

# The Prevalence of *H. Pylori cagA* Gene in Patients with Gastric Ulcer

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## ABSTRACT

**Background and Aim:** *Helicobacter Pylori* (*H. pylori*) is one of the reasons for the gastric inflammation and peptic ulcers. It is a predisposing factor of gastric adenocarcinoma. Cytotoxin A encoded by the *cagA* gene is one of the major virulence factors in bacterial pathogenicity, which is of special importance due to genetic diversity in different geographical areas. The purpose of this study was to evaluate the prevalence of the *cagA* gene in the patients suffering from gastric ulcers.

**Materials and Methods:** Seventy-five paraffin blocks of stomach biopsy samples from patients infected with *H. pylori* and suffering from gastric ulcers were randomly evaluated in the province of Kermanshah in the spring of 2016, Iran. DNA samples extracted by the boiling method were investigated using PCR by the use of particular primer pairs for a protected area in the *glmM* gene, and the existence of the *cagA* gene.

**Results:** From the 75 samples, 56 cases (%74.66) were *glmM*-positive and among them, 39 cases (%69.64) tested positive for *cagA* gene. Comparing patients in terms of gender, the frequency of the *cagA* gene was 22 (%39.28) and 17 (%30.35) in males and females, respectively. Finally, comparing patients in terms of age, 16 cases (%21.33) were younger than 40 years old and 23 cases (%41.07) were older than 40 years old.

**Conclusion:** The results of this study showed significant association between the frequency of the *cagA* gene and peptic ulcer in the studied patients.

**Keywords:** *CagA* gene, Gastric ulcer, *GlmM* gene, *H. Pylori*

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## Introduction

Gastric Ulcer is a painful injury in the internal gastric wall or the initial part of the small intestine. There is no clear reason for the gastric ulcers. Today, it is evident that gastric ulcer is due to an imbalance of gastric and duodenal ulcers. The main bacterium involved in gastric ulcers is *Helicobacter Pylori* (*H. pylori*) (1-4).

*H. pylori* is a gram-negative and spiral-shaped bacillus. The size of this bacterium is around 0.3×5.5 micrometers. These bacteria are colonized in the human stomach epithelium and most of the other animal species. They have an important role in pathogenicity of gastric inflammation, gastric ulcer, and gastric cancer. They are also etiologically

important in the illnesses of peptic ulcer and malignant stomach (5-7).

Antrum mucus, exotic, and cardia are equally sensitive to infection and the bacteria are colonized in the same way (8). The bacteria of *H. pylori* are colonized in the stomach of half of the population in the world. Tissue gastritis is a public complication among all individuals infected with these bacteria; however, it has evident clinical circumstances similar to gastric ulcer and gastric cancer just among a few of the patients (5-9).

The mechanisms responsible for different stages of the disease are not fully understood. However, two pathogenicity indices have been identified which have been introduced as virulence factors of different bacterial strains (10-11). These factors are cytotoxin-associated gene A (*cagA*) and vacuolating cytotoxin (*vacA*) (12-14).

The *cagA* gene is located in the region of I in the pathogenicity part (*CagA*, *PAI CagA*) and is one of the leading causes of pathogenicity. *Cag PAI* is a 40 kb locus and its percentage of guanine-cytosine (35% to 39%) is different from the other genomes (8) and is transmitted through some strains of *H. pylori*. This island has 31 genes out of which, 6 are coding the fourth type of secretion system and take the role in the association of the host bacteria with the pathogenicity process (1-10).

*CagA* is a 120–145 kDa protein encoded on the 40 kb *cag* pathogenicity island (PAI). The *cagA* protein is one of the superficial proteins of the external membrane in *H. pylori* that has great power in raising the safety. The products of this gene cause duckling in the host cell, after entering bacteria to the stomach epithelial cells. The *cagA* protein can increase the virulence of bacteria after entering the target cell directly and interference with the cell messaging systems. The existence of this gene in some strains of *H. pylori* shows the existence of such pathogenicity island of *cag PAI* (6-9).

*H. pylori* strains can be divided into *cagA*-positive or -negative strains. It seems that the severity of digestive illnesses such as peptic ulcer, gastric mucosal atrophy, and gastric cancer is because of *H. pylori* relating to *cag* pathogenicity island such as peptic ulcer (1-15). Also, studying the specific bacterial genes

of *glmM* was used to prove, control, and study the existence of *H. pylori*. According to the previous studies, the *glmM* gene had more sensitivity to recognize these bacteria in comparison with the other genes (10). Therefore, the purpose of the present survey was to study the frequency of the *cagA* gene of *H. pylori* gene among the patients suffering from gastric ulcers in the City of Kermanshah.

## Materials and Methods

### Samples

The present research was a case-observing study. The samples were 75 PA paraffin blocks; 40 men (47±28) and 35 women (47±26) of the patients suffering from gastric ulcer that were randomly collected from Imam Reza Hospital in the City of Kermanshah, Iran. Participants in research clearly expressed consent to being involved in the study. Then, the information related to each patient was collected from the registered Office of Pathology in the Hospital.

Selecting the patients was performed randomly, and the only inclusion criterion was gastric ulcer.

### DNA Extraction

DNA extraction was done by the boiling method according to the Protocol. Then, genomic DNA samples (4 µL) mixed with diluted loading dye (2 µL) were electrophoresed on 1.5% agarose gel.

### Measuring DNA Concentration

The optical absorption of extracted DNA samples was measured by NanoDrop (2000/2000C, Thermo, USA) using a ratio of OD260/OD280 to ensure the desired quality of DNA to be used in PCR.

### Molecular Studies

The primers sequences to amplify the *glmM* gene were selected from a previous study (16) and primers for the *cagA* gene were designed by Gene Runner software considering all the points of SNP. To ensure the accuracy of the primers, they were blasted in NCBI database (Table 1). Primers were synthesized by Cinnagen Company (Iran). Molecular study was performed by PCR method using the PCR detection Kit (Cinnaclon). The PCR cycles of the desired genes are presented in Table 2, separately.

Table 1. The primers sequences

Primer name	Primer sequence	Primer connection temperature	Length of the piece
<i>glmM-F</i>	AAGCTTTTAGGGGTGTTAGGGGTTT	58	294bp
<i>glmM-R</i>	AAGCTTACTTTCTAACACTAACGC		
<i>CagA-F</i>	TTGACCAACAACCACAACCGAAGC	55	183bp
<i>CagA-R</i>	TTCCCTTAATTGCGAGATTCC		

**Table 2.** PCR proliferation conditions

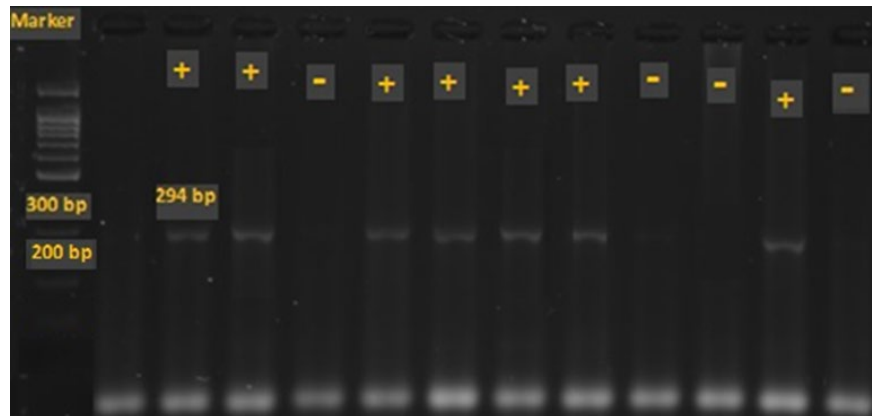
Genes	Cycling condition				
	Initial denaturation	Denaturation	Annealing	Extension	Final Extension
<i>glmM</i>	94 °C	94 °C	58 °C	72 °C	72 °C
	5 Min	30 Sec	60 Sec	30Sec	5 min
Repeated for 35 Cycles					
<i>cagA</i>	94 °C	94 °C	55 °C	72 °C	72 °C
	5 Min	30 Sec	30 Sec	30Sec	5 min
Repeated for 35 Cycles					

### Statistical Analysis

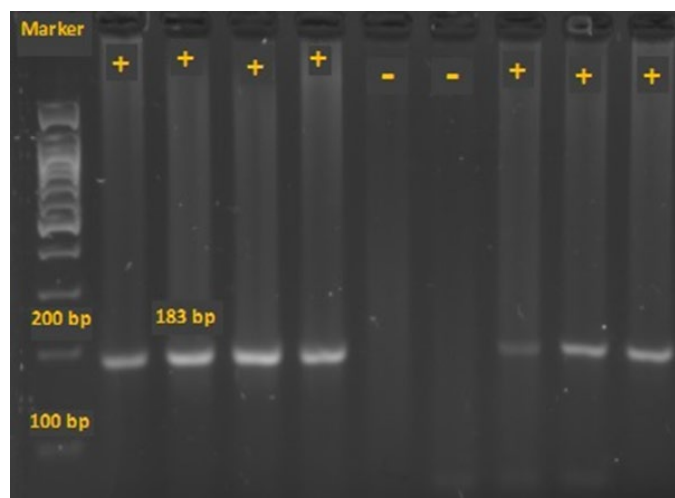
The data were analyzed by Excel software and SPSS 20 (SPSS Inc., Chicago, Ill., USA) and frequency of the genes were studied. Also, the logistic regression test was used to determine the association of each form of the disease with the virulence genes alone. A P-value < 0.05 was considered statistically significant.

The electrophoretic patterns of The PCR products of *glmM* and *cagA* genes were presented in [Figures 1](#) and [2](#), respectively. The length of amplified fragments related to *glmM* and *cagA* genes are 294 and 183 bp, respectively. After confirming *glmM*-positive samples, which indicate the presence of *H. pylori*, these samples were used to monitor and determine the presence of *cagA* gene.

### Results



**Figure 1.** The electrophoretic pattern of the PCR product of *glmM* gene. The length of amplified fragment by *glmM* primers is 294 bp. The first column shows the ladder of 100 bp. The samples of 2, 3 and 5-8 and 11 are positive for *glmM* and 1, 4 and 9, 10, 12 are negative for this gene.



**Figure 2.** The electrophoretic pattern of the PCR product of the *cagA* gene. The length of amplified fragment by *cagA* primers is 183 bp. The first column shows the ladder of 100 bp. The samples of 1,2,3 4 and 7,8,9 are positive for *cagA* and 5 and 6 are negative for this gene.

The results showed that out of 75 samples, 56 were positive for *glmM* gene and out of 56 positive samples (*glmM*-positive), 39 (69.64 %) were positive for *cagA* gene.

Among them, the frequency of *glmM* gene was more in men (29, 38.66%) compared to women (27, 36%), although not significant. Also, regarding *cagA* gene, its frequency was more in men (22, 39.28%) compared to women (17, 30.35%) but not significant (Table 3) (Figure 3).

The correlation between the clinical forms of the disease and the presence of *glmM* and *cagA* genes was found to be significant ( $P=0.005$ ).

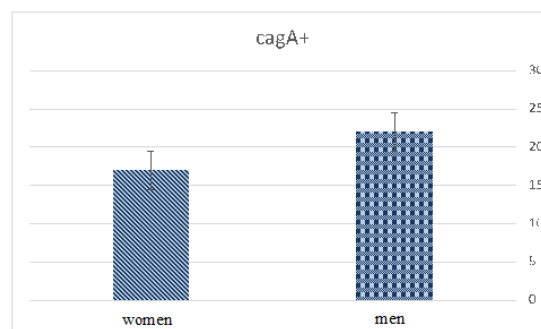


Figure 3. Comparing the frequency of positive *cagA* gene

Table 3. Comparing the frequency of positive *glmM* and *CagA* genes

	GlmM+	GlmM-	CagA+	CagA-
<b>Patients N=75</b>	56 (74.66%)	19 (25.33%)	39 (69.64%)	17 (30.35%)
<b>Man 40 (53.33%)</b>	29 (38.66%)	11 (14%)	22 (39.28%)	7 (12.5%)
<b>Woman 35 (46.66%)</b>	27 (36%)	8 (10.6%)	17 (30.35%)	10 (17.85%)

The study of *glmM* gene among women and men indicated that 23 patients (30.66%) under 40 years old, and 33 patients (44%) over 40 years old tested positive for *glmM* gene that shows the frequency of this gene is more among patients who were over 40 years old. Also, about *cagA* gene, 16 patients (21.33%) under 40 years old and 23 patients (41.07%) over 40 years old were positive for the *cagA* gene that shows

the frequency of this gene is higher in the patients over 40 years old (Table 4).

Also, the relation between age and sex of the patients with clinical forms of *H. pylori* disease was studied using Chi-square and Fisher's exact tests. No significant association was found in this regard.

Table 4. Comparing the frequency of the positive *glmM* and *cagA* genes among people with the age around 40 years old.

Patients	GlmM+	GlmM-	cagA+	cagA-
<b>N=75</b>	56 (74.66%)	19 (25.34%)	39 (69.64%)	17 (30.35%)
<b>Less than 40</b>	23 (30.66%)	11 (14.64 %)	16 (28.57 %)	7 (12.5 %)
<b>More than 40</b>	33 (44 %)	8 (10.70 %)	23 (41.07 %)	10 (17.85 %)

## Discussion

In this research, 75 paraffin blocks of stomach biopsy samples from the patients infected with *H. pylori* and suffering from gastric ulcers were investigated in the province of Kermanshah, Iran. Also, the extra information about these patients was achieved through registered Office of Pathology, Imam Reza Hospital.

The *glmM* gene, which has more sensitivity for *H. pylori* detection compared to the other genes, was used to identify positive *H. pylori* samples (10-11). Out of 75 samples, 56 (74.66%) were positive for the *glmM* gene that indicates the high frequency of *H. pylori*

among patients suffering from gastric ulcer that was in line with the previous studies outcome (8).

In the study of Megrow *et al.*, the prevalence of *H. pylori* or the prevalence of *glmM* gene in the biopsy specimens was 44% (12). In *glmM*-positive samples, the prevalence of *cagA* was 69.64% (39), indicating a high frequency of this gene in these samples. In previous study by Jafari *et al.*, on 280 gastric biopsy samples in Tehran, the frequency of the *cagA* gene was about 74.3% (13).

The prevalence of the *cagA* gene in the studies of Douraqi and Crashia were 84.02% (2) and 41.5% (14),

respectively. These results were different from the previous studies in Europe (66-73%) but identical with the majority of studies in Middle East countries (Turkey, Egypt, Israel, and Jordan) in which the outbreak of the *cagA* gene was between 26 to 44% (7).

In another study by Hussein *et al.*, the frequency of the *cagA* gene was 65.9% along with achieved results from gathered samples over the same gene in Tunisia (61.6%), Iran (76%), and Iraq (71%) (15), and was similar to the results of the present study. In the study by Essawi *et al.*, which was conducted to recognize the existence of *H. pylori* in biopsy samples through PCR, the frequency of this gene was 65.9% (16) that accords with my results.

Then, the patients were studied and compared based on the gender. The findings showed the frequency of *cagA* gene in man at 22 (39.28%) and in women at 17 (30.35%). The frequency of the *cagA* gene was more in men compared to women. It seems that high number of men samples or having more exposure of men to infection or even the gender and some other factors might be effective.

The patients were also compared in terms of their age. The results showed that the level of *cagA* gene frequency in the patients under 40 was 16 (21.33%)

and in the patients over 40, was 23 (41.07%). This determines that the frequency level of this gene among patients over 40 years old is higher. Thus, the frequency of *cagA* gene is higher among people over 40 years because of the existence of these bacteria among the elder patients.

## Conclusion

In the present study, the frequency of the *cagA* and *glmM* genes was investigated in the patients with gastric ulcer in the province of Kermanshah. The results showed high level of *glmM* gene in the studied patients. Therefore, *H. pylori* infection is probably an effective factor to cause gastric ulcer in these patients. Also, the high abundance of *cagA* gene emphasizes the pathogenicity of this gene in positive *H. pylori* patients. Therefore, monitoring this gene is important in assessing the possibility of gastric ulcer disease.

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## Conflict of Interest

The authors declared that they have no competing interests.

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## شیوع ژن *cagA* هلیکوباکتر پیلوری در بیماران مبتلا به زخم معده

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### چکیده

زمینه و اهداف: هلیکوباکتر پیلوری (*H. Pylori*) یکی از دلایل التهاب معده و زخم معده است. این باکتری یک عامل مستعدکننده آدنوکارسینومای معده است. سیتوتوکسین A رمزگذاری شده به وسیله ژن *cagA* یکی از عوامل اصلی شدت بیماری‌زایی این باکتری‌ها بوده و به دلیل تنوع ژنتیکی در مناطق مختلف جغرافیایی از اهمیت ویژه‌ای برخوردار است. هدف از این مطالعه بررسی شیوع ژن *cagA* در بیماران مبتلا به زخم معده بود.

**مواد و روش کار:** هفتاد و پنج نمونه بلوک پارافینی بیوپسی معده به‌طور اتفاقی مربوط به بیماران آلوده با *H. Pylori* و مبتلا به زخم معده در استان کرمانشاه در بهار ۱۳۹۵ بررسی شد. DNA نمونه‌ها با روش جوشاندن استخراج شد و در ادامه نمونه‌های DNA استخراج شده با استفاده از جفت آغازگرهای خاص برای یک منطقه محافظت شده در ژن *glmM* و وجود *cagA* با PCR بررسی شدند.

**یافته‌ها:** از ۷۵ نمونه، ۵۶ مورد (۷۴٪/۶۶) *glmM* مثبت بودند که از بین آنها ۳۹ بیمار (۶۹٪/۶۴) برای ژن *cagA* مثبت بودند. در مقایسه بیماران از نظر جنسیت، فراوانی ژن *cagA* در مردان و زنان به ترتیب ۲۲ (۳۹٪/۲۸ درصد) و ۱۷ (۳۰٪/۳۵ درصد) بود. سرانجام، با مقایسه بیماران از نظر سن، ۱۶ مورد (۲۱٪/۳۳ درصد) جوان‌تر از ۴۰ سال و ۲۳ مورد (۴۱٪/۷ درصد) بزرگتر از ۴۰ سال بودند.

نتیجه‌گیری: نتایج این مطالعه ارتباط معنی‌داری بین فراوانی ژن *cagA* و زخم معده در بیماران مورد مطالعه نشان داد.

**کلید واژه‌ها:** ژن *CagA*، زخم معده، ژن *GlmM*، *H. Pylori*

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