EFFICACY OF SONICATED AND ACID-EXTRACTABLE ANTIGENS IN THE SERODIAGNOSIS OF H. PYLORI INFECTION IN PEPTIC ULCER PATIENTS

*N Parimala, M Ishaq

Abstract

_Helicobacter pylori_ is implicated in causation of peptic ulcers and gastric cancer and plays a pivotal role in gastric pathophysiology. In the present study we evaluated the relative efficacy of sonicated and acid-extractable antigens in the serodiagnosis of _H. pylori_ infection in peptic ulcer patients by ELISA. In the present study we evaluated the relative efficacy of sonicated and acid-extractable antigens in the serodiagnosis of _H. pylori_ infection in peptic ulcer patients by ELISA. The two types of antigens mentioned above were prepared from _H. pylori_ subcultures following appropriate procedures. Sera were collected from 13 subjects of whom eight were diagnosed to be suffering from duodenal ulcer (DU) and five from non-ulcer dyspepsia (NUD) and screened for the presence of anti _H. pylori_ antibodies by ELISA. A case was considered seropositive, if the OD value was more than or equivalent to twice the mean OD value of blank. Analysis of our results showed that, with acid extractable antigen at a concentration of 2 mg/mL, 12 cases were seropositive. Contrastingly, with sonicated antigen, at a concentration of 2 mg/mL only eight cases were positive. It is concluded from this study, that the use of relatively purified antigens like acid extractable antigens enhances the sensitivity and specificity of this serodiagnostic test, indicative of its relatively higher efficacy over sonicated lysate containing multiple antigens.

Key words: _Helicobacter pylori_, sonicated antigen, acid extractable antigen, ELISA

The discovery of _Helicobacter pylori_ has brought a fundamental change in understanding the aetiopathogenesis of peptic ulcer disease. It is known to play a vital role in gastric pathophysiology. _H. pylori_ causes chronic active gastritis and is implicated as one of the prime pathogenic factors in peptic ulcer disease and is surmised to have a role in pathogenesis of gastric carcinoma.

Discovery of _H. pylori_ as gastric pathogen has provided much impetus for researchers to understand its complicity in causation of disease. _H. pylori_ infection causes chronic active gastritis in 70-80% of infected subjects and in substantial proportion of these subjects, _H. pylori_ induced inflammatory response contributes immensely to development of atrophic gastritis eventually leading over a period of time to gastric cancer and has also been classified as group I carcinogen.

The most crucial observation that ulcers stay healed for longer periods after eradication of _H. pylori_ and that its eradication alleviates the recurrence of ulcers convincingly indicates the essential role of _H. pylori_ in the development of gastroduodenal diseases. The importance of _H. pylori_ as an aetiological agent in gastroduodenal disease necessitates its treatment as the main target for elimination of infection. However, it is observed that, despite the availability of various antibiotic regimens, ulcer recurs and response to treatment is precarious, in that it does not give desired results as expected in a considerable proportion of cases.

Several invasive and non-invasive tests for diagnosing _H. pylori_ infection have been developed. With increasing availability of different invasive and non-invasive tests there is a continuous improvement in diagnosis of _H. pylori_ infection. Invasive biopsy based tests include rapid urease test, histology, culture and molecular diagnostic techniques.

Non invasive tests compromise on serology and urea breath test and are more pertinent, in that they appear to circumvent the problems surfacing in invasive tests, thereby obviating the need for endoscopy. Serology is now a highly sensitive and specific test (85%-95%) for current or past _H. pylori_ infection. Serological methods like ELISA have proven especially valuable in screening large number of individuals in epidemiological studies. The serological test ELISA is used as a global method of diagnosis as infected subjects develop elevated levels of IgG antibodies to _H. pylori_. Infection of gastric mucosa with _H. pylori_ results in systemic as well as local immune responses, including elevation of specific IgG and IgA levels in serum and elevated levels of secretory IgA and IgM in stomach. Screening of anti _H. pylori_ antibodies is recommended as pre-endoscopy or as a pre-treatment test. Thus, serological tests are more compliant in that they show positive result in a patient with gastric atrophy in whom the number of _H. pylori_ organisms is so small as to be undetectable by biopsy / breath test based methods. Detection of antibodies is
regarded as more sensitive as it also reveals the intracellular survival of *H. pylori* after treatment.11

In the present study, we examined the diagnostic value of detecting systemic (IgG) antibodies, against *H. pylori* using ELISA technique employing sonicated and acid extractable antigens from *H. pylori* suspension and also assessed the relative efficacy of these antigens in the serodiagnosis of *H. pylori* infection.

**Subjects and Methods**

*H. pylori* cultures obtained by inoculating biopsy extracts from different patients were subcultured on blood-agar medium (containing 5% sheep blood) and incubated at 37°C for 5 days under strict microaerophilic conditions. Characteristic translucent tiny colonies were subjected to different morphological and biochemical tests for establishing identification of *H. pylori*.

**Preparation of acid extractable antigen**

Acid extractable antigens were prepared from *H. pylori* subcultures by the method described by Goodwin *et al.*12

*H. pylori* cells from the subcultures were harvested in sterile distilled water. Cells were washed twice, centrifuged and the pelleted cells were suspended in 0.2M glycine hydrochloride buffer (pH 2.2) at a concentration of 0.1 gm w/w of cells to 1.5 mL of buffer. The suspension was stirred at 25°C for 15 minutes and then centrifuged at 11000 g for 15 minutes at 4°C. The supernatant was carefully removed and its pH was made neutral with sodium hydroxide. It was then dialysed against sterile distilled water for 24 hours. Subsequently, this supernatant was concentrated and stored at -20°C until further use as an antigen for ELISA.

**Preparation of sonicated antigen**

*H. pylori* cells were harvested in sterile distilled water, washed twice in sterile distilled water and subjected to sonication for 6 minutes.

**Collection of blood**

Subjects visiting gastroenterology unit of Gandhi hospital were selected for present study. The test group comprised of 15 cases of which eight were diagnosed to be suffering from duodenal ulcer (DU) and five from non-ulcer dyspepsia (NUD) based on upper gastrointestinal endoscopy. Blood (5 mL) was collected from these patients. Sera obtained were stored at –70°C, which were later used for serodiagnosis using ELISA.

**Procedure**

Antigens extracted from *H. pylori* using sonicated and acid extractable methods were diluted to a final concentration of 2 mg/mL and 5 mg/mL in the coating buffer (0.1 M carbonate buffer, pH 9.6) respectively. Microtitre tray wells were coated with 100 ml of each of these antigens and the plates were incubated overnight at 4°C. Antigen solution was then flicked from the plates and the plates washed thrice with phosphate buffer saline tween (PBST). The non-specific binding sites were then saturated by addition of 150 ml of 1% bovine serum albumin (BSA) in PBST and the plates incubated for one hour at 37°C followed by washing with PBST thrice.

Patient’s sera were subjected to 1:100 dilution in PBST – BSA and 100 µL of these patients sera were added to the wells separately. The plates were incubated for 2 hours at room temperature and washed with PBST thrice. 100 µL of

<table>
<thead>
<tr>
<th>Case number</th>
<th>Category</th>
<th>Sonicated antigen OD values 2 mg/mL</th>
<th>5 mg/mL</th>
<th>Acid extractable antigen OD values 2 mg/mL</th>
<th>5 mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>DU</td>
<td>0.339</td>
<td>0.410</td>
<td>0.223</td>
<td>0.276</td>
</tr>
<tr>
<td>2.</td>
<td>DU</td>
<td>0.326</td>
<td>0.339</td>
<td>0.188</td>
<td>0.211</td>
</tr>
<tr>
<td>3.</td>
<td>DU</td>
<td>0.261</td>
<td>0.234</td>
<td>0.135</td>
<td>0.130</td>
</tr>
<tr>
<td>4.</td>
<td>DU</td>
<td>0.429</td>
<td>0.390</td>
<td>0.176</td>
<td>0.174</td>
</tr>
<tr>
<td>5.</td>
<td>NUD</td>
<td>0.505</td>
<td>0.558</td>
<td>0.413</td>
<td>0.306</td>
</tr>
<tr>
<td>6.</td>
<td>NUD</td>
<td>0.376</td>
<td>0.421</td>
<td>0.190</td>
<td>0.213</td>
</tr>
<tr>
<td>7.</td>
<td>NUD</td>
<td>0.391</td>
<td>0.368</td>
<td>0.268</td>
<td>0.175</td>
</tr>
<tr>
<td>8.</td>
<td>DU</td>
<td>0.487</td>
<td>0.490</td>
<td>0.230</td>
<td>0.238</td>
</tr>
<tr>
<td>9.</td>
<td>DU</td>
<td>0.400</td>
<td>0.431</td>
<td>0.185</td>
<td>0.193</td>
</tr>
<tr>
<td>10.</td>
<td>DU</td>
<td>0.412</td>
<td>0.413</td>
<td>0.200</td>
<td>0.198</td>
</tr>
<tr>
<td>11.</td>
<td>NUD</td>
<td>0.412</td>
<td>0.420</td>
<td>0.206</td>
<td>0.195</td>
</tr>
<tr>
<td>12.</td>
<td>NUD</td>
<td>0.520</td>
<td>0.561</td>
<td>0.213</td>
<td>0.279</td>
</tr>
<tr>
<td>13.</td>
<td>DU</td>
<td>0.284</td>
<td>0.285</td>
<td>0.187</td>
<td>0.191</td>
</tr>
<tr>
<td></td>
<td>BLANK</td>
<td>0.191</td>
<td>0.252</td>
<td>0.084</td>
<td>0.104</td>
</tr>
<tr>
<td></td>
<td>BLANK</td>
<td>0.193</td>
<td>0.252</td>
<td>0.082</td>
<td>0.102</td>
</tr>
</tbody>
</table>
antihuman IgG-HRP conjugate diluted 1:500 with PBST-BSA was dispensed in each well and incubated for 2 hours at 37°C and washed with PBST thrice. 100 µL of substrate solution (58 mg of tri-methyl benzene was dissolved in 10 mL of dimethyl sulfoxide containing 0.015% of hydrogen peroxide) was added to each well and incubated at 37°C for 30 minutes for development of colour. The reaction was stopped by adding 50 µL of stopping solution (IN H₂SO₄) and OD values recorded at 405nm in an ELISA reader.

Results

The principal focus in this study was on assessment of relative efficacy of sonicated and acid – extractable *Helicobacter pylori* antigens in the serodiagnosis of *H. pylori* infection in peptic ulcer patients by ELISA.

The test group comprised of 13 cases of which 8 were diagnosed to be suffering from DU and the remaining were NUD cases. Anti *H. pylori* systemic (IgG) antibodies were screened in sera from 13 *H. pylori* infected subjects. OD values representing antibody titres in each case are given in table. A case was considered seropositive for *H. pylori* if its OD value was more than twice the mean OD value of blank (lacking patient’s sera). The corresponding mean OD values of blank were 0.192, 0.252 for sonicated antigen at a concentration of 2 mg/mL and 5 mg/mL respectively and 0.083, 0.103 for acid extractable antigen at a concentration of 2 mg/mL and 5 mg/mL respectively.

Discussion

ELISA is characterized by its sensitivity and is widely used as a global method of diagnosis as infected subjects develop elevated levels of IgG antibodies to *H. pylori*. In the present study, sonicated and acid extractable antigens were employed after adjusting the protein concentration to 3 mg/mL. Two different concentrations of antigen viz, 2 mg/mL and 5 mg/mL were used to determine optimum concentration to be employed for regular screening.

Considering acid-extractable antigen at a concentration of 2 mg/mL, a perusal of individual OD values of *H. pylori* infected subjects reveals that, excepting one case (case no. 3), individual OD values of the remaining 12 cases are more than twice the mean OD value of blank. Contrarily, with sonicated antigen, at a concentration of 2 mg/mL, there is a marked deviation, in that only eight cases display OD values greater than twice the mean OD value of blank. Thus it is concluded that acid – extractable antigen is relatively more effective and at a concentration of 2 mg/mL, can be routinely employed in serodiagnosis of *H. pylori* infection.

Conclusively, we recommend the use of relatively purified antigens like acid-extractable antigen at a concentration of 2 mg/mL for serodiagnosis of *H. pylori* infection by ELISA owing to its relatively higher efficacy over sonicated lysate containing multiple antigens.

References