Endoscope versus microscope in the diagnosis of esophageal non-erosive reflux disease: a study of 71 cases

ARUL P MD, Manjiri PHANSALKAR MD, Thomas ALEXANDER* MD, DM, Somanath PADHI MD, and VINOTH B* MD, DM

Departments of Pathology and *Gastroenterology, Pondicherry Institute of Medical Sciences, India

Abstract

Background: Non-erosive reflux disease (NERD) is a type of gastroesophageal reflux disease (GERD) defined as symptomatic GERD without mucosal breaks on endoscopy. There is no gold standard for diagnosis of NERD till date. Biopsy from the distal esophagus in patients of GERD is said to reveal characteristic, although non-specific injury patterns such as squamous cell hyperplasia and intraepithelial inflammatory cell infiltration, collectively known as microscopic esophagitis. Recently, dilated intercellular spaces (DIS) are also shown to be associated with NERD. The present study was undertaken to evaluate the role of biopsy in NERD cases. Materials and methods: Two mucosal biopsies were taken at 3 cm above the squamocolumnar junction (SCJ) from 71 cases of NERD having symptom scores of more than 10. Biopsies were evaluated for features of microscopic esophagitis and DIS, and the results were analyzed. Results: Fifty out of 71 (70.4%) patients with symptomatic NERD had features of microscopic esophagitis. DIS was noted in 46/71 (64.8%) of cases, and correlated significantly with microscopic esophagitis (p<0.0001). Basal cell hyperplasia, papillary elongation, intraepithelial neutrophils, and intraepithelial eosinophils were seen in 83.1%, 76.1%, 40.8%, and 12.7% of NERD cases respectively. Conclusion: In symptomatic patients of GERD, when endoscopy does not show mucosal breaks (so called NERD), histopathological evaluation of distal esophageal mucosa may have a diagnostic value. The present study also reinforces the diagnostic utility of DIS in symptomatic NERD.

Keywords: Non-erosive reflux disease, microscopic esophagitis, dilated intercellular spaces, white light endoscopy.

INTRODUCTION

Gastroesophageal reflux disease (GERD) is defined as a condition which develops when the reflux of stomach contents cause troublesome symptoms and/or complications. This is considered troublesome when mild symptoms occur two or more days in a week, or moderate/severe symptoms occur one or more days in a week. Characteristic symptoms of GERD are heartburn and regurgitation. GERD is classified into erosive reflux disease (ERD) and non-erosive reflux disease (NERD). ERD is defined as GERD with esophageal mucosal breaks evident on routine white light endoscopy (WLE), whereas NERD is defined as those with symptoms, but without mucosal breaks or erosions on endoscopy. Biopsy from the distal esophagus in patients of GERD is said to reveal characteristic, although non-specific, injury patterns such as squamous hyperplasia and intraepithelial inflammatory cell infiltration. The term “microscopic esophagitis” (ME) refers to a group of histological lesions observed in most patients with GERD, both ERD and NERD. Some authors noted a histological feature called dilated intercellular space (DIS) as a useful tool in diagnosis, as well as in explanation of heartburn in NERD patients as they lack erosions.

The present study was undertaken to evaluate the proportion of NERD cases showing features of ME. On routine endoscopy, none of these cases showed mucosal breaks. Furthermore, the significance of DIS was also evaluated.
MATERIALS AND METHODS

The present prospective study was carried out in the Departments of Pathology and Gastroenterology, Pondicherry Institute of Medical Sciences, Puducherry from February 2012 to May 2013, after obtaining approval from the Institutional Ethical Committee. Informed consent was obtained from all patients recruited in the study.

Inclusion criteria: All cases with symptoms of GERD as defined by GERD health-related quality of life (HRQL) heartburn score of more than 10, but endoscopically negative for erosive reflux disease on routine WLE were included in our study. The total number of eligible cases was 71.

Exclusion criteria: (i) cases on acid suppression therapy and/or proton pump inhibitors for 4 weeks before endoscopy, (ii) history of upper gastrointestinal surgeries viz: gastrectomy, distal esophagectomy, gastro-jejunostomy, fundoplication, (iii) severe gastroparesis, (iv) contraindication to biopsy viz: esophageal varices, bleeding disorders, (v) history of pill use (doxycycline, quinidine, and alendronate, etc.) and impaction.

Method of collection of data: Baseline characteristics such as age, gender, smoking and alcohol history, history of prior NSAIDs usage, and other relevant history were collected from medical records. All cases (n=71) underwent routine WLE by using gastrointestinal videoscope (Olympus GIF-H 180). WLE findings such as erythema, edema, friability, and granularity were recorded by the gastroenterologist. In each case, two mucosal biopsies were taken at 3 cm above the SCJ.

Histopathological study: All mucosal biopsy specimens were fixed in 10% neutral buffered formalin, embedded in paraffin, and stained routinely with Hematoxylin and Eosin (H&E) (for histopathological study) and Periodic Acid Schiff (PAS) (to rule out fungal esophagitis). The histopathological slides were evaluated by a pathologist blinded for the clinical characteristics, GERD-HRQL heartburn score, and endoscopic findings.

The diagnosis of ME was based upon the presence of one or more of the following criteria:13 (i) basal zone hyperplasia (defined as basal cells exceeding 15% of the whole thickness of epithelium) along with elongation of the connective tissue papillae from lamina propria to the upper third of the whole epithelial thickness (Figs.1 & 2), (ii) focal or diffuse infiltration of the epithelium by polymorphonuclear (PMN) leucocytes (Fig. 3) (>2 PMN leucocytes per tissue section) or eosinophils (Fig. 4) (>5 eosinophils per tissue section), (iii) dense non-follicular infiltration of mononuclear inflammatory cells and/or an easily recognized infiltrate of neutrophils in lamina propria.

Microscopic esophagitis was further graded into three categories based on the degree and type of above mentioned three criteria:13 (i) mild esophagitis- presence of basal zone hyperplasia and papillary elongation, (ii) moderate esophagitis-small number of PMN leukocytes and/or eosinophils and those with moderate number of intraepithelial lymphocytes and/or moderate non-follicular lymphocytic infiltration of lamina propria with/without elongation of rete ridges, (iii) severe esophagitis- presence of severe neutrophil infiltration in lamina propria and/or epithelium usually

FIG. 1: Distal esophageal squamous mucosa showing basal cell hyperplasia (>15% of the epithelial thickness) (black line) (Hematoxylin and eosin, x400).
associated with erosion and ulceration. Dilated intercellular spaces (DIS) were also evaluated (Fig. 5).

Statistical analysis:
Chi Square test was used to assess the significance of the association of ME with WLE findings other than erosions, and DIS. A p value <0.05 was considered statistically significant. SPSS version 20 was used for statistical analysis.

RESULTS
The mean age of the recruited symptomatic
NERD cases was 38.59 years, with a range from 18 to 63 years. Of the 71 cases, 35 (49.3%) were males and 36 (50.7%) were females. Twelve (16.9%) were smokers and 12 (16.9%) were alcoholics. Fifty (70.4%) patients had evidence of ME.

Among the 50 cases with ME, 19 (38%) were graded as mild; 30 (60%) were graded as moderate; and 1 (2%) was graded as having severe. WLE findings such as erythema, edema, friability, and granularity were analyzed to check for correlation with histopathological findings. However, none of these WLE findings showed statistically significant correlation with ME as shown in Table 1.

Individual histological parameters considered to detect ME were analyzed to find out their individual frequency. Among all the patients with symptomatic NERD (n=71), basal cell hyperplasia was the most frequent finding noted in 59 (83.1%) cases, papillary elongation in 54 (76.1%), intraepithelial neutrophils in 29 (40.8%), and intraepithelial eosinophils were seen in 9 (12.7%) cases. Dense infiltration of

Table 1: Correlation of WLE findings with histological findings among symptomatic NERD patients (n=71)

<table>
<thead>
<tr>
<th>WLE findings</th>
<th>Microscopic esophagitis</th>
<th>P* value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>present</td>
<td>absent</td>
</tr>
<tr>
<td>Erythema</td>
<td>Present</td>
<td>16(76.2%)</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>34(68%)</td>
</tr>
<tr>
<td>Edema</td>
<td>Present</td>
<td>14(73.7%)</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>36(69.2%)</td>
</tr>
<tr>
<td>Friability</td>
<td>Present</td>
<td>1(100%)</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>49(70%)</td>
</tr>
<tr>
<td>Granularity</td>
<td>Present</td>
<td>6(50%)</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>44(74.6%)</td>
</tr>
</tbody>
</table>

*: P value <0.05 was considered statistically significant
mononuclear inflammatory cells or of neutrophils in lamina propria was not seen in any of these cases. Dilated intercellular spaces (DIS) were seen in a total 46 (64.8%) cases. On correlation of DIS finding with ME, statistically highly significant association (p<0.0001) was noted (Fig. 6).

DISCUSSION

The epidemiological characteristics of our cases and their comparison with other studies are presented in Table 2. In the present study, symptoms of GERD were noted at younger age (mean age=38.59 years) as compared to the other studies. However, the mean age of our study group was comparable with that of a recent Indian study by Bhatia et al.17 There was no significant difference between male and female distribution, as noted in many other studies. The prevalence of smoking was less common, similar to the studies done by Tseng et al16 and Bhatia et al,17 and alcohol consumption was less common, similar to study done by Bhatia et al.17

Reflux disease is associated with a constellation of histological features, representing changes secondary to acid injury and mucosal healing. Histologically, reflux changes are typically distributed over the distal 8 to 10 cm of the esophagus in a patchy fashion, which indicates that multiple biopsies are often necessary to consistently demonstrate histological abnormalities. Furthermore, because of a high level of discordance between endoscopy and histology, it is recommended that all symptomatic patients should undergo biopsy, regardless of the presence or absence of endoscopic abnormalities. No individual or group of histological features is diagnostic of GERD. Each feature is limited to some degree by practical use or diagnostic sensitivity or specificity and although these numerous features, alone or in combination, can contribute to establishing the diagnosis of GERD, none is an absolutely reliable criterion.2-5,11, 18-21

Table 2: Comparison of epidemiological characteristics of patients of present study with other studies

<table>
<thead>
<tr>
<th></th>
<th>Tadiparthi et al14</th>
<th>Fock et al15</th>
<th>Tseng et al16</th>
<th>Bhatia et al17</th>
<th>Present study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>57.4</td>
<td>45.1</td>
<td>51.1</td>
<td>40</td>
<td>38.59</td>
</tr>
<tr>
<td>Male: Female (%)</td>
<td>92.8:8</td>
<td>50.6:49.4</td>
<td>59.7: 40.3</td>
<td>45.7:54.3</td>
<td>49.3:50.7</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>-</td>
<td>-</td>
<td>13.4</td>
<td>8.6</td>
<td>16.9</td>
</tr>
<tr>
<td>Alcoholics (%)</td>
<td>-</td>
<td>-</td>
<td>34.1</td>
<td>16.4</td>
<td>16.9</td>
</tr>
</tbody>
</table>
Histological features of reflux esophagitis can be seen in other rare forms of esophagitis caused by fungal, viral infections, pill impaction etc. However, careful study of H&E, and PAS stained biopsy sections along with properly taken clinical history, helps to rule out these conditions. The above said factors were meticulously considered in analyzing our cases; hence, the histological features of esophagitis in the present study were highly suggestive of reflux esophagitis.

In the distal most 2cm of the esophagus, the presence of epithelial cell hyperplasia represent normal findings related to physiologic reflux. However, some authors found biopsies from the SCJ to be more sensitive than more proximal biopsies, but less specific. However, studies have shown that biopsies taken at 1 to 2cm proximal to the SCJ demonstrate significant differences with respect to epithelial hyperplasia and DIS among patients and controls, suggesting that these more proximal biopsies have the greatest potential to discriminate patients with and without GERD.3,5 Because biopsy specimens from the lower 1 to 2 cm of the esophagus, even in asymptomatic subjects, often reveal evidence of mild squamous hyperplasia, diagnostic biopsy specimens should generally be obtained more than 2.0 cm above the level of the Z line to diagnose esophagitis reliably.11

Kasap et al,22 correlated WLE, narrow band imaging (NBI) endoscopy, and histopathological findings in the diagnosis of NERD. Among 40 NERD cases, 23 (57.5%) showed abnormal histology. In another study, Zentilin et al11 evaluated the diagnostic utility of histology in patients with GERD (n=119), using multiple biopsy sites and compared them with an appropriate control group (n=20). Among 59 NERD cases, in their study, a very high proportion (76%) had abnormal histology.11 Zuberi et al23 prospectively evaluated the correlation between clinical, endoscopic and histological findings at SCJ in 196 patients with GERD (109 NERD). Seventy of 109 (64.2%) NERD cases showed abnormal histology. In comparison, a very significant number of cases (50/71, 70.4%), in our study, had evidence of ME at 3 cm above SCJ.

In symptomatic NERD cases:

Frequency of individual histopathological parameters in the symptomatic NERD cases: A brief comparative review on histological features in NERD is presented in Table 3. In the present study, among the 71 symptomatic NERD cases, basal cell hyperplasia was seen in 59 (83.1%), papillary elongation in 54 (76.1%), intraepithelial neutrophils in 29 (40.8%), and intraepithelial eosinophils in 9 (12.7%) cases. In this context, studies have shown a marked variation in the frequency of various histological features currently utilized in the histological diagnosis of ME.11,21,28,29 Variation in the site of biopsy and variation in criteria to define the histological features may be the reason for such differences.

Dilated intercellular spaces (DIS): A few studies showed that DIS is a useful and promising light microscopical (LM) criterion for early mucosal injury in GERD. DIS occurs due to loss of tight junctions between squamous cells and leads to increased paracellular permeability that may facilitate leaking of the acid through
the mucosa and direct contact with terminal dendritic processes of underlying sensory neurons in epithelium.\textsuperscript{6,30} Slight acidification of intercellular spaces can trigger symptoms, which explains the occurrence of typical symptoms of GERD in the absence of an endoscopic lesion.\textsuperscript{8-10} Prevalence of DIS varies from 67\% to 94\% depending on the presence or absence of clinical symptoms, endoscopic lesions, and pH monitoring abnormalities.\textsuperscript{11} Ravelli \textit{et al} studied the DIS as a major morphological feature of esophagitis among 48 GERD patients. Among the 48 patients, 40 (83.3\%) patients showed evidence of esophagitis at histology; and all these 40 patients showed DIS indicating a high statistical significance (p<0.0001).\textsuperscript{8} Similarly, Solcia \textit{et al} studied DIS by light microscopy and noted it in 68\% of NERD cases.\textsuperscript{21} Zentilin \textit{et al} showed DIS in 80\% of NERD cases with 70\% specificity.\textsuperscript{11} In another LM study by Villanacci \textit{et al}, DIS was observed in 71\% of NERD cases.\textsuperscript{31} In a transmission electron microscopic (TEM) study by Caviglia \textit{et al}, DIS was observed in 100\% cases of NERD.\textsuperscript{32} In comparison to other studies, our study demonstrated a slightly lower frequency of DIS (64.8\%) on light microscopy. However, on correlation of DIS with ME, a statistically highly significant association (p<0.0001) was noted.

To summarize, in symptomatic patients of GERD, when endoscopy does not show mucosal breaks (a condition known as NERD), histopathological evaluation of distal esophageal mucosa may have promising diagnostic value. The present study also reinforces the diagnostic utility of DIS in NERD. However, our study has a few drawbacks. Since ME can be found in asymptomatic individuals,\textsuperscript{33} the lack of enrollment of a control group was a major limitation of our study; for which the outcome of our study need to be interpreted with caution. We used LM criteria for the evaluation of DIS, and morphometric study may help to increase accuracy and objectivity. Furthermore, considering the patchy nature of the disease process, comparison of multiple random mucosal biopsies from distal 1/3rd esophagus might have been more useful. Besides these, the small number of cases might have been the reason behind the lack of correlation between endoscopic signs and histological findings. Therefore, the outcome of our study needs to be validated by future larger prospective studies involving histopathological correlation.

\textbf{REFERENCES}


