Diagnostic Value of Housekeeping [glmM] Gene Expression in Antral Biopsies in Comparison to Rapid Urease Test and Histological Detection of Helicobacter Pylori Infection

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ABSTRACT

Helicobacter pylori (H. pylori), a common infectious bacterium, has been linked to chronic gastritis, peptic ulcer and gastric cancer. Several invasive and noninvasive techniques are currently used for detecting H. pylori infection. Molecular approaches based on DNA amplification by polymerase chain reaction (PCR) have been developed for the detection of H. pylori in clinical samples but are used only in the research setting. Our objective is to comparatively evaluate the efficiency of using PCR technique for diagnosis of H. pylori infection through determination of glmM gene, and the rapid urease test (RUT), with reference to histological detection of H. pylori as the gold standard. Two hundred gastric biopsy specimens from the antrum of 50 dyspeptic patients were subjected to histology, RUT and PCR; targeting the glmM gene. No age or gender difference regarding H. pylori infection was observed, H. pylori colonization was detected in 82% of cases. The sensitivity and specificity of the RUT and PCR for glmM methods in comparison to histology are as follows: 87.80% and 88.89%; 65.9% and 44.4%, respectively. Histology demonstrating the highest detection rate among cases with superficial gastritis and gastroduodenitis (82% and 80%) compared to RUT (65.5% and 70%) and glmM gene that was expressed in 41.4% and 40% respectively. Colonization of the gastric mucosa by H. pylori was detected in 61.9% of the patients with chronic superficial gastritis, while, RUT was positive in 38.1% of them. Nearly the same percentages were observed for histology and RUT in the other histopathological findings. Similar percentage for glmM gene as RUT was detected in chronic superficial gastritis and in 38.1%, 33.3% of intestinal metaplasia and gastric dysplasia, respectively, while it is not expressed in chronic gastric ulcer and atrophic gastritis. Therefore, we conclude that PCR for glmM gene could be a method with diagnostic value. Nevertheless, histology and RUT were found to be relatively reliable methods for examining gastric biopsy specimens. The low percentage of glmM gene reported here may be attributed to polymorphism and diversity of glmM gene of H. pylori. Therefore, further studies are required to search for the specific sequence of the glmM gene or the presence of mutation in H. pylori strains in Egypt.

Key Words: H. pylori; glmM gene; rapid urease test; PCR.

INTRODUCTION

Helicobacter pylori (H. pylori) is a gram-negative microaerophilic bacterium that infects human gastric epithelial cell surfaces and the overlying gastric mucus, which is a highly specialized niche. It is the major cause of peptic ulcers and a contributor to other illnesses, ranging from childhood malnutrition to gastric cancer.[1-3] H. pylori is chronically infect more than half of the human population worldwide. In developing countries, 70-90% of the populations carry H. pylori, while in developed countries; the prevalence is lower ranging from 20 to 50%, due to the improved socioeconomic conditions.[4-6]

Several invasive and noninvasive techniques are currently used for detecting H. pylori infection, such as rapid urease test (RUT), urea breath test, culture, serological tests and histological methods.[7] All the invasive methods utilize gastric mucosal biopsies for histology, culture, and/or the urease test. However, conventional culture methods is tedious and time consuming and usually underestimates the number of these organisms.[8] Histology has long been considered the “gold standard” in H. pylori diagnosis.[9,10] The major advantage of using it is the assessment of morphological changes of the gastric mucosa.[11]

H. pylori produces substantial amount of a highly active enzyme urease. H. pylori infection is commonly diagnosed by testing of gastric biopsy specimens by the RUT.[11] The sensitivity of testing by RUT is generally
greater than 90% and provides a useful tool for screening of gastric biopsy specimens for the presence of *H. pylori*. However, the interpretation of results can be difficult when ambivalent results are obtained.\(^{(12)}\)

Molecular approaches based on DNA amplification by polymerase chain reaction (PCR) have been developed for the detection of *H. pylori* in clinical samples but are used only in the research setting.\(^{(13)}\) Several genes have been used to detect and identify *H. pylori*. However, *H. pylori* is a microorganism with marked genetic diversity. Therefore, genetic identification by using housekeeping *H. pylori* genes is needed to accurately identify *H. pylori* and evaluate the gastroduodenal diseases resulting from *H. pylori* infection. For the genetic identification of *H. pylori*, several PCR methods that employ the 16S rRNA gene, *rpoD*, *ureA*, *ureB*, and *ureC* have been used. Among them, *ureC* PCR is known to be specific to *H. pylori*.\(^{(14,15)}\)

PCR-based detection of the *ureC* gene appears to be the most promising for detection of *H. pylori*.\(^{(15)}\) The *ureC* gene, renamed *glmM* by De Reuse et al., in 1997\(^{(16)}\), encodes phosphoglucomutase mutase, which is unrelated to urease production. It is an enzyme catalyzing the inter-conversion of glucosamine-6-phosphate into glucosamine-1-phosphate, which is subsequently transformed into N-acetylglycosamine. Therefore, *H. pylori* *glmM* is essential for cell growth. *glmM* gene is a housekeeping gene that is present in all *H. pylori* strains. Its sequence appears to be relatively well conserved between strains.\(^{(15,16)}\)

The goal of the research described here is to comparatively evaluate the efficiency of using PCR technique for diagnosis of *H. pylori* infection through determination of *glmM* gene and the other diagnostic methods (RUT and histology) in order to determine whether it is of value in detecting *H. pylori* in gastric biopsy specimens.

**SUBJECTS AND METHODS:**

**Patients**

The study group was formed of fifty patients (30 males, 20 females; age range: 25-68 years, mean age 44.4 ± 10.17 years) attending the Endoscopy Unit, Medical Research Institute, Alexandria University between October 2007 – 2008. They underwent endoscopy owing to clinical symptoms attributable to upper gastrointestinal tract. Patients were fasted overnight or for at least four hours before endoscopy. Patients who received antibiotics, H2-receptor antagonists or proton pump inhibitor during the last three weeks prior to endoscopy, were excluded from the study to avoid inconclusive or false negative results. An informed consent was taken from each patient and the study was approved by the Ethics Committee of the Medical Research Institute-University of Alexandria. All clinical specimens were tested under code.

**Endoscopic examination of the upper gastrointestinal tract**

Upper gastrointestinal endoscopy was performed with a long forward viewing instrument (Fuji:-EG 250D video – endoscopy). The stomach was completely examined and all the endoscopic findings were registered.

**Gastric biopsies sampling and processing**

Four biopsies were taken from the antral mucosa of the stomach of each patient from an area that is not eroded, denuded or ulcerated. One biopsy was used for carrying out a commercially available RUT (Pronto Dry®, Medical Instrument Corp, France). Pronto Dry consists of a dry filter paper containing urea, phenol red (a pH indicator), buffers and a bacteriostatic agent in a sealed plastic slide. If the urease enzyme of *H. pylori* is present in an inserted tissue sample, the resulting decomposition of urea causes the pH to rise and the color of the dot turns from yellow to a bright magenta. The second biopsy was used for histological examination to grade the severity of gastritis and *H. pylori* density.\(^{(10)}\) It was fixed directly in 10% buffered formalin and embedded in paraffin. The paraffin sections were stained using Hematoxylin and Eosin (H&E) stain. The mucosal specimens were evaluated histopathologically for the presence of neutrophil infiltration, chronic lymphocytic inflammation, surface epithelial damage, atrophy, intestinal metaplasia and lymphoid follicles. *H pylori* density based on histological examination. The density of *H. pylori* colonization and morphotypes in each specimen were evaluated and graded as no bacteria (grade 0), occasional bacteria found after searching and scattered bacteria in most high power fields (grade 1) and occasional groups of numerous bacteria (grade 2). The other two biopsies were required to carryover the conventional PCR assay for detection of *glmM* gene of *H. pylori*. All biopsies tested by PCR were stored directly at -20°C in 70% ethanol in eppendorf tubes until processed. The frozen biopsy specimens were
crushed and suspended in 200 µl of TE buffer for DNA extraction.

**PCR for glmM gene amplification and detection**

**DNA extraction:** DNA was extracted from the frozen gastric biopsy specimen using Genomic DNA purification kit, Fermentas Co., Lithuania according to the manufacturer’s instructions.

**PCR amplification:** The presence of the glmM gene was assessed by PCR using primers, the reaction mixture {2x PCR Master mix (dNTPs [0.4 mM of each dATP, dCTP, dGTP, dTTP], 0.05u/µl Taq DNA polymerase, reaction buffer) and Nuclease-free water}, and thermal cycling [Bioer- little Genius thermal cycler]. A set of primers (forward primer, 5`-AGG CTT TTA GGG GTG TTA GGG GTT T-3`; and reverse primer, 5`-AAG CTT ACT TTC TAA CAC TAA CGC-3`) were used to amplify the glmM DNA (294 bp) (17). To each PCR tube the following were added (final volume, 25µl): 6µl of the extracted DNA, 12.5 µl of master mix, 1.5 µl of forward primer, 1.5 µl of reverse primer, and 3.5 µl of nuclease free H2O. Then tubes were transferred to the thermal cycler for amplification. The PCR mixture was subjected to 35 amplification cycles. PCR conditions were as follows: An initial denaturation (94°C, 2min), followed by 35 cycles of denaturation (94°C, 2min), annealing (55°C, 2min), and extension (72°C, 2min), with a final extension (72°C, 10 min).

**Detection:** The amplicons were analyzed with 2% (wt/vol) ethidium bromide stained agarose gel. The DNA bands were visualized on a 302 nm UV transilluminator. The gel was examined for bands of 294 bp as determined by the molecular weight marker runs at the same time.

**Statistical analysis**

Statistical analysis was done using statistical package for social sciences (SPSS Inc., version 17). The data were analyzed by Fisher's exact and chi-square tests, p values less than 0.05 was considered to be statistically significant. Results are presented as mean ± standard deviation for quantitative variables and numbers (percentages) for qualitative variables. Sensitivity, specificity, positive and negative predictive values were calculated by two by two standard method.

**RESULTS**

**Endoscopic findings**

Endoscopic examination was done for esophagus, stomach and duodenum. Lesions as gastric ulcers, gastritis, duodenal ulcers, reflux esophagitis and masses were noted and recorded. The endoscopic findings for the 50 patients included in the present study were as follows: Superficial gastritis was recorded in most of the cases (58%), while gastric, duodenal ulcers and mild esophagitis were recorded each in 4% of cases. On the other hand; gastroduodenitis, duodenal ulcer associated with reflux esophagitis (GERD) and atrophic gastritis were reported in 20%, 8% and 2% respectively.

**Histological grading of H. pylori infection of the studied antral gastric biopsies:**

*H. pylori* colonization was detected histologically in 41/50 (82%) of cases. The highest percentage of them 31/41 (75.6%) showed grade 1. While 10/41 (24.4%) showed grade 2. On the other hand, *H. pylori* colonization was not detected in 9/50 (18%) of cases. *H. pylori* in gastric pits are demonstrated in Figure (1).

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**Fig.1.** Demonstration of *H. pylori* by H&E stain x1000 (High power view, immersion). Gastric pits show numerous spiral- shaped and coccoid forms of *H. pylori*. 
Age distribution of *H. pylori* positive and negative cases according to the histological findings:

The highest percentage of *H. pylori* infection was among both age groups, from 40 to 49 and from 50 to 59 (87.5%), while the lowest percentage of infection was in the age group from 30 to 39 (66.7%). This difference was found to be statistically insignificant (P > 0.05) [Table 1].

Gender distribution of *H. pylori* positive and negative cases according to the histological findings:

Out of 30 studied males; 25(83.3%) showed positive histological results, while 5(16.7%) showed negative results. For the studied females; 16/20 (80%) showed positive histological results, while 4(20%) showed negative results. This difference was found to be statistically insignificant (P> 0.05) [Table 2].

### Table (1): Relation between Age and Histological findings of *H. pylori* infection

<table>
<thead>
<tr>
<th>Age</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>20 - 29</td>
<td>3</td>
<td>75</td>
<td>1</td>
</tr>
<tr>
<td>30 - 39</td>
<td>6</td>
<td>66.7</td>
<td>3</td>
</tr>
<tr>
<td>40 - 49</td>
<td>21</td>
<td>87.5</td>
<td>3</td>
</tr>
<tr>
<td>50 - 59</td>
<td>7</td>
<td>87.5</td>
<td>1</td>
</tr>
<tr>
<td>60 - 69</td>
<td>4</td>
<td>80</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>82</td>
<td>9</td>
</tr>
</tbody>
</table>

### Table (2): Relation between Gender and Histological findings of *H. pylori* infection

<table>
<thead>
<tr>
<th>Gender</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Female</td>
<td>16</td>
<td>60</td>
<td>4</td>
</tr>
<tr>
<td>Male</td>
<td>25</td>
<td>83.3</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>82</td>
<td>9</td>
</tr>
</tbody>
</table>

Association between RUT results and histological detection of *H. pylori*

Out of 41 positive cases by histology, only 36 (87.80%) were also positive by RUT. On the other hand, out of 9 that were negative by histology, 8 (88.89%) were negative by RUT. This association was found to be statistically significant. (p=0.0001). By comparing the results of RUT to the histological results, it was found that, this test showed sensitivity of 87.80%, specificity of 88.89%, positive predictive value (PPV) of 97.23%, negative predictive value (NPV) of 61.54%, and accuracy of 88.3% [Table 3].
Table (3): Rapid Urease Test results versus Histological detection of H. pylori.

<table>
<thead>
<tr>
<th>Rapid Urease Test</th>
<th>Histology (H. pylori)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>36 (87.8)</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>Negative</td>
<td>5 (12.2)</td>
<td>8 (18.9)</td>
</tr>
<tr>
<td>Total</td>
<td>41 (82)</td>
<td>9 (18)</td>
</tr>
</tbody>
</table>

**Amplification of glmM gene of H. pylori by PCR**

The amplification of glmM gene of *H. pylori* was done for the extracted DNA from all tissue biopsies. The results revealed that 18 (36%) out of 50 tested samples showed clear bands at 294 bp using 2% agarose gel electrophoresis (Fig. 2).

![Fig. 2. Ethidium bromide stained agarose gel showing bands of amplified PCR products for glmM gene of H. pylori from the gastric biopsies: Lane (1) marked DNA molecular weight marker, lanes (2, 3 and 5) marked negative cases, while lanes (4, 6) marked positive cases showing amplified bands of 294 bp.](image)

**Association between glmM gene amplification results and histological detection of H. pylori**

Out of 41 positive cases by histology, 14 (34.1%) were also positive by the PCR test for glmM gene. On the other hand, the 5 negative cases for glmM gene by PCR test were also negative by histology. This association was found to be statistically insignificant (p>0.05).

As the PCR test results for glmM gene compared to the histological ones, it was found that, glmM gene detection showed sensitivity of 65.9%, specificity of 44.4%, positive predictive value (PPV) of 84.4%, negative predictive value (NPV) of 22.2%, and accuracy of 55% [Table 4].
Table (4): PCR amplification of glmM gene versus Histological detection of H. pylori.

<table>
<thead>
<tr>
<th>PCR amplification of glmM gene</th>
<th>Histology (H. pylori)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>n(%)</td>
<td>n(%)</td>
</tr>
<tr>
<td>Positive</td>
<td>14(34.1)</td>
</tr>
<tr>
<td>Negative</td>
<td>27(65.9)</td>
</tr>
<tr>
<td>Total</td>
<td>41(82)</td>
</tr>
</tbody>
</table>

Endoscopic findings in relation to histology, RUT and glmM amplification results

Histology demonstrating the highest detection rate among cases with superficial gastritis and gastroduodenitis (82.14% and 80% respectively). On the other hand, glmM was expressed in these cases in 41.4% and 40% respectively and in 50% of cases with duodenal ulcers associated with GERD, while it is not expressed in the other endoscopic findings [Table 5].

Table (5): Endoscopic findings in relation to histology, RUT and glmM amplification

<table>
<thead>
<tr>
<th>Endoscopic Findings</th>
<th>Histology (H. pylori)</th>
<th>RUT</th>
<th>glmM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superficial Gastritis</td>
<td>23(82.14)</td>
<td>19(65.5)</td>
<td>12(41.4)</td>
</tr>
<tr>
<td>Gastroduodenitis</td>
<td>8(80)</td>
<td>7(70)</td>
<td>4(40)</td>
</tr>
<tr>
<td>Gastric ulcers</td>
<td>2(100)</td>
<td>2(100)</td>
<td>0</td>
</tr>
<tr>
<td>Duodenal ulcers</td>
<td>2(100)</td>
<td>2(100)</td>
<td>0</td>
</tr>
<tr>
<td>Atrophic Gastritis</td>
<td>1(100)</td>
<td>1(100)</td>
<td>0</td>
</tr>
<tr>
<td>Duodenal ulcers with GERD</td>
<td>4(100)</td>
<td>4(100)</td>
<td>2(50)</td>
</tr>
<tr>
<td>Mild esophagitis</td>
<td>2(100)</td>
<td>2(100)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>37</td>
<td>18</td>
</tr>
</tbody>
</table>

Histopathological findings in relation to histology, RUT and glmM amplification results

Histopathological examination for the antral biopsies revealed that 21(42%) cases had chronic superficial gastritis, 2(4%) chronic active gastritis, 1(2%) atrophic gastritis, 21(42%); intestinal metaplasia, 3(6%); gastric dysplasia and 2(4%) gastric ulceration. Colonization of the gastric mucosa by H. pylori was detected in 61.9% of the patients with chronic superficial gastritis, while it was detected in all the other histopathological findings. On the other hand, RUT showed positive results in 38.1% of chronic superficial gastritis cases and nearly the same findings as histology were observed for the RUT in the other histopathological findings. Regarding glmM gene, similar percentage as RUT was detected among chronic superficial gastritis, however, it was expressed in 50% with chronic active gastritis, 38.1% with intestinal metaplasia and 33.3% with gastric dysplasia, while it is not expressed in chronic gastric ulcer and atrophic gastritis [Table 6].
DISCUSSION

*H. pylori* is one of the most prevalent infections in the world. Infection occurs mainly in childhood and infected individuals usually carry it for life unless treated. Epidemiology of infection by *H. pylori* has been characterized by a linear increase with age in western industrial countries and by a large number of children and juveniles being infected in developing countries. In the developed world, the prevalence rates vary from 1.2% to 12.2%. The incidence of new *H. pylori* infections among adults in the Western world is less than 0.5% per year. The infection is steadily declining in developed countries. In developing countries, the prevalence rates are much higher almost 90% of the population acquires infection with *H. pylori* is almost within the first 5 years of life, before the age of 10 years. The prevalence of the infection is correlated with low socioeconomic status during childhood, high density of living, and low household income. The incidence of *H. pylori* in healthy people in their 3rd decades is 10% while the incidence of *H. pylori* rises to over 60% among people in their 6th decades. The present work was carried out on 50 patients, their age ranges between 25 to 68 years. The prevalence of *H. pylori* was 82%. This is in accordance with the previously mentioned studies and verifies that infection in developing countries is seen nearly in middle age people. Similar result also reported in Japan by Yamagata et al. The present study also revealed that *H. pylori* infection can be acquired at any age regardless sex difference. Similar results were reported by Naomi et al. who found that there is no significant difference in infection at any age and between both sex groups. On the other hand, Replogle et al. and Hafez et al. reported that male sex was a significant risk factor for infection. It is possible that the risk of acquisition is not different between the two sexes but that females are more likely to have *H. pylori* infection eradication with antimicrobials used for other illnesses, such as genitourinary infections.

*H. pylori* infection results in chronic gastritis, ulcer, mucosal associated lymphomas and gastric carcinomas. However, only a minority of infected patients develops such severe diseases, and most infected individuals are asymptomatic throughout life. Such variations in clinical outcome may be due to the considerable genetic diversity of the *H. pylori* strains that cause infection, although host factors may also be important for the development of disease.

In view of the role played by *H. pylori* in the various processes mentioned above, it is critical to set up diagnostic tests which are both sensitive and specific enough to enable detection of *H. pylori*. There are several tests available for the diagnosis of *H. pylori* colonization, each with their specific advantages.
and limitations. Currently, histology is considered the gold standard for diagnosing *H. pylori* infection.\(^{(3,9)}\)

In the present study, invasive tests were applied including, rapid urease test, histology and conventional PCR for *glmM* gene of *H. pylori* with respect to the detection of *H. pylori* in the histological sections as a gold standard. The rapid urease tests are the most widely used method to detect *H. pylori* infection. They can be performed readily in the endoscopy suite and give a rapid result. There are different urease media commonly utilized in bacteriology.\(^{(37)}\)

In the current study, the sensitivity and specificity of rapid urease test was 87.8% and 88.9% respectively. Our results are in accordance with that of Morio et al.,\(^{(38)}\) who found that the same test showed a sensitivity of 84.4% and a specificity of 98%. Nearly similar results were reported by Cutler et al.,\(^{(39)}\) and Monteiro et al.,\(^{(40)}\) who found that the sensitivity of rapid urease test was 85% and 90% respectively. A higher sensitivity and specificity of rapid urease test was reported by Said et al.,\(^{(41)}\) who record sensitivity of 98%, specificity of 100% and accuracy of 97% also, by Chiu et al.,\(^{(42)}\) and van Keeken et al.,\(^{(43)}\) who reported that RUT is considered highly accurate with a sensitivity and specificity over 90%. On the other hand, a lower sensitivity of rapid urease test was reported by Ho et al.,\(^{(44)}\) (62.7%), also by Adesanya et al.,\(^{(45)}\)

In recent years, diagnostic laboratories have been concerned with reducing the time required for diagnosis of *H. pylori* infections. Different methods based on molecular biology techniques have therefore been developed for identification of *H. pylori* from clinical specimens, with those based on DNA amplification being the most rapid and sensitive. PCR analysis of *H. pylori* has several advantages. First, it does not rely on culturing. Second, many samples can be analyzed quickly. The *glmM* is a housekeeping gene that is present in *H. pylori* strains. There is one copy of the *glmM* gene per *H. pylori* organism.\(^{(15,16)}\)

In the present study, *glmM* gene was detected in 36% of cases and show sensitivity of 44.4% and specificity of 65.85%, recording a percent detection of 41% in patients with superficial gastritis. Similar results were reported by Lage et al.,\(^{(46)}\) who detect *glmM* gene in 38.5% of 104 American consecutive dyspeptic patients. Also, Brooks et al.,\(^{(47)}\) found that PCR, targeting the *glmM* gene detected *H. pylori* in the biopsy specimens from 44% of studied patients. Moreover, Lim et al.,\(^{(48)}\) detected *glmM* by PCR in 48.8% of the studied cases. On the other hand, Linpisarn et al.,\(^{(49)}\) reporting that in comparison to the results obtained from histology and the urease test, sensitivity was 61.5% and 84.0% for the *glmM* gene, respectively. In addition, Salehi et al.,\(^{(50)}\) reported that among patients with gastric ulcer, gastritis, and duodenal ulcer, 91.7% *glmM* PCR-positive biopsy specimens were detected. Several authors\(^{(51-53)}\) reporting higher sensitivity and specificity and concluding that *glmM* is of the most specific and most appropriate diagnostic method for *H. pylori* detection in clinical specimens.

The diagnosis of *H. pylori* in atrophic gastritis is a challenge since all invasive and almost all non-invasive tests have been shown to have poor sensitivity in this connection\(^{54-57}\) and this agree with our finding as\(^{(58)}\) who showed that even simple careful visual evaluation of the mucosa and the diagnoses of erythema and oedema correlated well with *H. pylori* infection.

Go et al.,\(^{(59)}\) reporting that the genetic diversity in *H. pylori* may be sufficient to classify *H. pylori* strains into four or more species. However, the similarity of strains within a cluster was rather low and varied between 30 and 70%. In addition, a similar analysis revealed that no clustering among 74 *H. pylori* isolates occurred and a very high mean genetic diversity was found.

Almost every *H. pylori* strain from the patients living in different geographic regions had its own unique DNA sequence. Multicolonization is more frequent in countries in which *H. pylori* infection is highly prevalent. Furthermore, genetic recombination events occurred between strains. Intragenic recombination events occurred during the evolution of the *glmM* gene, which resulted in mosaicism as has previously been reported for some housekeeping genes.\(^{(60)}\)

Based on the previous studies, the low percentage of *glmM* gene reported in the present work may be attributed to a high polymorphism and diversity of *glmM* in different species of *H. pylori*.
Several mechanisms, such as point mutations, intragenic recombination and introduction of foreign alleles may enhance this strain diversity.

Therefore, we conclude that infection with *H. pylori* can be acquired at any age with no age or gender difference. PCR for *glmM* gene could be a method with diagnostic value. Nevertheless, histology and RUT were found to be relatively reliable methods for examining gastric biopsy specimens. The low percentage of *glmM* gene reported in the present work may be attributed to polymorphism and diversity of *glmM* gene of *H. pylori*. Therefore, further studies are required to search for the specific sequence of the *glmM* gene or the presence of mutation in *H. pylori* strains in Egypt.

REFERENCES


59. Go MF, Kapur V, Graham DY, Musser JM. Population genetic analysis of