MATERIAL
&
METHODS
MATERIAL AND METHODS

The present study was conducted in the Gastroenterology section, Department of Medicine, M.L.B. Medical College Jhansi, in active collaboration with the Department of Pathology, M.L.B. Medical College, and Jhansi.

A. Selection of study group

Study group was constituted by the diabetic (NIDDM) and non-diabetic (controls) dyspeptic patients attending medicine in and out patient department of all age groups belonging to different strata of society who agreed to undergo upper gastrointestinal endoscopy.

*Dyspepsia* being defined as nausea, bloating, sour eructation, early satiety, heart burn or epigastric discomfort for at least one month.

The following exclusion criteria were applied in both diabetic and non-diabetic dyspeptic patient groups.

1. Treatment during previous month with gastro erosive medications, antibiotics, proton pump inhibitors or NSAIDS corticosteroids bismuth derived drugs.

2. Patients who received anti *H. pylori* treatment before.

3. The patients with cholelithiasis, previous cholecystectomy, or major gastrointestinal surgery were excluded.
B. Clinical evaluation of patients / subjects

The detailed history including age, sex, occupation, socio-economic status, total duration of illness, any treatment received was taken.

A physical examination to assess the general condition (pulse rate, blood pressure, pallor, icterus, edema, lymph node enlargement, cyanosis and clubbing) was carried out.

The presence of autonomic neuropathy was evaluated using standard test (postural hypotension, hand grip test).

Postural hypotension defined as sustained drops in systolic (> 20 mm Hg) or diastolic (> 10 mm Hg) BP after standing from supine position for at least 2 min that are not associated with an increase in pulse rate of > 15 beats per minute.

Hand grip test – patient was asked to make a sustained tight handgrip for 5 min. This act normally increases the heart rate and the systolic and diastolic pressures by 15 mm Hg or more. The reduced or absent response was considered to indicate autonomic neuropathy.
General investigations like Hemogram, Blood sugar, BT.CT, Boold Urea, Serum creatinine were done before subjecting patients to biopsy.

C. Procedure

The invasive tests including endoscopy followed by antrum biopsy were done.

ENDOSCOPY AND BIOPSY

a) Preparation for Endoscopy:

1. All patient were examined thoroughly to exclude other systemic disease.

2. All patients were asked for overnight fasting and endoscopy was performed in morning.

3. Every patient was explained about the procedure and given reassurance.

4. End viewing flexible OLYMPUS (model GIF 10Q.) fibreoptic oesophagogastrroduodenoscope was used. Proper consent was taken before each endoscopic procedure.

5. To facilitate the passage of instrument oropharyngeal anaesthesia was achieved with 2% Xylocaine gargle and lubricating jelly was applied on the tube.
b) **Position of Patient:**

Patient was placed in left lateral decubitus position with a single pillow under patient's head. An assistant placed a mouth gag in position and controlled the patient's head.

c) **Procedure:**

Endoscope was placed in oropharynx and patient was asked to assist passage by making swallowing movement. After passing the cricopharynx the tip was further guided forward under direct vision. Air insufflation and suction were done as required. The oesophagus, stomach and duodenum were examined carefully during passing endoscope as well as during withdrawing the instrument.

The biopsy forceps were sterilized after each patient by soaking in a 2% solution of gluteraldehyde (Cidex) and allowing them to dry. They were rinsed in tap water before the first biopsy specimen was taken.

Three antral biopsy specimens were taken from each patient, the first one for rapid urease test and two other for histopathological studies. Biopsy specimens were taken from mucosa 5 cm of the pylorus, one histology specimen from the greater curve and the other from the lesser curve. It was not necessary to obtain red mucosa to demonstrate either bacteria or gastritis.
DIAGNOSIS OF H. PYLORI INFECTION

The biopsy specimen were then subjected to:

1. Rapid urease test.

2. Histological demonstration of the organism.

1. RAPID UREASE TEST:

Heliobacter pylori has a very high endogenous urease activity and split urea rapidly by the action of enzyme urease forming ammonia which causes change in pH (i.e) towards alkaline). This change in pH is indicated by appearance of intense pink red colour.

**Rapid urease solution:**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>In gms/ liter purified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtered water</td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>20.0</td>
</tr>
<tr>
<td>Mono sodium phosphade</td>
<td>0.7</td>
</tr>
<tr>
<td>Phenol red</td>
<td>0.1</td>
</tr>
<tr>
<td>Agar</td>
<td>4.0</td>
</tr>
</tbody>
</table>

pH = 6.4± 0.2 at 25°C

**Storage:**

The prepared media was stored at 2-8°C away from direct light.
Specimen Collection

Biopsy specimen from antrum is placed directly into rapid urease medium at the time of endoscopy.

Method of use

Biopsy specimen was submerged in the medium, the inoculated tube was incubated aerobically at room temperature for up to 20 hours to 3 days. Colour change usually occurs within 4 hours but can occur upto 24 hours. Colour change from yellow to pink indicates presence of H. pylori.

Interpretation.

Positive: Intense pink (red – violet) colour.

Negative: No Colour change (yellow).

False reaction:

Can be given by proteus species which are also capable of splitting urea at a much slower rate.

False –negative findings

The possible causes of false negative findings are listed as follows

Endoscopy

- Lignocaine swallowed
- Simethicone given before biopsy
Patient taking bismuth preparations or antibiotic drugs
• Cimetidine or ranitidine tablets in the stomach.
• Biopsy forceps contaminated with gluteraldehyde.
• Biopsy specimen containing no antral epithelium.

Histology
• Specimen containing mainly intestinal metaplasia.
• Specimen containing mainly acid secreting mucosa.
• Low number of bacteria and poor Silver stain.

2. HISTOLOGY:

Biopsy specimen from antrum, preserved in formalin is sent to Department of Pathology for direct visualization / demonstration of Helicobacter pylori and other histological additional information including the degree and pattern of inflammation, atrophy, metaplasia and dysplasia in Giemsa staining.

3. ENDOSCOPIC FINDINGS

Endoscopic findings were assessed in the following headings:
Reflux esophagitis/ gastritis/gastric ulcer/duodenal ulcer/ duodenitis/malignancy/normal findings.