RESEARCH ARTICLE

**Editorial Process:** Submission:02/27/2023 Acceptance:12/06/2023

# Methylation Status of *CDH1* Gene in Gastric Biopsy Specimens and Its Association with *H. Pylori* Infection

**Karima Ali Hamid[1], Rayan Sidig Adam Abdalgalil[1], Yousif Abdelhameed Mohammed[2], Rania Mahgob[1], Khalid Abdelsamea Mohamedahmed[3]*, Bakri Yousif Mohamed Nour[4]**

## Abstract

**Background:** *Helicobacter pylori (H. pylori)* have been accepted as having an etiologic role in gastro-duodenal diseases as chronic gastritis, peptic ulcer, and gastric carcinoma. Methylation of CGI has been correlated with the tumorigenic process since it can inactivate tumor suppressor genes. *CDH1* is a tumor suppressor gene that encodes the E-cadherin protein, which is preserving cell-cell connections. Early stages of gastric carcinogenesis may be affected by the promoter methylation-mediated inactivation of this gene. **Objective:** This study aimed to investigate the methylation status of *CDH1* using Methylation-Specific PCR (MSP) technique in clinical suspected patients with *H. pylori* infection who undergoing upper gastrointestinal endoscopy and correlated it with *H. pylori* detection by glmM PCR test. **Methods:** Fifty gastric mucosal biopsies were selected from one hundred and five samples included in this study. The detection of *H. pylori* was performed with the PCR primers specific to *glmM* gene. Bisulfite modification was done and the methylation status of the *CDH1* gene was detected using MSP reaction. **Results:** *H. pylori* was detected in 36% (18/50) of study population using glmM gene PCR test, 89% (16/18) of *H. pylori* positive cases were *CDH1* methylated positive (chi-square, p-value=0.002). *CDH1* methylation can be present in cancerous and noncancerous gastric mucosa, where 60% (18/30) of *CDH1* methylation positive gastric mucosa showed gastritis as an endoscopy finding and gastric cancer in 6% (2/30). There was a significant correlation between and *CDH1* methylation positive results and age group (P-value = 0.02). There was no significant correlation between *CDH1* methylation positive results and participants gender (p-value=0.431) and clinical symptoms (all P-value > 0.05). **Conclusion:** This work suggested strong significance association between *H. pylori* infection and *CDH1* methylation.

**Keywords:** *CDH1* methylation- gastric cancer- *H. Pylori*

*Asian Pac J Cancer Prev,* **24 (12)**, 4071-4076

## Introduction

In mammals, DNA methylation occurs when a methyl or hydroxymethyl group is added to the cytosine at position C5, forming 5-methylcytosine., It mostly only occurs in CpG dinucleotide (Kurdyukov and Bullock, 2016; Schubeler, 2015). DNA methylation is a major epigenetic mechanism and plays a critical part in numerous important biological processes such as embryonic development, gene imprinting, and gene expression regulation. Aberrant DNA methylation has been demonstrated to be a mechanism for the development of many malignancies and is linked to further complicated disorders. (e.g diabetes and cardiovascular diseases, neurodegenerative and psychiatric disorders) (Jin and Liu, 2018; Nakajima et al., 2006).

Methylation of CpG islands has been attributed to the tumorigenic process because it can inactivate tumor suppressor genes (Zhang et al., 2014). Therefore, DNA methylation profiles are considered as markers for disease assessment, prognosis, and response to treatment (Šestáková et al., 2019).

Gastric cancer (GC) is one of the most prevalent cancers in the world; it continues to be the major cause of cancer death in Asia and certain European countries. As the main cause of death among malignant tumors, gastric carcinoma has an average yearly mortality rate of up to 16 per 100,000 people (Jemal et al., 2004). Gastric cancer is known to be associated with tumor-suppressor or tumor-related genes that are

*[1]Department of Histopathology and Cytopathology, Faculty of Medical Laboratory Sciences, University of Gezira, Wad Medani, Sudan. [2]Department of Clinical Chemistry, Faculty of Medical Laboratory Sciences, University of Gezira, Wad Medani, Sudan. [3]Department of Hematology and Immunology, Faculty of Medical Laboratory Sciences, University of Gezira, Wad Medani, Sudan. [4]Department of Medical Parasitology, Faculty of Medical Laboratory Sciences, University of Gezira, Wad Medani, Sudan. *For Correspondence: khalid.gu89@gmail.com*

inactivated more frequently by CGI hyper methylation than by mutations. Additionally, CGI hyper methylation has been observed in the non-neoplastic gastric mucosa of persons without GC as well as in the surrounding non-cancerous tissues of GC patients (Shin et al., 2010). *Helicobacter pylori* colonize the human gastrointestinal tract and has been acknowledged as contributing to the development of gastro duodenal disorders such chronic active gastritis, peptic ulcers, and gastric cancer. It has been classified by the International Agency for Research on Cancer (IARC) as a grade-I carcinogen (Ohata et al., 2004; Ahsan et al., 2008; Fakhrjou et al., 2011). Numerous publications have revealed a connection between certain gene promoter methylation and *H. pylori* infection (Perri et al., 2007; Kaise et al., 2008). Promoter CGI hypermethylation appears to be triggered by active inflammation due to *H. pylori* (Ushijima, 2007; Chan et al., 2006; Qian et al., 2008). It is not yet known, nevertheless, whether persistent aberrant DNA hypermethylation will remain after the active *H. pylori* infection stops (Shin et al., 2008). *CDH1* is a tumor suppressor gene that encodes the E-cadherin protein, which is a critical in maintaining cell-cell contacts. The chance of metastasis may rise if the *CDH1* gene is inactivated. A calcium-dependent transmembrane adhesion glycoprotein called E-cadherin is codified by *CDH1* (Tamura, 2002). Epithelial cells produce the tumor suppressor gene *CDH1*, which is crucial for maintaining tissue integrity and cellular activities such as adhesion, morphology, migration, and development (Ashour et al., 2002). The inactivation of *CDH1* gene is related with cancer growth and involving in invasion and metastasis and it is seen in a variety of cancers, including gastric cancer (Bartchewsky et al., 2009). Gastric cancer of the diffuse histological type carries a *CDH1* mutation in about 50% of cases. Moreover, *CDH1* hyper methylation was discovered to be the second factor in two kin with familial gastric cancer and a germ line *CDH1* mutation (Kague et al., 2010). This study aimed to investigate the methylation status of *CDH1* using MSP technique in clinical suspected patients with *H. pylori* infection who undergoing upper gastrointestinal endoscopy and correlated it with *H. pylori* detection by glmM gene PCR method.

## Materials and Methods

*Patients and specimens*

Fifty gastric mucosal biopsies from 105 clinically suspected patients for *H. pylori* infection were included in this study. The samples selected according to concentration of DNA, which should be more than 200 µg. The specimens were collected from endoscopy unit, Wad Medani Teaching Hospital, Gezira state, Sudan, between March 2018 and December 2019. The presence of *H. Pylori* was detected by: histopathology and (*glmM*) gene PCR analysis in gastric biopsies. In histopathology we used hematoxylin and eosin stain and Warthin-Starry stains. We calculated the sensitivity, specificity, negative predictive value and positive predictive value of each method for detection of *H. Pylori*. We depended on glmM gene molecular method as a gold standard for detection of *H. pylori* (the sensitivity was 92%). All molecular analyses were performed in the Molecular Biology Laboratory, Faculty of Medical Laboratory Sciences, University of Gezira.

*Molecular identification*

*DNA extraction and PCR of glmM gene*

The innuPREP DNA Mini Kit DNA extraction protocol (analyticjena, Germany, LOT:023-17, REF:845-KS-1040050) was followed for extraction of DNA from gastric biopsy. Extracted genomic DNA with concentration more than 200 µg was amplified using glmM H pylori specific primers. Primer sequences, amplicon sizes and the annealing temperature are showed in Table 1. PCR reactions were performed in a volume of 20 µL containing 5 µL genomic DNA, 1.5 µL forward primer (10µM in concentration), 1.5 µL reverse primer (10µM in concentration), 5 µL master mix and 7µl Deionized sterile water. Cycling for primers was as follows: primary denaturation (94°C for 5 min) then 30 cycles of alternating denaturation (94°C for 45 sec), annealing of primers (60°C for 45 sec) and extension by the thermo-stable polymerase (72°C for 1 min) with final extension of 10 min. The resulted amplicon was visualized by UV in a 2% agarose gel electrophoresis method.

*CDH1 methylation technique*

*Bisulfite modification*

Purified DNA was subjected to bisulfite modification. Bisulfite treatment converts unmethylated cytosine in uracil and methylated cytosine is unchanged. Briefly, 20 µl of DNA sample was added to 130 µl of the CT Conversion Reagent in a PCR tube then Placed in a thermal cycler at 98°C for 10 minutes followed by 64°C for 2.5 hours. 600 µl of M-Binding Buffer was added to a Zymo-Spin™ IC Column and place the column into a provided Collection Tube then Loaded the sample into the Zymo-Spin™ IC Column containing the M-Binding Buffer. Centrifuge at full speed (>10,000 x g) for 30 seconds. Discard the flow-through. 100 µl of M-Wash Buffer was added to the column. Centrifuge at full speed for 30 seconds. 200 µl of M-Desulphonation Buffer was added to the column and let stand at room temperature (20-30°C) for 15-20 minutes, centrifuge at full speed for 30 seconds. 400 µl of M-Wash Buffer was added to the column. Centrifugation and elution the DNA by adding 10 µl of M-Elution Buffer directly to the column matrix. Centrifuge for 30 seconds at full speed. The DNA was stored below -20°C. (ZYMO RESEARCH CORP. EZ DNA Methylation-Gold™ Kit Catalog Nos. D5005 & D5006).

*MSP (methylation-specific PCR) for CDH1 gene*

Bisulfite-modified DNA was amplified in two PCR reactions. The first reaction with primers specific for methylated gene, and the second one for unmethylated gene. All primer sequences, product size and annealing temperatures were summarized in Table 1. PCR was performed in 20µL reaction volumes, containing 1µl of Bisulfite-modified DNA, master mix 4µl, foreword primer 0.5µl, reveres primer 0.5µl and the reaction volume was

Table 1. Primer Sequences, Amplicon Sizes and the Annealing Temperature Used in This Study

| Primer | Primer sequence (5′-3′) forward Primer sequence (5′-3′) reverse | Product size (bp) | Annealing T (°C) | Reference |
|---|---|---|---|---|
| glmM | 5'- AAGCTTTTAGGGGTGTTAGGGGTTT-3′<br>5′-AAGCTTACTTTCTAACACTAACGC-3′ | 294 | 60 | (Bickley et al., 1993) |
| E-cadherin methylated | 5'-TTA-GGT-TAG-AGG-GTT-ATC-GCG-T-3′<br>5'-TAA-CTA-AAA-ATT-CAC-CTA-CCG-AC– 3' | 115 | 62 | (Kague et al., 2010) |
| E-cadherin unmethylated | 5'- TAA-TTT-TAG-GTT-AGA-GGG-TTA-TTG-T– 3'<br>5'- CAC-AAC-CAA-TCA-ACA-ACA-CA– 3' | 97 | 58 | |

completed to 20µl by adding 14µ Deionized sterile water. Amplicons were screened using 2% agarose gel and gel documentation system.

*Statistical analysis*

Statistical analysis was performed using SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for windows, Version18.0 Armonk, NY: IBM Corp)

The descriptive statistics of categorical variables were presented as frequency and percentage. Chi-squared tests were used for categorical variables. Associations between variables were examined by using Pearson correlation.

## Results

*Patient characteristics*

This study included fifty participants from a total of one hundred and five clinically suspected patients for *H. pylori* infection who undergoing upper gastrointestinal endoscopy. Among these, 46% were women and 54% were men. The age range between 20- 72 years with the mean age 47 years and SD 16.

Most study population suffering from Epigastric pain (76%) followed by vomiting in 44% of patients followed by lack of appetite in 38%, gastric reflex and nausea in 32%, other symptoms were founded among participants includes (black feces, chest pain, vomiting of blood and dysphagia). Endoscopy findings diagnosed 62% of patients as chronic gastritis, 22% had esophagitis, 6%, had stomach cancer, 4% had duodenal ulcer and normal endoscopy result in 6%.

*Detction of H. pylori in the study population using glmM gene*

Detection of *H. pylori* was found in 18 (36%) of study population, 94% of them show gastritis as an endoscopy finding and 6% showed gastric cancer.

*Methylation status of CDH1 Gene*

Using MSP technique the methylation of *CDH1* using methylated primers was observed in 30 (60%) of study samples while 20 (40%) of study samples showed negative results. Unmethylated primers gave positive results in 10 (20%) of study samples (Figure 1). Applying cross tabulation analysis: 27 samples gave positive results with methylated primer and negative to unmethylated primer, 7 samples positive with unmethylated and negative with methylated primer. 3 samples gave positive results with both primers, 13 samples negative with the both primers (Table 2).

*CDH1 methylation and H. pylori*

Among *H. pylori* positive cases 89% (16 from 18) were *CDH1* methylated primer positive (p = 0.002). 44% (14 from 32) showed positive *CDH1* methylation

Table 2. Methylated * Unmethylated Cross Tabulation

| | | Unmethylated | | Total |
|---|---|---|---|---|
| | | Positive | Negative | |
| Methylated | Positive | 3 | 27 | 30 |
| | Negative | 7 | 13 | 20 |
| Total | | 10 | 40 | 50 |

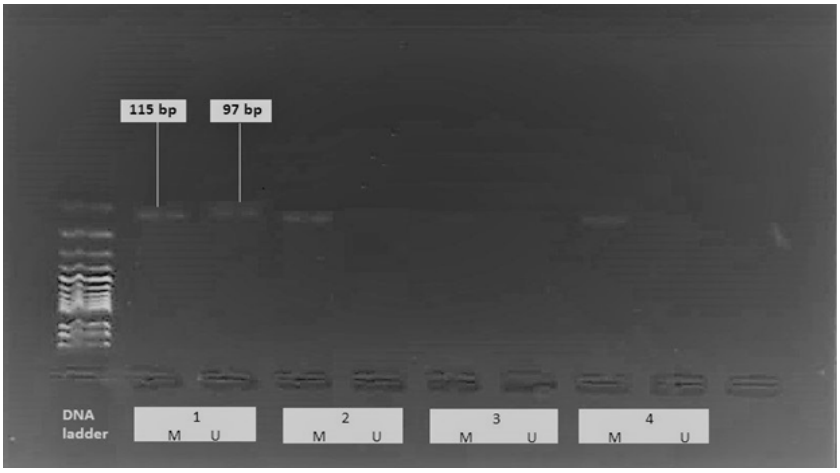


Figure 1. Gel Electrophoresis Representative MSP PCR Products of *CDH1* Gene. DNA ladder = 100 bp, samples 1 was positive in both methylated (M) and unmethylated (U), sample 2, 3 and 4 were positive just only in methylated (M).

Table 3. *CDH1* Methylation* *H. pylori glm*M PCR Results Cross Tabulation

| | | *glm*M PCR results | | | | | p-value |
|---|---|---|---|---|---|---|---|
| | | Positive | | Negative | | Total | |
| | | F | % | F | % | | |
| CDH1 Methylation | Positive | 16 | 89 | 14 | 44 | 30 | 0.002 |
| | Negative | 2 | 11 | 18 | 56 | 20 | |
| Total | | 18 | 100 | 32 | 100 | 50 | |

Table 4. Methylation of *CDH1* and Age Group, Gender, Symptoms, Endoscopy Results Cross Tabulation

| | | *CDH1* Methylation | | | | P-value |
|---|---|---|---|---|---|---|
| | | Positive | | Negative | | |
| | | F | % | F | % | |
| Age/Years | 20 - 35 years | 9 | 30 | 8 | 40 | 0.025 |
| | 36 - 50 years | 2 | 7 | 7 | 35 | |
| | 51 - 65 years | 12 | 40 | 3 | 15 | |
| | 66 - 80 years | 7 | 23 | 2 | 10 | |
| | Total | 30 | 100 | 20 | 100 | |
| Gender | Male | 17 | 57 | 10 | 50 | 0.431 |
| | Female | 13 | 43 | 10 | 50 | |
| | Total | 30 | 100 | 20 | 100 | |
| clinical symptoms | Gastric reflex | 9 | | 7 | | 0.472 |
| | Epigastric Pain | 22 | 73 | 16 | | 0.425 |
| | lack of appetite | 11 | 37 | 8 | | 0.522 |
| | Dysphagia | 3 | 10 | 1 | | 0.472 |
| | Nausea | 11 | 37 | 5 | | 0.291 |
| | Vomiting | 14 | 47 | 8 | | 0.432 |
| | Blood vomiting | 3 | 10 | 3 | | 0.456 |
| | Chest pain | 6 | 20 | 4 | | 0.645 |
| | Black feces | 8 | 27 | 5 | | 0.582 |
| Endoscopy findings | Normal | 1 | 3 | 2 | 10 | 0.769 |
| | Gastritis | 18 | 60 | 13 | 65 | |
| | Eosophagitis | 8 | 27 | 3 | 15 | |
| | Duodenal ulcer | 1 | 3 | 1 | 5 | |
| | Stomach cancer | 2 | 6 | 1 | 5 | |
| | Total | 30 | 100 | 20 | 100 | |

and diagnosed as *H. pylori* negative (Table 3). From this study, the relationships between *CDH1* methylation and participants age showed significance association (P-value 0.025), while gender, clinical symptoms and endoscopy findings were not have statistical significance (All P-value <0.05) (Table 4).

## Discussion

*Helicobacter pylori* infection causes stomach cancer through numbers of pathogenic pathways. In particular, several driver genes are influenced by aberrant DNA methylation (Such as *p16*, *CDH1*, *hMLH1* and *RHOA*) which inactivate them. Even noncancerous stomach mucosae can have aberrant DNA methylation, creating an "epigenetic field for cancerization" (Maeda et al., 2016).

In the present study we analyzed 50 samples from clinical suspected patients with *H. pylori* infection who undergoing upper gastrointestinal endoscopy to investigate the methylation status of *CDH1* gene using MSP technique and correlated it with *H. pylori* infection. *H. pylori* was detected in 36% (18/50) of study population using *glmM* gene PCR test, 89% (16/18) of *H. pylori* positive cases were *CDH1* methylated primer positive (chi-square, p-value=0.002). This suggested strong association between *H. pylori* infection and *CDH1* methylation. This data is consistent with Chan et al., (2006) who reported this association for the first time in 2003. Various results about this association have been reported. The finding of Kague et al., (2010)'s group demonstrated a positive association between *H. pylori* and *CDH1* methylation. Maekita et al., (2006) and other researchers indicated that *H. pylori* infection potently induces methylation of CpG Island to various degrees.

In addition, Niwa et al., (2013)'s group studied

Prevention of Helicobacter pylori-induced gastric cancers in gerbils, their results showed that, elimination of *H. pylori* clearly resulted in a decrease in methylation levels and a reduction in histological inflammatory responses. Our results disagree with other studies demonstrated that, Epithelial *CDH1* expression is significantly reduced in *H. pylori* infected gastric tissues (Aguilar et al., 2001; Tahara et al., 2009). The techniques used to detect the prevalence of *H. pylori* infection were very difference, these may justify these contradiction.

Findings from MSP technique showed that, the unmethylated primer was positive in 20% (10/50) of study cases and negative in 80% (40/50) of study case. Appling cross tabulation analysis between methylated and unmethylated *CDH1* primers results: 26% (13/50) of study cases showed negative results with both primers. In this study no techniques were used to check the complete bisulfite conversion was occur and excluded the possibility that successful amplification could be attributable to incomplete bisulfite conversion. Kague et al., (2010) used Bisulfite genomic sequencing of the representative PCR products of *CDH1* gene and showed that all cytosine at non-CpG sites were completely converted to thymine.

Other findings from MSP technique showed that, 6% (3/50) of study cases have band with both primers. Many researchers suggested the use of real-time methylation specific-PCR when methylation level was calculated as the fraction of methylated molecules in the total number of DNA molecules (Vaissie et al., 2009; Ando et al., 2008; Kikuyama et al., 2012).

Although there was no statistical significant correlation between *CDH1* methylation positive gastric mucosa and endoscopy finding (p-value = 0.76). The current study found that DNA methylation can even be present in cancerous and noncancerous gastric mucosa, 60% (18/30) of *CDH1* methylation positive gastric mucosa showed gastritis as an endoscopy finding, 27%(8/30) showed eosophagitis, 3%(1/30) showed duodenal ulcer and gastric cancer in 6% (2/30). This supported by the study of Kikuyama et al., (2012) discovered that, diverse expression levels of genes like *p16*, *CDH1*, and MLH1 are found in the gastric mucosa, and they are methylated in cancer cells and chronic inflammation. Recently, a multicenter prospective cohort study for predicting the risk of metachronous gastric cancer firstly proved the usefulness of an epigenetic cancer risk marker (Maeda et al., 2016).

In view of epidemiological results among study participants, the age group 20 - 35 years showed higher percentage 34%, while 40% of *CDH1* positive cases have an age group 51 - 65 years and there was a significant correlation between and *CDH1* methylation positive results and age group p-value = 0.02. There was no significant correlation between and *CDH1* methylation positive results and gender p-value=0.431. These findings agree with Yu et al., (2012) who found no correlations between *CDH1* methylation in preoperative peritoneal washes from Gastric Cancer patients and clinicopathologic parameters (all P > 0.05).

Although most study population suffering from Epigastric pain (76%) there was no significant association between clinical symptoms and *CDH1* positive methylation (all P-value > 0.05). Wide range of clinical symptoms and low prevalence of *CDH1* positive methylation among study population may justifies this insignificance. Several researches discovered that, the DNA methylation can even be present in noncancerous gastric mucosa, and its levels are influenced by *H. pylori* infection this is related to an increase in inflammatory mediators in the stomach mucosa, including IL-8, GRO, MIP-1, ENA-78, and MCP-1. And this is responsible for most clinical symptoms (Maeda et al., 2016).

In conclusion, *CDH1* gene methylation is associated with *H. pylori* infection in gastric mucosa specimens and it was indicated that *H. pylori* infection potently induces methylation of CpG islands. *CDH1* methylation is common occurrences in samples with chronic gastritis and gastric cancer and the correlation between *H. pylori* infection and *CDH1* inactivation involved in early gastric tumorigenesis.

## Author Contribution Statement

All authors contributed equally in this study.

## Acknowledgements

Great thanks to Dr. Adil Mergani who help in the primers acquisition.

*Funding Statement*

We acknowledge the support of the German Academic Exchange Service (DAAD) for funding this Study.

*Ethical Declaration*

The ethical approval of this study was obtained from Faculty of Medical Laboratory Sciences, University of Gezira, Sudan. Permission was obtained from Ministry of Health, Gezira State.

*Conflict of Interest*

The authors declare that they have no conflict of interests.

## References

Aguilar GR, Ayala G, Zarate FG (2001). *Helicobacter pylori*: recent advances in the study of its pathogenicity and prevention. *Salud Publica Mex*, **43**, 237-7.

Ahsan K, Hossain MZ, Uddin MR (2008). Role of crush cytology for the detection of the Helicobacter pylori in gastroduodenal disease. *J Dhaka Med Coll*, **17**, 89-1.

Ando T, Yoshida T, Enomoto S, et al (2008). DNA methylation of microRNA genes in gastric mucosae of gastric cancer patients: Its possible involvement in the formation of epigenetic field defect. *Int J Cancer*, **124**, 2367-4.

Ashour AA, Magalhaes PP, Mendes EN, et al (2002). Distribution of vacA genotypes in Helicobacter pylori strains isolated from Brazilian adult patients with gastritis, duodenal ulcer or gastric carcinoma. *FEMS Immunol Med Microbiol*, **33**, 173-8.

Bartchewsky W, Martini MR, Masiero M, et al (2009). Effect

of Helicobacter pylori infection on IL-8, IL-1beta and COX-2 expression in patients with chronic gastritis and gastric cancer. *Scand J Gastroenterol*, **44**, 153-1.

Bickley J, Owen RJ, Fraser AG, Pounder RE (1993). Evaluation of the polymerase chain reaction for detecting the urease C gene of Helicobacter pylori in gastric biopsy samples and dental plaque. *J Med Microbiol*, **39**, 338-4.

Chan AO, Peng JZ, Lam SK, et al (2006). Eradication of Helicobacter pylori infection reverses E-cadherin promoter hypermethylation. *Gut*, **55**, 463-8.

Fakhrjou A, Somi MH, Fattahi E, et al (2011). Rapid urease test, touch cytology and Histological assessment in determining infection by Helicobacter Pylori in outpatient setting. *Pakistan J Biological Sci*, **14**, 698-2.

Jemal A, Tiwari RC, Murray T, et al (2004). "Cancer Statistics". *CA Cancer J Clin*, **54**, 8-9.

Jin Z, Liu Y (2018), DNA methylation in human diseases. *Genes Dis*, **5**, 1-8.

Kague E, Thomazini C, Pardini MC, et al (2010). Methylation Status of *Cdh1* Gene in Samples of Gastric Mucous from Brazilian Patients with Chronic Gastritis Infected by Helicobacter pylori. *Arq Gastroenterol*, **47**, 2-7.

Kaise M, Yamasaki T, Yonezawa K, et al (2008). CpG island hypermethylation of tumor-suppressor genes in *H. pylori*-infected non-neoplastic gastric mucosa is linked with gastric cancer risk. *Helicobacter*, **13**, 1-35.

Kikuyama M, Takeshima H, Kinoshita T, et al (2012). Development of a novel approach, the epigenome-based outlier approach, to identify tumor-suppressor genes silenced by aberrant DNA methylation. *Cancer Lett*, **322**, 204-2.

Kurdyukov S, Bullock, M (2016). DNA Methylation Analysis: Choosing the Right Method. *Biology*, **5**, 1-2.

Maeda M, Moro H, Ushijima T (2016). Mechanisms for the induction of gastric cancer by Helicobacter pylori infection: aberrant DNA methylation pathway. *Gastric Cancer*, **20**, 5-8.

Maekita T, Nakazawa K, Mihara M, et al (2006). High Levels of Aberrant DNA Methylation in Helicobacter pylori Infected Gastric Mucosae and its Possible Association with Gastric Cancer Risk. *Clin Cancer Res*, **12**, 989-5.

Nakajima T, Maekita T, Oda I, et al (2006) Higher Methylation Levels in Gastric Mucosae Significantly Correlate with Higher Risk of Gastric Cancers. *Cancer Epidemiol Biomarkers Prev*, **15**, 2317-1.

Niwa T, Toyoda T, Tsukamoto T, et al (2013). Prevention of Helicobacter pylori-induced gastric cancers in gerbils by a DNA demethylating agent. *Cancer Prev Res (Philadelphia, PA)*, **6**, 263.

Ohata H, Kitauchi S, Yoshimura N, et al (2004). Progression of chronic atrophic gastritis associated with Helicobacter pylori infection increases risk of gastric cancer. *Int J Cancer*, **109**, 138-3.

Perri F, Cotugno R, Piepoli A, et al (2007). Aberrant DNA methylation in nonneoplastic gastric mucosa of *H. pylori* infected patients and effect of eradication. *Am J Gastroentrol*, **102**, 1-1361.

Qian X, Huang C, Cho CH, et al (2008). E-cadherin promoter hypermethylation induced by interleukin-1beta treatment of *H. pylori* infection in human gastric cancer cell lines. *Cancer Lett*, **263**, 107–3.

Schubeler D (2015). Function and information content of DNA methylation. *Nature*, **517**, 321.

Šestáková S, Šálek C, Remešová H (2019). DNA Methylation Validation Methods: a Coherent Review with Practical Comparison. *Biol Procedures Online*, **21**, 1-9.

Shin CM, Kim N, Jung Y, et al (2010). Role of Helicobacter pylori infection in aberrant DNA methylation along multistep gastric carcinogenesis. *Cancer Sci*, **101**, 1337-6.

Tahara T, Arisawa T, Shibata T, et al (2009). Increased number of methylated CpG islands correlates with Helicobacter pylori infection, histological and serological severity of chronic gastritis. *Eur J Gastroenterol Hepatol*, **21**, 613-9.

Tamura G (2002). Genetic and epigenetic alterations of tumor suppressor and tumor-related genes in gastric cancer. *Histol Histopathol*, **17**, 323-9.

Ushijima T. (2007) Epigenetic field for cancerization. J Biochem Mol Biol, 40, 142–0.

Vaissie T, Hung RG, Zaridze D, et al (2009). Quantitative Analysis of DNA Methylation Profiles in Lung Cancer Identifies Aberrant DNA Methylation of Specific Genes and Its Association with Gender and Cancer Risk Factors. *Cancer Res*, **69**, 243-2.

Yu Q, Wang X, Luo J, et al (2012). CDH1 Methylation in Preoperative Peritoneal Washes Is an Independent Prognostic Factor for Gastric Cancer. *J Surg Oncol*, **106**, 765-1.

Zhang J, Xing B, Song J, et al (2014). Associated Analysis of DNA Methylation for Cancer Detection Using CCP-Based FRET Technique. *Anal Chem*, **86**, 346.