PCR for Diagnosing *Helicobacter pylori* Infection in Moroccan Patients: Comparison with ELISA and Histopathological Tests

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Abstract: *Helicobacter pylori* (H. pylori) infection is a real public health problem. In Morocco, 70% of the populations are infected by this pathogen. Several techniques are used for the detection of *H. pylori* infection. In Morocco, histopathological examination and enzyme-linked immunosorbent assay (ELISA) are the most commonly used tests. Our aim is to compare the sensitivity and the specificity of these two techniques with the polymerase chain reaction (PCR) which is not used currently in Morocco. In this study, biopsies of 60 chronic gastritis patients were used for histopathological examination and for the detection of ureA gene by Nested-PCR. Blood samples were used for ELISA. *H. pylori* was detected in 57 (95%) patients by histopathological examination, 60 (100%) by PCR and 46 (77%) by ELISA. Sensitivity and specificity are calculated using Histopathological examination and PCR findings as gold standard. ELISA test had the lowest specificity (79%), followed by histopathological examination (95%), whereas PCR had the highest specificity (100%). The use of PCR test showed a good result in term of sensitivity and detection. Then, it should be recommended to use PCR for routine diagnosis of *H. pylori* infection.

Keywords: *Helicobacter pylori*, Histopathological examination, PCR, ELISA, ureA gene

1. Introduction

*H. pylori* is considered as a causative agent in the development of several gastric diseases such as chronic gastritis, peptic and gastric ulcers, gastric carcinoma, and mucosa associated lymphoid tissue (MALT)-type lymphoma. It’s a gram negative, spiral-shaped, microaerophilic bacterium that is present in the human stomach of approximately 50% of the world’s population [1]. Recently in 2017, the World Health Organization classified *H. pylori* among the 12 families of bacteria that represent the greatest threat to human health due to their increasing resistance to antibiotics [2]. In Morocco, *H. pylori* infection represents a real public health problem and affects about 70% of the population and its resistance to clarithromycin reached 28.2% [3]-[4]-[5].

In fact, the detection of *H. pylori* even before or after treatment is a key to avoid the severity of gastric mucosa inflammation, leading to the development of preneoplastic lesions and then gastric cancer. For this purpose, numerous invasive and non-invasive methods have been developed for the diagnosis of *H. pylori* infection. Invasive methods need biopsy samples obtained by gastrointestinal endoscopy and they include histopathological examination, culture, rapid urease test, and molecular detection of *H. pylori* using polymerase chain reaction (PCR). Non-invasive methods don’t need biopsies and include urea breath test, serological tests, and stool antigen test. There is no gold standard method for the detection of *H. pylori* infection, however, culture and histopathological examination remain the most widely used techniques [6]-[7]-[8].

The aim of this study is to compare the performance of three methods for the detection of *H. pylori* infection; histology examination, PCR and ELISA, in term of sensitivity and specificity, in Moroccan patients with chronic gastritis.

2. Material and methods

2.1. Patients and ethics

A total of 60 patients consulting in gastroenterology service of Ibn Roch University Hospital Center and suffering from chronic gastritis were included in this study. All individuals participant were informed about their inclusion in the study and agreed to it in a writing form.

From all the patients, 6 biopsies (2 antrum, 2 fundus, 2 lesser curvature) and 1 blood sample have been sampled. Biopsies were used for histopathological examination and for detection of ureA gene by PCR. Blood samples were used to detect IgG antibodies against *H. pylori*.

2.2. Histopathological analysis

The biopsy samples were transported in 10% formalin. Paraffin embedded and multiple histological sections were obtained from each biopsy. Biopsy sections were then obtained and stained with hematoxylin and eosi and then with Giemsa for the detection of *H. pylori*. The blades were read by a pathologist.

2.3. Extraction of genomic DNA

Human and bacterial DNA was extracted from gastric biopsies using a PureLink Genomic DNA kit (Invitrogen, Thermofisher) according to manufacturer’s instructions. The
quantity and purity of DNA were checked by 0.8% agarose gel electrophoresis and also by the ratio of optical density (OD) at 260 nm and 280 nm using Nanodrop (NanoVue plus). Then it was stored at -20 °C until use.

2.4. Detection of H. pylori ureA gene by Nested-PCR

A Nested-PCR was used to detect H. pylori ureA gene in biopsy samples. Two sets of primers were used in order to amplify the ureA gene (Table 1). The first PCR amplified a 314 bp fragment of the ureA gene and the second PCR amplified a 206 bp fragment [9].

The two PCRs were performed in a 20 µl final volume with 2 µl PCR Buffer (1X), 0.6 µl MgCl₂ (50 mM) 0.4 µl dNTP (10 mM), 0.3 µl of each primer (20 µM) and 1U Taq polymerase (Invitrogen). 2 µl DNA samples were added to the PCR reaction mixture, and the tubes were placed in a thermal cycler (Applied Biosystems).

The amplification conditions for the two PCRs were: one cycle of initial denaturation at 94°C for 10 min, 35 cycles of denaturation at 94°C for 30 sec, annealing at 57°C for 30 sec, one cycle of elongation at 72°C for 1 min, and one cycle of final elongation at 72°C for 7 min.

2.5. Serum ELISA

Blood samples were centrifuged, and sera were stored at -20 °C for later analysis. IgG commercial ELISA kit (EUROIMMUN) was used to detect the presence of H. pylori IgG antibodies according to the manufacturer’s instructions.

2.6. Statistical analysis

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for all tests. All analyses were performed using IBM SPSS Statistics software (version 22).

3. Results

In the present study, histopathological analysis findings and PCR findings were accepted as gold standard. The detection of H. pylori infection in at least one of the two tests was considered as H. pylori positivity. Negative result in both tests was accepted as H. pylori negativity.

The population is constituted by 29 (48%) males and 31 (52%) females. The mean age was 47.73 ± 17 (Table 2).

According to histopathological analysis and PCR, H. pylori infection was determined in 60 (100%) patients in both of the two tests. For histopathological analysis, H. pylori positivity was determined in 57 (95%) patients and H. pylori negativity was determined in 3 (5%) patients. For the PCR, H. pylori infection was diagnosed in 60 (100%) patients (Table 3).

3.1. Histopathological analysis

Among 60 patients, H. pylori positivity was detected in 57 (95%) patients and H. pylori negativity was detected in 3 (5%) patients. The sensitivity and positive predictive values were 95% and 100%, respectively.

3.2. Detection of ureA status

The presence of ureA gene was detected in all the 60 patients. The use of PCR for the detection of H. pylori in the 3 patients, judged negative by histopathological analysis, showed the positivity of all these 3 negative patients. The sensitivity and PPV of PCR were both 100%.

3.3. ELISA

H. pylori positivity by serology was detected in 46 (77%) patients and H. pylori negativity was detected in 14 (23%) patients. The sensitivity, specificity, positive and negative predictive values were 79%, 67%, 98% and 14%, respectively (Table 4).

4. Discussion

As H. pylori infection becoming a serious issue for human health, many diagnostic methods have been developed for its diagnosis. The use of non-invasive methods, have been suggested for the detection and treatment of H. pylori in patients with dyspeptic complaints and without alarm.

**Table 1: PCR primers sets**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR1 (314 bp)</td>
<td>F- 5’-ATATTATGAAAGAGGAGCCGAGAC-3’ R- 5’-ATGGAAGTGATGAGGCGATTTG-3’</td>
</tr>
<tr>
<td>PCR2 (206 bp)</td>
<td>F- 5’-CATGAAGTGGGTATTGAAGC-3’ R- 5’-ATGGAAGTGATGAGGCGATTTG-3’</td>
</tr>
</tbody>
</table>

**Table 2: Description of the population**

<table>
<thead>
<tr>
<th>Gender</th>
<th>Number of cases (%)</th>
<th>Mean ± SD</th>
<th>Min - Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>29 (48%)</td>
<td>49.70 ± 18</td>
<td>23 – 85</td>
</tr>
<tr>
<td>Female</td>
<td>31 (52%)</td>
<td>45.76 ± 16</td>
<td>21 – 81</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>47.73 ± 17</td>
<td>21 – 85</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tests</th>
<th>Sensitivity (%)</th>
<th>Specificity* (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histopathology</td>
<td>95</td>
<td>100</td>
<td>100</td>
<td>–</td>
</tr>
<tr>
<td>PCR</td>
<td>100</td>
<td>–</td>
<td>100</td>
<td>–</td>
</tr>
<tr>
<td>ELISA</td>
<td>79</td>
<td>67</td>
<td>98</td>
<td>14</td>
</tr>
</tbody>
</table>

*Specificity could not be calculated in histopathology and PCR because there were no true-negative cases. 

**Table 3: Distribution of H. pylori infection according to different methods of detection**

<table>
<thead>
<tr>
<th>Tests</th>
<th>n* (%)</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histopathology</td>
<td>57 (95)</td>
<td>3 (5)</td>
</tr>
<tr>
<td>PCR</td>
<td>60 (100)</td>
<td>0</td>
</tr>
<tr>
<td>Histopathology + PCR</td>
<td>60 (100)</td>
<td>0</td>
</tr>
<tr>
<td>ELISA</td>
<td>46 (77)</td>
<td>14 (23)</td>
</tr>
</tbody>
</table>

n: number of cases.
symptoms in the populations with high seroprevalence rates. The use of invasive methods requiring endoscopic biopsy has been suggested in the presence of alarm symptoms (namely epigastric pain, severe vomiting, unexplained weight loss, unexplained anemia), in case of impossibility to eradicate the bacteria despite treatment, and in the presence of family history of gastric cancer[10]-[11]. Histopathology among invasive methods and ELISA among noninvasive methods are the most frequently used methods in the world and in Morocco.

In our study, histopathological analysis was evaluated as a gold standard together with PCR. We found that sensitivity of histopathological analysis was 95%. According to several studies, the sensitivity of the histopathological examination varies from 90 to 100%. For instance, Monteiro et al and Cosgun et al found that the sensitivity of the histopathological examination was 95.6% and 94.8%, respectively[12]-[13]. Our findings are consistent with these studies. Among the 60 gastritis patients included in this study, H. pylori positivity was found in 57 (95%) patients, whereas H. pylori negativity was found in 3 (5%) patients. Of this 3 histopathological negative patients, H. pylori ureA gene was detected by PCR in all of this 3 patients. Then, these patients were evaluated as false negative results for histopathological analysis. We can explain this by the fact that two of these 3 patients were receiving an eradication treatment against H. pylori one year ago, which could lead to a decrease in bacterial load, and thus complicated the detection of H. pylori by the pathologist.

The use of molecular tests, particularly the PCR, for the diagnosis of infectious diseases became frequent in many laboratories. PCR offer many advantages such as rapidity, specificity and also enable to identify H. pylori in several types of samples (gastric biopsy, gastric juice, saliva, and dental plaque). To date, many PCR methods have been developed to detect H. pylori and a variety of genes including the ureA gene have been used as targets. In our study, the use of Nested-PCR for the amplification of ureA gene revealed 100% of sensitivity and PPV. These results are superior to those found by Cosgun et al who determined 88.7% and 97.1% of sensitivity and specificity, respectively. They used a Nested-PCR for the amplification of the 23S rRNA [13]. Ramis et al, who used the ureA gene for the detection of H. pylori, found that the sensitivity and PPV of the PCR were 100 and 66.67%, respectively [14]. All these results clearly show that the sensitivity of PCR varies from one study to another. This difference can be explained by the use of different sets of primers as well as the established protocol. Nevertheless, PCR remains a reliable technique for the diagnosis of H. pylori infection since its sensitivity is above 70%.

It should be noted that in our study, the three patients diagnosed negative for H. pylori infection by histopathological examination were detected positive by Nested-PCR. This shows that PCR is a technique that we recommend to use in addition to histopathological examination to confirm the negativity of the infection.

Noninvasive testing for H. pylori has been strongly recommended as it is less expensive and more patient-friendly than invasive testing that requires endoscopy [15]. Serology was one of the first methods used for diagnosis of H. pylori infection. In the populations with high seroprevalence rates, serological methods have been suggested to be used as the first method if urea breath test and fecal antigen test are not available. In our study, the sensitivity, specificity, PPV and NPV of ELISA IgG method were 79%, 67%, 98% and 14%, respectively. In the study conducted by Cosgun et al, the sensitivity and specificity of ELISA IgG method were determined to be 86.2% and 40%, respectively[13]. In the study by Pourakbari et al, in which H. pylori was diagnosed in children and adults, the sensitivity and specificity of ELISA IgG assay were found to be 29% and 91% in children and 62% and 80% in adults, respectively[16]. All these different results show that serology cannot be used alone for the diagnosis of H. pylori infection since the sensitivity and the specificity of this technique vary widely from one study to another.

5. Conclusion

Among the techniques we used, histopathological analysis and PCR showed good results in terms of sensitivity. Serology was the lowest among all tests in term of sensitivity. Therefore, it would be recommended to combine the histopathological analysis and PCR together in order to establish more accurate diagnosis of H. pylori infection. It is also advisable to use ELISA as a last resort, if all other tests are not available.

6. Future scope

Increasing the size of the population is desirable in order to further confirm the obtained results.

References


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