



Comparing Diagnostic Accuracy of the *fliD* Gene and the *glmM* Gene in *Helicobacter pylori*

Ahmad Hormati ¹, Mohammad Khalifeh Gholi ², Alireza Sharifi ³, Mahdiieh Ghoddoosi ⁴, Mehdi Pezeshgi Modarres ⁵, Pooya Jafari ³, Mahdi Zarei ⁶, Mojde Bagheri ⁷ and Mohaddeseh Zojaji ^{5,*}

¹Gastroenterology and Hepatology Diseases Research Center, Iran University of Medical Sciences, Tehran, Iran

²Department of Microbiology and Immunology, School of Medicine, Qom University of Medical Sciences, Qom, Iran

³Qom University of Medical Sciences, Qom, Iran

⁴Department of Pathology, Shahid Beheshti Medical Centre, Qom University of Medical Sciences, Qom, Iran

⁵Gastroenterology and Hepatology Diseases Research Center, Qom University of Medical Sciences, Qom, Iran

⁶Tabriz University of Medical Sciences, Tabriz, Iran

⁷Shiraz University of Medical Sciences, Shiraz, Iran

*Corresponding author: Gastroenterology and Hepatology Diseases Research Center, Qom University of Medical Sciences, Qom, Iran. Email: Email: mohaddeseh.zojaji@gmail.com

Received 2021 December 19; Revised 2022 March 27; Accepted 2022 April 19.

Abstract

Background: *Helicobacter pylori* is one of the most common human bacterial infections, accounting for the infection of half of the world's population. The polymerase chain reaction (PCR) has high specificity and sensitivity in diagnosing this bacterial infection.

Objectives: The present study aimed to compare the sensitivity and specificity of the *fliD* gene and the most widely used *glmM* gene in the PCR technique.

Methods: The research population encompassed patients with indications for upper endoscopy. This cross-sectional study compared the sensitivity and specificity of a proposed gene (*fliD*) with the most widely used *glmM* gene to detect the *H. pylori* infection in tissue samples.

Results: The participants encompassed ninety-nine participants aged above 18 years. Their median age was 45.92 ± 13.63 years. The most common complaints of the patients were epigastric pain and heartburn. Our described gold standard detected 61.6% and 38.4% as positive and negative, respectively. The sensitivity and specificity were 72.1% and 100.0% for the routine PCR (*glmM* gene) and 80.3% and 94.7% for the proposed PCR (*fliD* gene).

Conclusions: Different genes have been used to detect *H. pylori* in PCR. The *glmM* gene is easily used to diagnose the *H. pylori* infection; however, according to the present findings, the *fliD* gene has higher sensitivity than the *glmM* gene. Accordingly, the former can be used as a screening gene for the *H. pylori* infection in the PCR technique.

Keywords: *Helicobacter pylori*, Polymerase Chain Reaction, *fliD*, *glmM*, *ureC*, Sensitivity, Specificity

1. Background

Helicobacter pylori is one of the most common human bacterial infections, accounting for the infection of half of the world's population (1). The prevalence of this bacterial infection varies with regard to region, race, age, and socioeconomic status. Accordingly, the prevalence of the *H. pylori* infection varies from 50.8% in developing countries to 34.7% in developed countries (2). The prevalence of the *H. pylori* infection is reported to be minimum and maximum in the Kurdistan and Ardabil provinces (36%, 90%, respectively) (3). Most infected individuals exhibit asymptomatic chronic gastritis; however, the infection in some individuals causes peptic ulceration, atrophy, and active chronic

gastritis. This bacterial infection plays a crucial role in developing mucosa-associated lymphoid tissue (MALT) lymphoma, primary gastric non-Hodgkin's lymphoma, and gastric adenocarcinoma (4). *H. pylori* eradication can prevent gastric cancer in humans; however, it needs to be screened by a sensitive and specific diagnostic test (5).

The diagnostic tests for the *H. pylori* infection are classified into two categories: (1) Invasive tests such as histopathology, culture, rapid urease test (RUT), and molecular methods, which are based on endoscopy; and (2) non-invasive tests, including urea breath tests, serological tests, and fecal antigens. During the past few decades, molecular diagnostic methods have had remarkable ef-

fects on the clinical management of many infections (6). The polymerase chain reaction (PCR) technique is one of the most common molecular methods for clinical applications such as the evaluation of emerging infections, broad-spectrum infection detection, epidemiological studies, antibiotic resistance, and genotypic bacterial identification (7). Moreover, this method has high specificity and sensitivity in diagnosing the *H. pylori* infection (8).

Different genes (eg, *vacA*, *cagA*, *glmM*, *23SrRNA*, *16SrRNA*, and *ureC*) can diagnose *H. pylori* in the PCR technique (9). The *ureC* (*glmM*) gene is one of the main factors in *H. pylori* pathogenicity, which can easily be detected in gastric biopsy samples; therefore, it has been widely employed in the diagnosis of the *H. pylori* infection in the PCR technique (10). Labigne et al. reported that the *16SrRNA* and *glmM* genes have higher detection sensitivity in gastric biopsy samples than other genes (11). In the present study, we used the *fliD* gene as a novel gene in the PCR technique to diagnose the *H. pylori* infection.

The *H. pylori* *FliD* protein as a *fliD* gene product plays a crucial role in the functional flagella assembly leading to bacterial motility, colonization, and persistence of the *H. pylori* infection. It is noted that the motility of *H. pylori* plays a central role in gastric mucosal injury (12, 13). Accordingly, the *fliD* gene mutant induces morphologically abnormal flagellar appendages. The *H. pylori* *fliD* gene is a structural gene having a role in genetic regulation (14). Khalifeh Gholi et al. (15) mentioned that the *fliD* gene product in the serological diagnosis of the *H. pylori* infection revealed a high sensitivity by 97% and a specificity by 99%. In other words, it is a cost-effective, simple, and highly-efficient tool for detecting the *H. pylori* infection.

2. Objectives

All aforementioned benefits are in favor of using this protein as a screening method in developing countries where the prevalence of the *H. pylori* infection is high. Accordingly, the present study aimed to investigate the sensitivity and specificity of the *fliD* gene in comparison to the *glmM* gene in the PCR technique.

3. Methods

3.1. Study Design and Participants

This cross-sectional study was carried out at the Shahid Beheshti Hospital, Qom, Iran, from April 2019 to April 2020. The participants encompassed all patients referred to the gastrointestinal clinic of the Shahid Beheshti Hospital and had complaints of gastrointestinal symptoms such

as dyspepsia, nausea, vomiting, chronic epigastric and abdominal pain, decreased appetite, failure to thrive, and weight loss. The participants were above 18 years old. In the last month, we excluded individuals with a history of antibiotics, bismuth, and proton pump inhibitors (PPIs) therapies as such the number of patients decreased to 99 subjects. Based on the gastroenterologist suspicion, we performed endoscopy and obtained biopsy for all 99 patients. Experienced endoscopists performed the upper endoscopic examination, and they obtained biopsy specimens. Research data were collected from the clinical records of eligible patients. The data were registered in researcher-made checklists with sections addressing demographic and pre-treatment clinical signs.

3.2. Determination of *Helicobacter pylori*

For each participant, four biopsies were taken from the antrum, corpus-antrum junction, greater curvature of the antrum, and greater curvature of the corpus during endoscopy.

Two common diagnostic techniques were used simultaneously as the gold standard to detect the *H. pylori* infection: (1) histopathological detection (6) of *H. pylori* using Giemsa special stain of formalin-fixed paraffin-embedded (FFPE) tissue sections and determination of density (mild, moderate, severe) based on the Sydney system (6) and (2) rapid urease test (RUT) (6).

Therefore, our gold standard described as follows:

- (1) If the histopathology result was moderate to severe, regardless of the RUT result, it was considered positive.
- (2) If the histopathology result was mild, and the RUT result was positive, it was considered positive.
- (3) If the histopathology result was mild, but the RUT result was negative simultaneously, it was considered negative.
- (4) If the histopathology result was negative, it was considered negative.

3.3. DNA Extraction and PCR Assay

The bioinformatics analysis was performed by software such as CLC Genomic Workbench 12, Gene Runner, and AlleleID to consider gene arrangement and the schematic maps of the *fliD* gene in diagnosing the *H. pylori* infection. Then a pair of primers was designed, and their specificity and sensitivity were examined by Gene bank and (basic local alignment search tool) BLAST. Moreover, we used a specific primer targeting the *glmM* gene based on previous studies (Table 1). DNA was extracted from tissue samples using GeneAll- Exgene™ Kit according to the manufacturer's instructions. Furthermore, the quantity and quality of the extracted DNA were evaluated by the

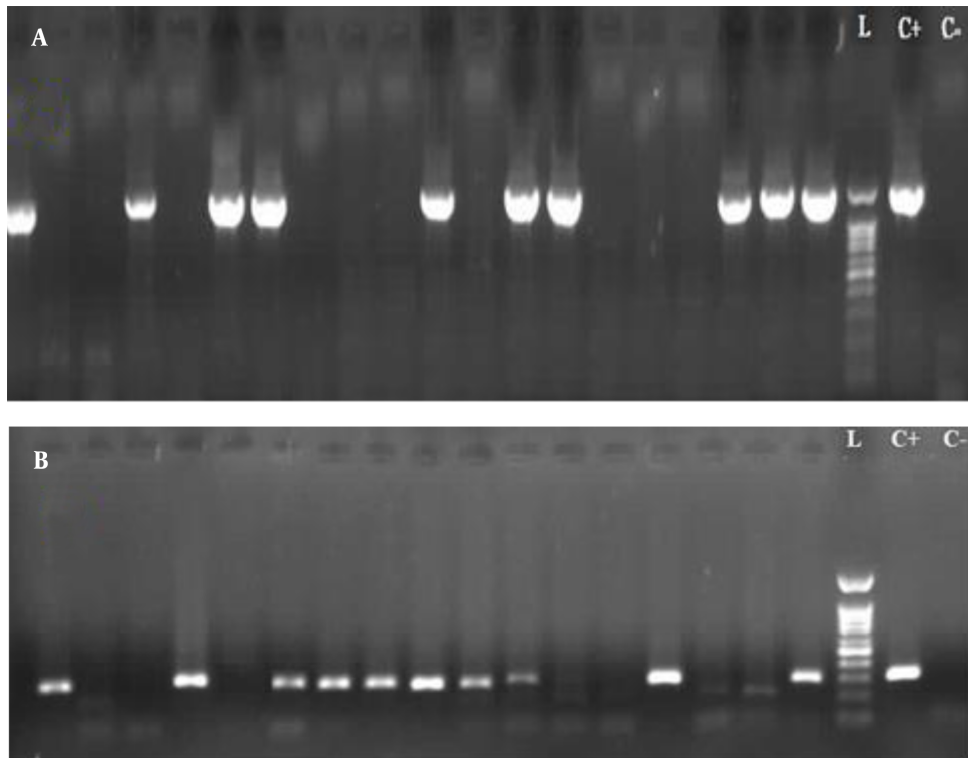


Figure 1. (A) The PCR product of the *fliD* gene is visualized in 1% agarose gel (band size: 1447 bp), (B) The PCR product of the *glmM* gene is visualized in 1% agarose gel (band size: 294 bp).

Nanodrop. Then all biopsy samples were re-examined using two genes to compare their specificity and sensitivity in diagnosing the *H. pylori* strain.

Amplification was conducted in a total volume of 20 μ L. The reaction mixture contained 10 μ L, 2X ready PCR mix (Biofact Master Mix), 0.8 μ L of each forward and reverse primers (Table 1), 2 μ L DNA template, 5.8 μ L PCR-grade H₂O, and 0.6 μ L nuclease-free distilled water to a total volume of 20 μ L. The PCR amplification was performed according to the following protocol: Initial denaturation at 95°C for 2 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 90 s and 40 s (for *fliD* and *glmM*, respectively), a final extension at 72°C for 7 min, and final storing at 4°C for 10 min (Table 2). The amplified PCR products were electrophoresed using 1% agarose gel (Figure 1), and they were then evaluated for a specific band of each gene to detect the *H. pylori* infection.

3.4. Statistical Analysis

All statistical analyses were performed using SPSS software version 20. Descriptive statistics are presented as frequencies and percentages for categorical variables, and

mean \pm standard deviation, and minimum-maximum values for numeric variables. Moreover, chi-square tests and the Kappa coefficient were used to analyze the collected data.

4. Results

The present study encompassed 99 participants aged above 18 years, among whom 47.5% were male ($n = 47$) and 52.5% were female ($n = 52$). The mean age of the patients was 45.92 ± 13.63 years. The most common indications for endoscopy were epigastric pain and heartburn (Figure 2). The *H. pylori* infection was detected using the concerned gold standard (Figure 3). Table 3 shows the *H. pylori*-positive and -negative cases based on RUT and histopathology separately. Finally, the gold standard detected 61.6% ($n = 61$) of the cases as *H. pylori*-positive and 38.4% ($n = 38$) as *H. pylori*-negative. All biopsy samples were re-examined using PCR (with *fliD* and *glmM* genes) to detect *H. pylori*. In this regard, the routine PCR (*glmM* gene) reported that 44.5% ($n = 44$) of the cases were positive and 55.5% ($n = 55$) were negative. In contrast, the proposed PCR (*fliD* gene) reported 51.5% ($n = 51$) positive cases and 48.5% ($n = 48$) negative

Table 1. Primer Set and Conditions in PCR

Gene Name	Nucleotide Sequence (5'→3')	Length	TM	GC%	Band Size (bp)
<i>fliD</i>	F: CTTTAGGCGATGGCTCAA	21	58.92	47.62	1447
	R: GCGTCTCTGTAGGGAATCT	21	58.70	52.38	1447
<i>glmM</i>	F: CTTTAGGCGATGGCTCAA	21	58.92	44	294
	R: GCGTCTCTGTAGGGAATCT	21	58.70	37.5	294

Abbreviations, TM, temperature of melting; GC%, percentage of G and C nucleotides in primer sequences.

Table 2. Amplification Protocol for Two Genes in PCR

Type	Temperature (°C)	Time	Cycle (n)
Initial denaturation	95	2 min	1
Denaturation	95	30 s	40
Annealing	58	30 s	
Extension			
<i>fliD</i>	72	90 s	
<i>glmM</i>	72	40 s	
Final extension	72	7 min	1
Store	4	10 min	1

cases (Table 4). The results of the two PCR techniques were compared with those of the proposed gold standard. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for the routine PCR technique were 72.1, 100, 100, and 69%, respectively. In contrast, they were 80.3, 94.7, 96, and 75% in the proposed PCR, respectively (Table 5).

Table 3. Results of Samples Tested by RUT and Histopathology^a

RUT Results	Histopathology Results	
	Negative	Positive
Positive	3	50
Negative	35	11
Total number	38 (38.4)	61 (61.6)

Abbreviation: RUT, rapid urease test.

^a Values are expressed as No. (%). Total numbers in this tables are equal to our described gold standard.

5. Discussion

Recently, various studies have focused on molecular PCR techniques for the diagnosis of *H. pylori* because of the high sensitivity of this method in detection of a small number of these microorganisms in the obtained biopsies and the high ability of this method in amplifying the target

DNA of the coccoid form of *H. pylori*, which is difficult to identify and cultivate histologically (16, 17). According to many studies, different genes are used to detect *H. pylori* in PCR. For example, Khalifeh Gholi et al. (15) used the *FliD* protein, a product of the *fliD* gene, to diagnose the *H. pylori* infection and reported acceptable results for the concerned protein in detecting this infection (15). On the other hand, the mutation in the *fliD* gene causes the complete immobility of bacteria and the complete cessation of the *FliD* protein expression (14). Accordingly, this study aimed to evaluate the sensitivity and specificity of the gene producing this protein for the diagnosis of the *H. pylori* infection by the PCR technique.

According to the present study, 61.6% (n = 61) of the participants were diagnosed with the *H. pylori* infection using the defined gold standard, but routine PCR and proposed PCR detected 72.1% (44 cases) and 80.3% (49 cases) of participants, respectively, as positive in terms of *H. pylori* infection. Moreover, 8.2% (5 cases) of the cases considered healthy in PCR using the *glmM* gene were reported to be infected in PCR using the *fliD* gene, indicating the higher sensitivity of the *fliD* gene than the common *glmM* gene in the diagnosis of the *H. pylori* infection. The present findings also showed that the NPV of the proposed PCR was higher than the routine PCR (75% vs. 69%); however, the former had a lower specificity and PPV (94.7% and 96% vs. 100% and 100%, respectively). The *ureC* (*glmM*) gene, a major pathogen factor in the *H. pylori* infection, is easily detectable in gastric biopsy specimens for *H. pylori* and is commonly used to diagnose this infection by the PCR technique (10). For example, in Labigne et al.'s study (11), the *glmM* gene was more sensitive than the other genes in the PCR technique to detect this bacterium. This finding is not in line with those of the present study because the diagnostic sensitivity of the *fliD* gene is higher than the *glmM* gene in the present study.

In 2013, Khalifeh Gholi et al. (15) included 318 samples in their study based on a histopathological examination of *H. pylori*. Then, using the *FliD* protein, patients were examined for this bacterium, and their results also showed that the sensitivity and specificity of the protein

Indications for upper endoscopy(%)

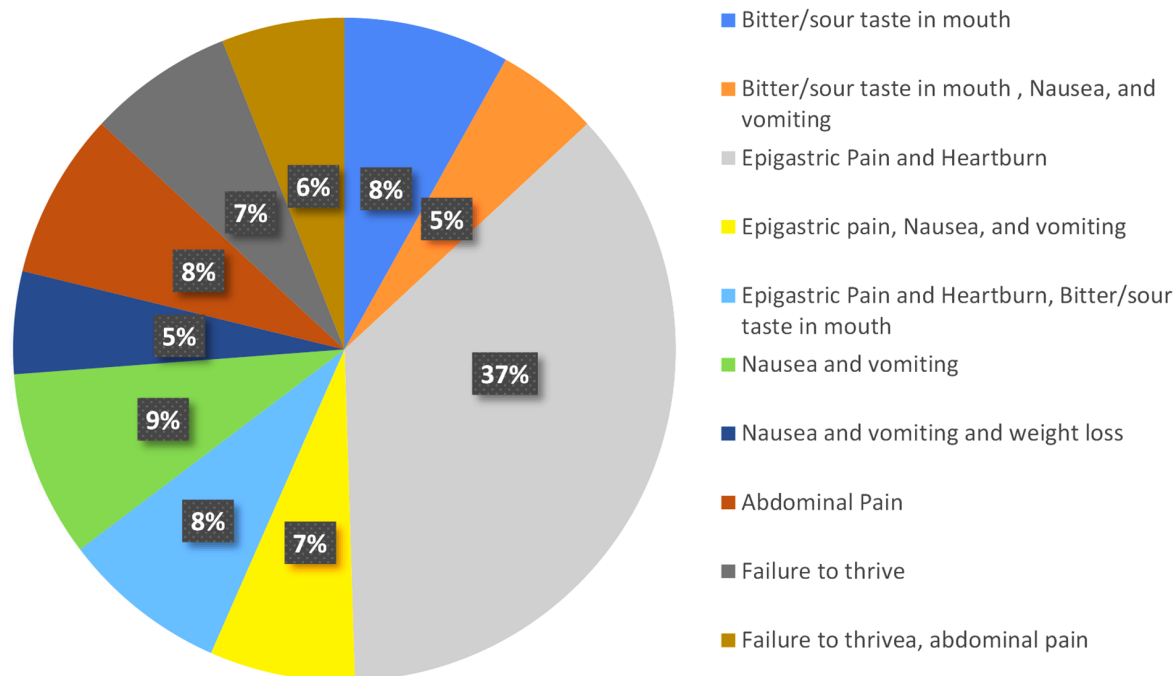


Figure 2. Upper endoscopy indications

Table 4. Results of Two Genes Compared to Gold Standard Results^a

	Gold Standard Results		Total Number
	Positive	Negative	
Routine PCR (<i>glmM</i> gene) results			
Positive (n)	44	0	44 (44.5)
Negative (n)	17	38	55 (55.5)
Suggestive PCR (<i>fliD</i> gene) results			
Positive (n)	49	2	51 (51.5)
Negative (n)	12	36	48 (48.5)
Total numbers (n)	61 (61.6)	38 (38.4)	99

Abbreviation: PCR, polymerase chain reaction.

^a Values are expressed as No. (%). Total numbers in this tables are equal to our described gold standard

translated from the *fliD* gene in diagnosing this bacterium are high (97% and 99%, respectively). In their study, only histopathology was used as the gold standard. In contrast, in the present study, we used two common diagnostic techniques (namely RUT and histopathology) as the gold standard simultaneously to minimize the diagnostic error caused by false positive and negative results. This is another advantage of our study compared to previous

studies. Moreover, in their study, the translated protein of the *fliD* gene was used and not compared with any other diagnostic method. However, the *fliD* gene was used in the present study, and the sensitivity and specificity of the PCR technique were compared between the mentioned gene and the common *glmM* gene. In another study by Cho et al. (18), the researchers stated that the *FliD* protein could be used as a molecular basis in vaccine production and the di-

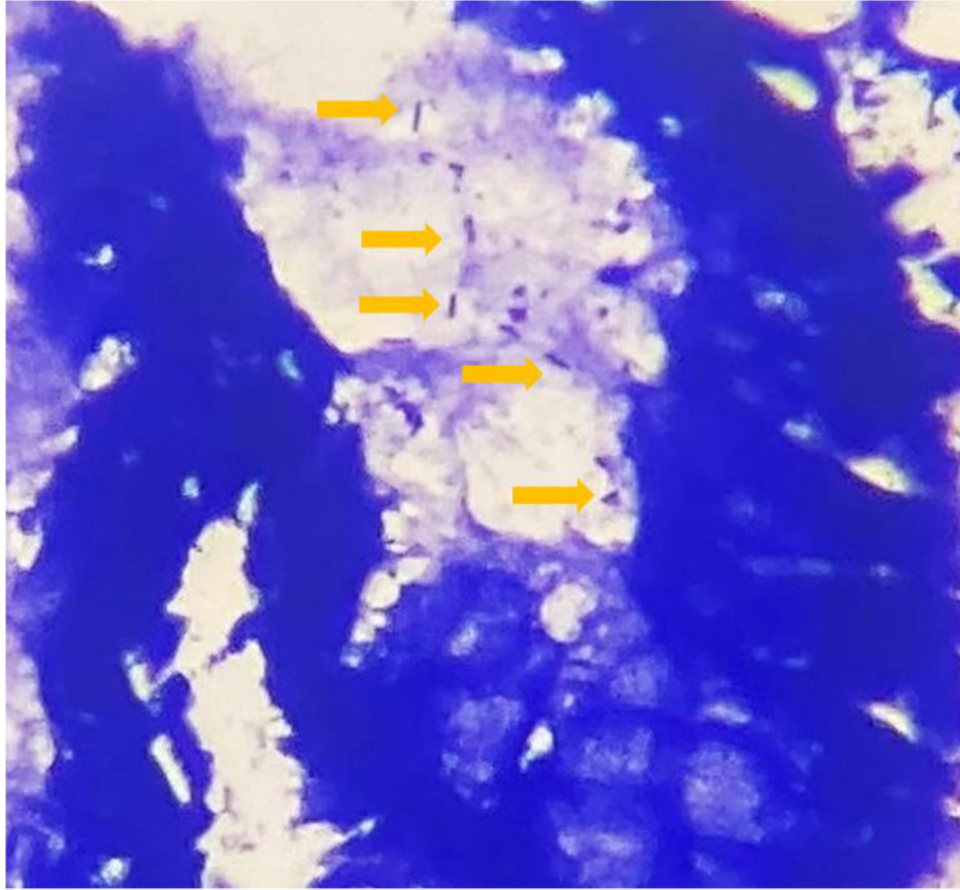


Figure 3. *Helicobacter pylori* detection on gastric biopsy. *H. pylori* organisms are blue-stained curved bacilli (arrows) on the tissue section (Giemsa stain, $\times 400$).

Table 5. Sensitivities, Specificities, Negative Predictive Values, and Positive Predictive Values of Two PCRs

Specifications	Routine PCR (<i>glmM</i> Gene) (%)	Proposed PCR (<i>fliD</i> Gene) (%)
Sensitivity	72.1	80.3
Specificity	100	94.7
Negative predictive value	69	75
Positive predictive value	100	96

agnosis of *H. pylori*. Although there are differences between the present study and Khalifeh Gholi and Cho's studies, the findings of their study, in line with those of the present study, showed that the *fliD* gene could play a crucial role in the *H. pylori* infection diagnosis and screening.

The present findings showed that the most common indications for upper endoscopy in the patients were epigastric pain and heartburn. The same results have been

mentioned in other studies (19). Since no study has been performed to evaluate the proposed *fliD* gene in the *H. pylori* infection using the diagnostic PCR technique and compare it with common genes used in PCR, future researchers are recommended to conduct further studies to provide a more accurate diagnostic value of this gene as the proposed gene can also be used for the PCR technique regarding its cost-effectiveness, simplicity, and efficiency.

5.1. Conclusions

The present findings indicate that the *fliD* gene has more sensitivity and negative predictive value than the most widely used *glmM* gene, suggesting that the *fliD* gene can play a key role in the patients regarding the *H. pylori* infection in gastric biopsy samples. It is worth noting that the specificity and positive predictive value of the *fliD* gene are approximately closed to the *glmM* gene. Accordingly, future researchers are recommended to perform further studies on the sensitivity and specificity of this proposed

gene to detect the *H. pylori* infection.

Acknowledgments

The authors would like to thank all the endoscopists for their assistance in performing endoscopy and obtaining biopsy samples.

Footnotes

Authors' Contribution: Study concept and design and the analysis and interpretation of results: A. H. and M. KH. GH.; histopathological examination: M. GH.; Data processing, collection, performing experiment: A. H., M. P. M., M. Z., M. Z., and M.B.; Draft manuscript preparation, visualization: M.Z. and A.SH.; Statistical analysis and interpretation of data: M. Z. and M. B.; Critical revision or editing: A. SH., P.J., and M. Z., Final Approval of the final version: M. KH.GH., M.GH., and M.P.M, Supervision and funding: M. KH. GH.

Conflict of Interests: There are no conflicts of interest.

Data Reproducibility: The dataset in the study is available on request from the corresponding author during submission or after its publication. The data are not publicly available to observe the patients' privacy and information confidentiality.

Ethical Approval: This study was approved by the Ethics Committee of the Qom University of Medical Sciences. IR.MUQ.REC.1399.052 (ethics.research.ac.ir/EthicsProposalView.php?&code=IR.MUQ.REC.1399.052).

Funding/Support: We received no funding and support for this study.

Informed Consent: Informed consent was obtained from all participants before conducting the study.

References

- Wang YK, Kuo FC, Liu CJ, Wu MC, Shih HY, Wang SS, et al. Diagnosis of Helicobacter pylori infection: Current options and developments. *World J Gastroenterol*. 2015;**21**(40):11221-35. doi: [10.3748/wjg.v21.i40.11221](https://doi.org/10.3748/wjg.v21.i40.11221). [PubMed: [26523098](https://pubmed.ncbi.nlm.nih.gov/26523098/)]. [PubMed Central: [PMC4616200](https://pubmed.ncbi.nlm.nih.gov/PMC4616200/)].
- Zamani M, Ebrahimitabar F, Zamani V, Miller WH, Alizadeh-Navaei R, Shokri-Shirvani J, et al. Systematic review with meta-analysis: the worldwide prevalence of Helicobacter pylori infection. *Aliment Pharmacol Ther*. 2018;**47**(7):868-76. doi: [10.1111/apt.14561](https://doi.org/10.1111/apt.14561). [PubMed: [29430669](https://pubmed.ncbi.nlm.nih.gov/29430669/)].
- Zojaji M, Sharifi A, Hormati A, Alemi F, Afifian M, Abedi SH. Antibiotic resistance of Helicobacter pylori infection and its eradication rate in different regions of Iran; a review. *Govaresh*. 2020;**25**(3):198-207.
- Suganuma M, Kurusu M, Okabe S, Sueoka N, Yoshida M, Wakatsuki Y, et al. Helicobacter pylori membrane protein 1: a new carcinogenic factor of Helicobacter pylori. *Cancer Res*. 2001;**61**(17):6356-9.
- Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, et al. Helicobacter pylori infection and the development of gastric cancer. *N Engl J Med*. 2001;**345**(11):784-9. doi: [10.1056/NEJMoa001999](https://doi.org/10.1056/NEJMoa001999). [PubMed: [11556297](https://pubmed.ncbi.nlm.nih.gov/11556297/)].
- Sabbagh P, Mohammadnia-Afrouzi M, Javanian M, Babazadeh A, Kopolu V, Vasigala VR, et al. Diagnostic methods for Helicobacter pylori infection: ideals, options, and limitations. *Eur J Clin Microbiol Infect Dis*. 2019;**38**(1):55-66. doi: [10.1007/s10096-018-3414-4](https://doi.org/10.1007/s10096-018-3414-4). [PubMed: [30414090](https://pubmed.ncbi.nlm.nih.gov/30414090/)].
- Rimbara E, Sasatsu M, Graham DY. PCR detection of Helicobacter pylori in clinical samples. *PCR Detection of Microbial Pathogens*. **943**. Springer; 2013. p. 279-87.
- Lehours P, Megraud F. Helicobacter pylori molecular diagnosis. *Expert Rev Mol Diagn*. 2011;**11**(4):351-5. doi: [10.1586/erm.11.17](https://doi.org/10.1586/erm.11.17). [PubMed: [21545252](https://pubmed.ncbi.nlm.nih.gov/21545252/)].
- Thaker Y. Helicobacter pylori: A review of epidemiology, treatment, and management. *J Clin Gastroenterol Treat*. 2016;**2**(2):1-5. doi: [10.23937/2469-584x/1510019](https://doi.org/10.23937/2469-584x/1510019).
- Espinoza MG, Vazquez RG, Mendez IM, Vargas CR, Cerezo SG. Detection of the glmM gene in Helicobacter pylori isolates with a novel primer by PCR. *J Clin Microbiol*. 2011;**49**(4):1650-2. doi: [10.1128/JCM.00461-10](https://doi.org/10.1128/JCM.00461-10). [PubMed: [21289140](https://pubmed.ncbi.nlm.nih.gov/21289140/)]. [PubMed Central: [PMC3122814](https://pubmed.ncbi.nlm.nih.gov/PMC3122814/)].
- Labigne A, De Reuse H, Mengin-Lecreux D. The Helicobacter pylori ureC gene codes for a phosphoglucosamine mutase. *J Bacteriol*. 1997;**179**(11):3488-93. doi: [10.1128/jb.179.11.3488-3493.1997](https://doi.org/10.1128/jb.179.11.3488-3493.1997). [PubMed: [9171391](https://pubmed.ncbi.nlm.nih.gov/9171391/)]. [PubMed Central: [PMC179139](https://pubmed.ncbi.nlm.nih.gov/PMC179139/)].
- Eaton KA, Suerbaum S, Josenhans C, Krakowka S. Colonization of gnotobiotic piglets by Helicobacter pylori deficient in two flagellin genes. *Infect Immun*. 1996;**64**(7):2445-8. doi: [10.1128/iai.64.7.2445-2448.1996](https://doi.org/10.1128/iai.64.7.2445-2448.1996). [PubMed: [8698465](https://pubmed.ncbi.nlm.nih.gov/8698465/)]. [PubMed Central: [PMC174096](https://pubmed.ncbi.nlm.nih.gov/PMC174096/)].
- Watanabe T, Tada M, Nagai H, Sasaki S, Nakao M. Helicobacter pylori infection induces gastric cancer in Mongolian gerbils. *Gastroenterology*. 1998;**115**(3):642-8. doi: [10.1016/S0016-5085\(98\)70143-X](https://doi.org/10.1016/S0016-5085(98)70143-X).
- Kim JS, Chang JH, Chung SI, Yum JS. Molecular cloning and characterization of the Helicobacter pylori flhD gene, an essential factor in flagellar structure and motility. *J Bacteriol*. 1999;**181**(22):6969-76. doi: [10.1128/JB.181.22.6969-6976.1999](https://doi.org/10.1128/JB.181.22.6969-6976.1999). [PubMed: [10559162](https://pubmed.ncbi.nlm.nih.gov/10559162/)]. [PubMed Central: [PMC94171](https://pubmed.ncbi.nlm.nih.gov/PMC94171/)].
- Khalifeh Gholi M, Kalali B, Formichella L, Gottner G, Shamsipour F, Zarnani AH, et al. Helicobacter pylori FlhD protein is a highly sensitive and specific marker for serologic diagnosis of H. pylori infection. *Int J Med Microbiol*. 2013;**303**(8):618-23. doi: [10.1016/j.ijmm.2013.08.005](https://doi.org/10.1016/j.ijmm.2013.08.005). [PubMed: [24103649](https://pubmed.ncbi.nlm.nih.gov/24103649/)].
- Lin TT, Yeh CT, Yang E, Chen PC. Detection of Helicobacter pylori by polymerase chain reaction assay using gastric biopsy specimens taken for CLOtest. *J Gastroenterol*. 1996;**31**(3):329-32. doi: [10.1007/BF02355020](https://doi.org/10.1007/BF02355020). [PubMed: [8726822](https://pubmed.ncbi.nlm.nih.gov/8726822/)].
- Narikawa S, Kawai S, Aoshima H, Kawamata O, Kawaguchi R, Hikiji K, et al. Comparison of the nucleic acids of helical and coccoid forms of Helicobacter pylori. *Clin Diagn Lab Immunol*. 1997;**4**(3):285-90. doi: [10.1128/cdli.4.3.285-290.1997](https://doi.org/10.1128/cdli.4.3.285-290.1997). [PubMed: [9144365](https://pubmed.ncbi.nlm.nih.gov/9144365/)]. [PubMed Central: [PMC170520](https://pubmed.ncbi.nlm.nih.gov/PMC170520/)].
- Cho SY, Song WS, Oh HB, Kim HU, Jung HS, Yoon SI. Structural analysis of the flagellar capping protein FlhD from Helicobacter pylori. *Biochem Biophys Res Commun*. 2019;**514**(1):98-104. doi: [10.1016/j.bbrc.2019.04.065](https://doi.org/10.1016/j.bbrc.2019.04.065). [PubMed: [31023530](https://pubmed.ncbi.nlm.nih.gov/31023530/)].
- Arslan M, Balamtekin N, Gunal A. Efficacy of a novel sequential treatment regimen containing bismuth for Helicobacter pylori eradication in Turkish children. *Helicobacter*. 2020;**25**(6). e12757. doi: [10.1111/hel.12757](https://doi.org/10.1111/hel.12757). [PubMed: [32939900](https://pubmed.ncbi.nlm.nih.gov/32939900/)].