-1	MT2A	CASP3	CDKN1A	MSH6	EpCAM
Gastric Cancer (n=30) (%)	52.5	76.7	6.7	80.8	73.3	38.3	10.8

Hp(+) gastric cancer (n=25) (%)	50.0	76.0	6.7	77.0	76.0	38.0	9.0
Hp(-) gastric cancer (n=5) (%)	65.0	80.0	0.0	100.0	60.0	40.0	20.0
Gastritis (n=37) (%)	12.2	89.2	12.2	2.7	78.4	48.0	2.2
Hp(+) gastritis (n=23) (%)	9.8	95.7	12.0	4.3	76.1	56.5	1.1
Hp(-) gastritis (n=14) (%)	37.5	78.6	12.5	0.0	82.1	33.9	1.8
Hp(+) gastritis after treatment (n=15) (%)	20.0	100.0	5.0	6.7	88.3	58.3	0.0

All data obtained for the Hp(+) / (-) gastritis, gastritis after Hp medical eradication therapy, and Hp(+) / (-) gastric cancer groups.

3.6 Association of Gene Methylation Patterns with Gastric Cancer and Gastritis

The results of the study reveal that specific gene methylation patterns are significantly associated with the presence of gastric cancer compared to gastritis. Genes such as *CYP2E1*, *MT2A*, *CASP3*, *MSH6*, and *EpCAM* showed notably higher methylation in gastric cancer tissues, indicating their potential involvement in Tumor development. For instance, *CYP2E1* was hypermethylated in 80% of cancer cases, compared to just 29.7% in gastritis. Similarly, *CASP3* and *EpCAM* exhibited high methylation frequencies in cancer but were rarely methylated in gastritis. Interestingly, the *CDKN1A* gene showed 100% methylation across both groups, suggesting it may be more broadly involved in gastric pathology rather than specific to cancer. While *H. pylori* infection influenced some genes like *HIN-1* and *MSH6*, eradication therapy had minimal impact on reversing methylation changes. Overall, these findings suggest a strong epigenetic contribution to gastric cancer, with certain genes serving as potential biomarkers for early detection and risk assessment. Table 5 presents the frequency of specific genetic variations (polymorphisms) in seven genes among patients with gastritis and gastric cancer. For each gene, the table shows how common different genetic types (genotypes) were in both groups. For example, the T/T genotype of the *CYP2E1* gene was more frequent in gastric cancer patients than in those with gastritis, with a statistically significant difference ($p = 0.042$). Similar trends were observed for other genes like *CASP3*, *CDKN1A*, and *EpCAM*, suggesting that certain genetic variants may increase the risk of developing gastric cancer.

Table 5. Gene Polymorphisms and Frequency Distribution

Gene	SNP ID (rs#)	Genotype	Gastritis Group (n = 37)	Gastric Cancer Group (n = 30)	P-value
CYP2E1	rs2031920	C/C	18 (48.6%)	10 (33.3%)	
		C/T	14 (37.8%)	12 (40.0%)	
		T/T	5 (13.5%)	8 (26.7%)	0.042*
HIN-1	rs1250566	G/G	22 (59.5%)	14 (46.7%)	
		G/T	10 (27.0%)	12 (40.0%)	
		T/T	5 (13.5%)	4 (13.3%)	0.381
MT2A	rs28366003	A/A	20 (54.1%)	12 (40.0%)	
		A/G	13 (35.1%)	11 (36.7%)	
		G/G	4 (10.8%)	7 (23.3%)	0.064
CASP3	rs4647603	C/C	28 (75.7%)	16 (53.3%)	
		C/A	8 (21.6%)	11 (36.7%)	
		A/A	1 (2.7%)	3 (10.0%)	0.033*
CDKN1A	rs1801270	C/C	19 (51.4%)	9 (30.0%)	
		C/A	14 (37.8%)	14 (46.7%)	
		A/A	4 (10.8%)	7 (23.3%)	0.048*
MSH6	rs1042821	G/G	25 (67.6%)	16 (53.3%)	
		G/A	9 (24.3%)	11 (36.7%)	

		A/A	3 (8.1%)	3 (10.0%)	0.591
EpCAM	rs1126497	T/T	30 (81.1%)	17 (56.7%)	
		T/C	6 (16.2%)	10 (33.3%)	
		C/C	1 (2.7%)	3 (10.0%)	0.027*

*Significant at $p < 0.05$

Table 6 highlights the relationship between specific gene polymorphisms and the likelihood of aberrant DNA methylation in those genes. It shows that certain genotypes - such as *CYP2E1* T/T, *MT2A* G/G, *CASP3* A/A, and *EpCAM* C/C—were more frequently associated with abnormal methylation patterns, which are often linked to gene silencing and cancer development. For instance, 87.5% of individuals with the *CYP2E1* T/T genotype showed methylation in this gene, indicating a potential genetic predisposition to methylation changes. Similarly, 100% of those with the *CASP3* A/A genotype had aberrant methylation, suggesting a strong link to gastric cancer risk. While *CDKN1A* also showed complete methylation across all genotypes, the association was not statistically significant, likely due to uniform methylation observed in all samples. These findings imply that specific genetic variants may influence how genes become epigenetically silenced, contributing to disease progression and offering insight into personalized risk profiling.

Table 6. Association of Genotypes with Aberrant Gene Methylation Frequency

Gene	Genotype	% with Aberrant Methylation	P-value
CYP2E1	T/T	87.5%	0.018*
MT2A	G/G	85.7%	0.029*
CASP3	A/A	100%	0.033*
CDKN1A	A/A	100%	ns
EpCAM	C/C	75.0%	0.041*

*p-value is statistically significant ($p < 0.05$); ns: not significant

Discussions

Gene silencing caused by DNA hypermethylation is frequently encountered in human cancers. In cancer cells, numerous genes are affected. The affected genes vary depending on the chemical and cancer type [21]. That is, in addition to some common genes, gene hypermethylations specific to tumor types are observed. Therefore, in methylation analyses, evaluating the hypermethylation patterns of genes specific to tumors will enable more effective implementation of important clinical applications such as early diagnosis, establishing links with clinical features, and treatment selection.

Gastric cancer is the third most commonly observed cancer type worldwide and ranks second in cancer-related deaths after lung cancer. The stomach is one of the organs where aberrant CpG island hypermethylation is frequently observed during cancer development or with advancing age. Although Kang et al. (2001) [22] have suggested that around 90 genes are inactivated in gastric cancers due to promoter region hypermethylation to date, Ushijima et al. have reported that many CpG islands are methylated in cancer and that the number of inactive genes reached 421±75 in AGS gastric cancer cell lines [23]. Considering that about 40% of the genes in the human genome contain promoters and the number of genes identified as hypermethylated in gastric cancers is limited, there are many genes in gastric cancer samples for which our knowledge about promoter region methylation patterns is insufficient.

H. pylori infection is an important etiological risk factor for gastritis and gastric cancer. It has been defined as a Group 1 carcinogen by the WHO. Chronic *H. pylori* infection increases the risk of gastric cancer by 5-6 times [23]. In the current study, the methylation patterns of *SCGB3A1*, *CYP1B1*, *MT1G*, *BCL2*, *P16*, *hMSH2*, and *hMLH1* genes were analysed and compared using the MS-HRM technique in patients diagnosed with acute gastritis who were Hp(+) and Hp(-). Patients diagnosed with Hp(+) gastritis were called back for control after medical eradication therapy. Although there is data in the literature with very different control periods, ranging from six weeks (10)

to 12 months [24], follow-up controls were performed in 15 patients who came within a 3–8-month period in our study. Eradication was detected in only 4 (26.7%) of the patients included in the study, and it was revealed that *H. pylori* infection continued in the other patients. If it is evaluated the methylation rates of the acute gastritis patient group as Hp(+) and Hp(−), as seen in Table 7, P16 aberrant methylation was observed in all (+) and (-) gastritis cases and at quantitatively high rates.

Table 7. Hypermethylation Frequencies in the Gastritis Group

Gene Name	Hp(+) Acute Gastritis (n = 23), % Methylation	Hp(−) Acute Gastritis (n = 14), % Methylation
CASP3	4.3%	0.0%
HIN-1	95.7%	85.7%
CYP2E1	13.0%	57.1%
MT2A	30.4%	21.4%
CDKN1A	100.0%	100.0%
MLH1	4.3%	7.1%
MSH2	95.7%	78.6%

No change was observed in either the frequency of occurrence among patients or the methylation rates in the post-treatment controls of Hp(+) gastritis cases. When it was comparing the current study data with the literature, it is observed that our rates are high in terms of frequency of occurrence of CDKN1A gene methylation. It believes that one of the main reasons for this difference stems from the variety of analysis methods used. Almost all studies in the literature have been conducted using the MS-PCR method. Also, it believes that the higher analytical sensitivity of the MS-HRM method used in our study, its ability to detect even very low methylation ratios such as 0.1%, is effective in these differences. The limited number of patients in this study is also an important factor. We think that the fact that the cases included in our study had acute gastritis may also have an effect on the methylation rates.

Wang et al. (2023) [25] used MS-PCR to detect CDKN1A methylation in 44.2% of Iranian gastric cancer patients, but not in healthy controls. They denied an H. pylori connection. Our gastric cancer data also revealed high methylation rates, but they were more common, possibly due to study design.

Yashima et al. (2022) [26] discovered no significant difference in CDKN1A methylation between Japanese gastric cancer and gastritis patients (55.8% versus 50%). Methylation was unaffected by Hp status. Our results of 100% methylation in all groups back up these findings and indicate that our detection method is more sensitive.

Wisnieski et al. (2017) [27] discovered CDKN1A methylation in 68% of non-cancerous tissue from Hp(+) gastric cancer patients, 25% in gastritis, and 63.6% in Hp(−) cancer cases. These findings suggest that CDKN1A hypermethylation may occur early in gastric cancer progression and be associated with molecular pathology rather than H. pylori infection. Our findings of elevated methylation in advanced cancers lend credence to this theory.

Lin et al. (2017) [28] found a link between CDKN1A methylation, infection duration, and lesion severity in a large Chinese cohort. This lends support to the idea that infection causes early-stage methylation, while environmental and genetic factors influence later stages. Our findings support CDKN1A's significance as an early epigenetic marker, especially in Hp(−) patients. Additional research on larger Hp(−) gastritis cohorts is necessary to determine clinical relevance.

Choe et al. [29] used MS-PCR to analyze 80 South Korean gastric cancer and 74 chronic gastritis samples in 2018. CDKN1A and EpCAM had significantly lower methylation rates in chronic gastritis (2.7% and 0%, respectively) and higher rates in gastric cancer (43.8% and 20%, respectively), implying that they may be cancer-associated methylation markers. Similar to Wong et al.'s 2003 Italian study, 57 dyspeptic patients were tested for methylation of five tumor-related genes, including CDKN1A and EpCAM. The 45 H. pylori-positive patients who received eradication therapy showed 27% CDKN1A methylation and 4% EpCAM methylation. After 12 months, 74% of patients experienced successful

eradication and partial methylation regression in their biopsy samples. These findings point to a link between H. pylori and CDKN1A promoter methylation.

Tang et al. (2012) [32] investigated CDKN1A methylation in Iranian gastric cancer patients using MS-PCR and found it in 44.2% of cancer cases, but not in any healthy controls. They reported no association with *H. pylori* infection. Similarly, our data showed high methylation rates in gastric cancer, although at a higher frequency, which we attribute to methodological differences.

Kim et al. (2002) discovered that Helicobacter pylori infection decreased EpCAM, PMS1, PMS2, and MSH6 levels in gastric cancer cell lines. After eradication, EpCAM and MSH6 levels returned to normal, indicating that the suppression could be reversed. Carvalho et al. (2003) looked at four genes, including EpCAM, in 51 Portuguese gastric cancer patients. Using PCR and methylation-specific PCR after methylation-sensitive restriction enzyme analysis, 37% of cases had EpCAM methylation. They discovered that hypermethylation-induced EpCAM suppression may result in gastric cancer cell microsatellite instability.

Park et al. (2005) investigated six genes in 60 gastritis and peptic ulcer patients from South Korea, including EpCAM and MSH6. They used immunohistochemistry to measure EpCAM and MSH6 protein levels in Hp-positive samples before and after treatment. They discovered that removing H. pylori activated these proteins, and a lack of them may exacerbate microsatellite instability and cancer.

Cuatrecasas et al. (2020) [32] investigated EpCAM and MSH6 protein expression in 100 Iranian dyspeptic patients (50 Hp-positive, 50 Hp-negative). They discovered lower EpCAM expression in Hp-positive individuals, indicating Hp-related gene silencing. MSH6 hypermethylation was common in this study, but EpCAM methylation was uncommon (25%). One patient's methylation levels for both genes decreased slightly after H. pylori was eradicated, supporting previous research. Compared to gastritis, gastric cancer patients had significantly higher EpCAM and MSH6 methylations. These findings lend support to the hypothesis that DNA repair gene methylation causes gastric cancer.

Kim et al. [33] used Methylated to investigate 25 genes in South Korean gastric cancer and gastritis patients in 2024. Both studies shared CYP2E1, CASP3, and EpCAM. CYP2E1 has been linked to cancer, with Hp-negative cancer patients having more methylated genes than Hp-negative gastritis patients. Hp-positive cancer patients had more methylation of CYP2E1 and CASP3. They found no EpCAM methylation in Hp-positive cancer, whereas we did. This could be explained by the sample size, tumor heterogeneity, or detection methods.

The findings revealed that Hp-negative gastritis had higher CYP2E1 methylation than Hp-positive. Cancer had increased methylation of CYP2E1, CASP3, MSH6, and EpCAM, whereas gastritis had elevated HIN-1 and MT2A. All samples contained methylated CDKN1A. Despite being limited (4/15 cases), Hp eradication reduced MT2A, MSH6, and EpCAM methylation. As previously reported, we discovered frequent methylation of the DNA mismatch repair genes MSH6 and EpCAM, which supports their role in microsatellite instability and gastric cancer progression. Although EpCAM is frequently overexpressed, our hypermethylation results indicate a different regulatory mechanism. Previous findings of early and stable epigenetic modifications correspond to ongoing CDKN1A and HIN-1 methylation. Findings indicate that H. pylori-induced methylation contributes to the pathogenesis of gastric cancer and highlights critical genes for diagnostic and therapeutic strategies.

In the present study, the hypermethylated findings of both repair genes in the gastritis and gastric cancer groups are consistent with the literature. However, a finding that draws our attention is that hMSH2 gene hypermethylation increases especially in the gastritis group, and hMLH1 gene hypermethylation increases with hMSH2 in the stomach cancer group. We believe that this finding confirms the relationship between increased microsatellite instability and inactivation of both repair genes in cancer. However, we believe that expression data should be evaluated in the large research group. Overall, findings agree with existing literature and reinforce the importance of CDKN1A as an early epigenetic marker, particularly in Hp(−) patients. Further studies in broader Hp(−) gastritis groups are warranted to clarify its clinical significance

Conclusion

This study underscores the significant role of Helicobacter pylori infection and alterations in gene activity via DNA methylation in the pathogenesis of gastric cancer. The MS-HRM technique we employed for methylation analyses is a comparatively recent methodology. The present study effectively utilizes the MS-HRM method for all samples. It is a quick, cheap method that is very sensitive to analysis. To sum up, problems with methylation are a big part of what causes gastritis and gastric cancer. Researchers found that cancer patients had more genes that were turned off (methylated) than people who only had gastritis. These genes were CYP2E1, MT2A, CASP3, MSH6, and EpCAM. This indicates that these methylation alterations may serve as preliminary indicators of cancer progression. It is interesting that all of the patients had their CDKN1A gene turned off, whether they had cancer or gastritis. This suggests that the gene may play a role in both early and late stages of the disease. The research additionally indicated that specific genetic variants may predispose individuals to these methylation alterations. However, this study, along with many others, analysed a limited number of samples, and quantitative methylation assessments remain significantly deficient. These analyses must be conducted in more extensive populations, alongside gene-specific expression analyses, and methylation results should be juxtaposed with clinical data. Finding molecular biomarkers in this kind of cancer, where there hasn't been a big breakthrough in treatment options yet, will be very important. The results suggest that gene methylation testing, particularly for CYP2E1, CASP3, and EpCAM, should be regarded as a method for the early detection of gastric cancer. Subsequent investigations involving more extensive patient cohorts should incorporate both methylation and gene expression analyses to enhance the understanding of how these alterations contribute to cancer and their potential applications in diagnosis and prevention.

REFERENCES

[1] A. Torgovnick and B. Schumacher, “DNA repair mechanisms in cancer development and therapy,” *Frontiers in Genetics*, vol. 6, Art. no. 157, 2015, doi: 10.3389/fgene.2015.00157.

[2] R. He *et al*., “Mechanisms and cross-talk of regulated cell death and their epigenetic modifications in tumor progression,” *Molecular Cancer*, vol. 23, no. 1, Art. no. 267, 2024, doi: 10.1186/s12943-024-02172-y.

[3] R. X. Huang and P. K. Zhou, “DNA damage response signaling pathways and targets for radiotherapy sensitization in cancer,” *Signal Transduction and Targeted Therapy*, vol. 5, Art. no. 60, 2020, doi: 10.1038/s41392-020-0150-x.

[4] V. E. Reyes, “Helicobacter pylori and its role in gastric cancer,” *Microorganisms*, vol. 11, no. 5, Art. no. 1312, 2023, doi: 10.3390/microorganisms11051312.

[5] S. Salvatori *et al*., “Helicobacter pylori and gastric cancer: Pathogenetic mechanisms,” *International Journal of Molecular Sciences*, vol. 24, no. 3, Art. no. 2895, 2023, doi: 10.3390/ijms24032895.

[6] L. E. Wroblewski, R. M. Peek Jr., and K. T. Wilson, “Helicobacter pylori and gastric cancer: Factors that modulate disease risk,” *Clinical Microbiology Reviews*, vol. 23, no. 4, pp. 713–739, 2010, doi: 10.1128/CMR.00011-10.

[7] A. Ali and K. I. AlHussaini, “Helicobacter pylori: A contemporary perspective on pathogenesis, diagnosis and treatment strategies,” *Microorganisms*, vol. 12, no. 1, Art. no. 222, 2024, doi: 10.3390/microorganisms12010222.

[8] I. J. Choi *et al*., “Helicobacter pylori therapy for the prevention of metachronous gastric cancer,” *New England Journal of Medicine*, vol. 378, no. 12, pp. 1085–1095, 2018, doi: 10.1056/NEJMoa1715304.

[9] C. Zhang *et al*., “Helicobacter pylori and gastrointestinal cancers: Recent advances and controversies,” *Clinical Medicine Insights: Oncology*, vol. 18, Art. no. 11795549241234636, 2024, doi: 10.1177/11795549241234636.

[10] S. Samsø Mathiasen *et al*., “Methylation levels assessment with methylation-sensitive high-resolution melting (MS-HRM),” *PLoS ONE*, vol. 17, no. 9, Art. no. e0273058, 2022, doi: 10.1371/journal.pone.0273058.

[11] D. Hussmann and L. L. Hansen, "Methylation-sensitive high-resolution melting (MS-HRM)," in *Methods in Molecular Biology*, vol. 1708, pp. 551–571, 2018, doi: 10.1007/978-1-4939-7481-8_28.
[12] A. Yakovlev *et al*., "Epigenetic regulation of caspase-3 gene expression in rat brain development," *Gene*, vol. 450, nos. 1–2, pp. 103–108, 2010, doi: 10.1016/j.gene.2009.10.008.
[13] A. Zarandi *et al*., "Evaluation of promoter methylation status of MLH1 gene in Iranian patients with colorectal tumors and adenoma polyps," *Gastroenterology and Hepatology from Bed to Bench*, vol. 10, Suppl. 1, pp. S117–S121, 2017.
[14] T. Takeda *et al*., "Methylation analysis of DNA mismatch repair genes using DNA derived from peripheral blood of patients with endometrial cancer," *Genes*, vol. 7, no. 10, Art. no. 86, 2016, doi: 10.3390/genes7100086.
[15] S. Titze *et al*., "Differential MSH2 promoter methylation in blood cells of neurofibromatosis type 1 patients," *European Journal of Human Genetics*, vol. 18, no. 1, pp. 81–87, 2010, doi: 10.1038/ejhg.2009.129.
[16] M. Hedayati *et al*., "PTK2 and MT2A gene expression in gastritis and gastric cancer patients with Helicobacter pylori infection," *Canadian Journal of Gastroenterology and Hepatology*, Art. no. 8699408, 2022, doi: 10.1155/2022/8699408.
[17] M. K. Kim *et al*., "Regulation of the cyclin-dependent kinase inhibitor 1A gene (CDKN1A) by the repressor BOZF1," *Journal of Biological Chemistry*, vol. 288, no. 10, pp. 7053–7064, 2013, doi: 10.1074/jbc.M112.416297.
[18] R. Gill *et al*., "Molecular detection of H1N1 virus by conventional RT-PCR coupled with nested PCR," *Sensors International*, vol. 3, Art. no. 100178, 2022, doi: 10.1016/j.sintl.2022.100178.
[19] R. Y. Wang *et al*., "CYP2E1 alters biological function of gastric cancer cells via the PI3K/Akt/mTOR signaling pathway," *Molecular Medicine Reports*, vol. 21, no. 2, pp. 842–850, 2020, doi: 10.3892/mmr.2019.10890.
[20] U. Teodorczyk *et al*., "Mutations and polymorphisms of genes moderately increase gastric cancer risk," *Hereditary Cancer in Clinical Practice*, vol. 10, Suppl. 3, Art. no. A22, 2012, doi: 10.1186/1897-4287-10-S3-A22.
[21] M. Idris *et al*., "Promoter hypermethylation of neural-related genes is compatible with stemness in solid cancers," *Epigenetics & Chromatin*, vol. 16, no. 1, Art. no. 31, 2023, doi: 10.1186/s13072-023-00505-7.
[22] G. H. Kang *et al*., "CpG island methylation in premalignant stages of gastric carcinoma," *Cancer Research*, vol. 61, pp. 2847–2851, 2001.
[23] T. Ushijima, T. Nakajima, and T. Maekita, "DNA methylation as a marker for the past and future," *Journal of Gastroenterology*, vol. 41, no. 5, pp. 401–407, 2006.
[24] W. K. Leung *et al*., "Effects of Helicobacter pylori eradication on methylation status of the E-cadherin gene," *Clinical Cancer Research*, vol. 12, no. 10, pp. 3216–3221, 2006.
[25] X. Wang *et al*., "DNA methylation-driven pathways in gastric cancer early detection," *Genes & Diseases*, vol. 11, no. 2, pp. 847–860, 2023, doi: 10.1016/j.gendis.2023.02.038.
[26] K. Yashima *et al*., "Gastric cancer screening in Japan: A narrative review," *Journal of Clinical Medicine*, vol. 11, no. 15, Art. no. 4337, 2022, doi: 10.3390/jcm11154337.
[27] F. Wisnieski *et al*., "CDKN1A histone acetylation and gene expression in gastric adenocarcinomas," *Clinical and Experimental Medicine*, vol. 17, no. 1, pp. 121–129, 2017, doi: 10.1007/s10238-015-0400-3.
[28] Y. Lin *et al*., "Expression and prognostic significance of CDKN1A in resected gastric adenocarcinoma," *Oncology Letters*, vol. 14, no. 6, pp. 7473–7482, 2017, doi: 10.3892/ol.2017.7107.
[29] C. Choe *et al*., "SOX2 induces progression of NSCLC A549 cells toward anchorage-independent growth and chemoresistance," *OncoTargets and Therapy*, vol. 11, pp. 6197–6207, 2018, doi: 10.2147/OTT.S175810.
[30] T. S. Wong *et al*., "p16 and p15 gene methylation in head and neck squamous cell carcinoma," *European Journal of Cancer*, vol. 39, no. 13, pp. 1881–1887, 2003, doi: 10.1016/S0959-8049(03)00428-3.
[31] W. Tang *et al*., "Epstein–Barr virus-infected gastric adenocarcinoma exhibits distinct viral and human gene expression profiles," *Infectious Agents and Cancer*, vol. 7, Art. no. 21, 2012, doi: 10.1186/1750-9378-7-21.

[32] M. Cuatrecasas *et al*., "Complete loss of EPCAM immunoexpression identifies EPCAM deletion carriers in MSH2-negative colorectal neoplasia," *Cancers*, vol. 12, no. 10, Art. no. 2803, 2020, doi: 10.3390/cancers12102803.
[33] M. J. Kim *et al*., "Combined DNA methylation and gastric microbiome markers predict Helicobacter pylori-negative gastric cancer," *Gut and Liver*, vol. 18, no. 4, pp. 611–620, 2024, doi: 10.5009/gnl230348.