Gastroesophageal reflux disease (GERD) is a common disorder in the West and it affects approximately 20%–50% of adults in Western countries, causing huge economic burden.[1-3] The prevalence of GERD symptoms occurring once a week is approximately 8% in Indian subjects.[4] Nonerosive reflux disease (NERD) and erosive reflux disease (ERD) are types of GERD. NERD is defined as white light endoscopy (WLE) negative patients with reflux symptoms with or without abnormal esophageal acid exposure during ambulatory 24-h esophageal pH monitoring.[5] WLE cannot visualize superficial and small esophageal lesions in GERD. In view of the need for more diagnostic accuracy, high-definition endoscopy with narrow band imaging (NBI) was developed by Olympus Medical System in Japan.[6] It was first reported by Sano et al., in 1999 and introduced commercially in 2005.[6,7] NBI endoscopy is said to be more sensitive in detecting mucosal abnormalities as it is an optical image technology that enhances structural mucosal patterns (pit-pattern), as well as mucosal/submucosal vessels by employing the characteristics of light spectrum.[8] It is based on the optical phenomenon that the depth of light penetration into tissues is dependent on the wavelength (415 and 540 nm). The 415 nm image channel analyzes the fine surface architecture of the mucosa and superficial capillary network appear as brownish color; the 540 nm image channel analyzes the collecting vessels in the depth of the mucosa appearing as a bluish color.[6‑10] NBI endoscopy may help to improve diagnostic accuracy compared with white light endoscopy in the diagnosis of GERD.[9,10] Reflux disease is associated with a group of histologic features, which represents changes from secondary to acid injury and mucosal healing.[11] and these histologic findings have been well defined for the diagnosis of GERD.[12] Hence
Narrow band imaging endoscopy in non erosive reflux disease may be considered as a diagnostic tool for NERD. This study was undertaken to evaluate diagnostic utility of NBI endoscopy and biopsy study in NERD and also to correlate NBI endoscopy findings with histologic features of GERD.

**PATIENTS AND METHODS**

**Overview of study design**

The present prospective study was undertaken at the Departments of Pathology and Gastroenterology, Pondicherry Institute of Medical sciences, Puducherry, India, conducted during the period from February 2012 to May 2013, after obtaining approval from the Institutional Ethical Committee. Informed written consent was obtained from each patient before endoscopy and for biopsy.

**Study population**

Subjects were enrolled if they met the following inclusion criteria: More than 18 years of age and ability to provide written informed consent with symptoms of GERD as defined by GERD health-related quality of life (HRQL) heartburn score more than 10, but endoscopically negative for ERD on WLE. A total number of 71 cases were included in the study. Patients with one or more of the following criteria were excluded from this study: (1) Inability to provide written informed consent; (2) patients on acid suppression therapy and proton pump inhibitors for 4 weeks before endoscopy; (3) evidence of cancer or mass lesion in the esophagus; (4) gastric lesion, namely, ulcer, polyp, or cancer; (5) prior history of upper gastrointestinal surgeries, namely, gastrectomy, distal esophagectomy, gastro-jejunostomy, fundoplication; (6) severe gastroparesis; (7) esophageal varices; (8) severe uncontrolled coagulopathy; (9) inability to discontinue nonsteroidal anti-inflammatory drugs or aspirin prior to the study; and (10) history of pill use (doxycycline, quinidine, alendronate, and so on).

**Equipment and endoscopic procedure**

The endoscopic procedures of all 71 cases performed using the gastrointestinal videoscope (Olympus Evis Exera II GIF-H180). NBI endoscopic findings such as microerosions [Figure 1a], increased vascularity above squamocolumnar junction (SCJ) [Figure 1b], increased vascularity below SCJ [Figure 1c], mucosal brownish areas [Figure 1d], round pit pattern [Figure 2a], straight pit pattern [Figure 2b], mucosal islands [Figure 3] were recorded by gastroenterologists. Two mucosal biopsies were taken at 3 cm above SCJ from each patient. Methodology of study is shown in Flow Chart 1.

**Histopathologic evaluation**

Biopsy specimens fixed in 10% neutral buffered formalin, processed routinely, embedded in paraffin and 4 µm thick sections stained with hematoxylin and eosin were evaluated. Periodic Acid Schiff stain was also done to...
rule out fungal esophagitis. All biopsies were examined using a light microscope by pathologists blinded to the clinical characteristics, GERD–HRQL heartburn score and endoscopic findings.

The diagnosis of microscopic esophagitis was based on the presence of one or more of the following criteria: 

1. Basal cell zone exceeding 15% of the whole thickness of epithelium along with elongation of the connective tissue papilla from lamina propria to the upper third of the whole epithelial thickness [Figure 4]. 
2. Focal or diffuse infiltration of the epithelium by polymorphonuclear (PMN) leukocytes (≥2 PMN leukocytes per tissue section) or eosinophils [Figure 5] (≥5 eosinophils per tissue section), 
3. Dense nonfollicular infiltration of mononuclear inflammatory cells and/or an easily recognized infiltrate of neutrophils in lamina propria.

**Statistical analysis**
Chi-square test and correlation coefficient (kappa) were used to assess the correlation of NBI endoscopic findings with histologic features of GERD. Statistical tests were performed using SPSS version 20. A $P < 0.05$ was considered statistically significant.

**RESULTS**

The mean age of the study population was 38.59 years, with a range from 18 to 63 years. Of these patients, 35 (49.3%) were males and 36 (50.7%) were females. Smokers were 12 (16.9%) and nonsmokers were 59 (83.1%). Fifty (70.4%) patients had evidence of histologic features of GERD.

NBI endoscopic findings such as microerosions, increased vascularity above SCJ, increased vascularity below SCJ, round pit pattern, straight pit pattern, mucosal brownish areas, and mucosal islands in 71 NERD patients and in 50 cases of histologic GERD are summarized in Table 1. (More than one NBI endoscopic findings were noted in most of the cases.) These NBI endoscopy findings were analyzed for correlation with histologic features of GERD by Chi-square test and correlation coefficient (kappa). The $P$ values of Chi-square test are shown in Table 2. The correlation coefficient (kappa) value for histologic features of GERD with microerosions was $-0.058$, increased vascularity above SCJ was $0.048$, increased vascularity below SCJ was $-0.075$, round pit pattern was $0.116$, straight pit pattern was $-0.129$, mucosal brownish areas was $-0.117$, and mucosal islands was $0.015$.

**DISCUSSION**

Our study showed that NBI endoscopy findings such as straight pit pattern (56.3%), increased vascularity at SCJ (46.47%) (considering together the increased vascularity...
Narrow band imaging endoscopy in non erosive reflux disease

Table 1: NBI endoscopic findings in NERD (n=71) and histologic GERD cases (n=50)

| NBI endoscopic findings                  | NERD cases | Histologic GERD cases |
|------------------------------------------|------------|-----------------------|
|                                          | N=71 (%)   | N=50 (%)              |
| Microerosions                            | 9 (12.7)   | 5 (10)                |
| Increased vascularity above SCJ          | 17 (23.9)  | 13 (26)               |
| Increased vascularity below SCJ          | 29 (40.8)  | 19 (38)               |
| Round pit pattern                        | 31 (43.7)  | 24 (48)               |
| Straight pit pattern                     | 40 (56.3)  | 26 (52)               |
| Tubular pit pattern                      | 0          | 0                     |
| Villous/ridge pit pattern                | 0          | 0                     |
| Mucosal brownish areas                   | 15 (21.1)  | 8 (16)                |
| Mucosal islands                          | 18 (25.4)  | 13 (26)               |

NBI: Narrow band imaging; SCJ: Squamocolumnar junction; NERD: Nonerosive reflux disease; GERD: Gastroesophageal reflux disease.

Table 2: Correlation of NBI endoscopy findings with histologic findings among NERD patients (n=71)

| NBI findings                  | Histologic GERD (%) | P value |
|------------------------------|---------------------|---------|
|                              | Present | Absent |       |
| Microerosions                |         |        |       |
| Present                      | 5 (10)  | 4 (19) | 0.296 |
| Absent                       | 45 (90) | 17 (81)|       |
| Increased vascularity above SCJ |        |        |       |
| Present                      | 13 (26) | 4 (19) | 0.531 |
| Absent                       | 37 (74) | 17 (81)|       |
| Increased vascularity below SCJ |        |        |       |
| Present                      | 19 (38) | 10 (47.6) | 0.452 |
| Absent                       | 31 (62) | 11 (52.4)|       |
| Round pit pattern            |         |        |       |
| Present                      | 24 (48) | 7 (33.3) | 0.255 |
| Absent                       | 26 (52) | 14 (66.7)|       |
| Straight pit pattern         |         |        |       |
| Present                      | 26 (52) | 14 (66.7) | 0.255 |
| Absent                       | 24 (48) | 7 (33.3) |       |
| Mucosal brownish areas       |         |        |       |
| Present                      | 8 (16)  | 7 (33.3) | 0.102 |
| Absent                       | 42 (4)  | 14 (66.7)|       |
| Mucosal islands              |         |        |       |
| Present                      | 13 (26) | 5 (23.8) | 0.846 |
| Absent                       | 37 (74) | 16 (76.2)|       |

NBI: Narrow band imaging; SCJ: Squamocolumnar junction; GERD: Gastroesophageal reflux disease. *P value <0.05 was considered statistically significant.

above and below SCJ, increased vascularity at SCJ was noted in 33 cases) and round pit pattern (43.7%) were common in NERD cases. A study done by Fock et al.'s[9] observed increased prevalence of increased vascularity at SCJ (91.7%), microerosions (52.8%), and lower prevalence of round pit pattern (5.6%) suggestive of NERD compared with controls. Sharma et al.'s[10] evaluated the utility of NBI in patients with symptoms of GERD, found that NERD cases had more frequency of increased number and dilatation of intrapapillary capillary loops (IPCLs), microerosions, and increased vascularity at SCJ compared with the control subjects. Our results are partially similar to Fock et al.'s[9] and Sharma et al.'s[10] studies as we all noted increased vascularity at SCJ in NERD cases. In contrast to Fock et al.'s[9] and Sharma et al.'s[10] studies, we found more number of straight and round pit patterns and less number of microerosions as shown in Table 3. Studies done by Landell et al.[14] and Bytzer et al.[17] suggested that endoscopic features of edema and increased vascularity were unreliable for the diagnosis of GERD. However, studies by Fock et al.,[9] Sharma et al.,[10] and our data suggest that increased vascularity at the SCJ noted by NBI may be one of the endoscopic feature of NERD. Similar to NERD patients (n = 71), histologic GERD (n = 50) also showed the same NBI endoscopic findings [Table 1]. NBI endoscopic findings were analyzed for correlation with histologic features of GERD. There is no study on NERD cases till date to correlate all the parameters of NBI endoscopy with histologic GERD. Hence, in the present study, we attempt to correlate the NBI endoscopic findings with histologic features of GERD. However, none of these NBI endoscopic findings, including increased vascularity above and below SCJ showed statistically significant correlation with histologic features of GERD [Table 2]. The correlation coefficient (kappa) showed that there was poor agreement between NBI endoscopic findings and histologic features of GERD. We could not characterize individual morphology of IPCLs as our endoscope had digital magnification and not an optical magnification. Hence we were unable to confirm the NBI endoscopic features such as more frequency of increased number and dilatation of IPCLs as markers of NERD.

The histologic parameters of GERD include squamous cell hyperplasia such as basal cell hyperplasia and papillary elongation, intraepithelial neutrophils and eosinophils, mucosal erosion/ulceration, and dilated intracellular spaces occur due to lower esophageal epithelial injury and repair. Endoscopic examination of ERD can reveal erosions, ulcers, strictures. However, approximately 50%–60% of symptomatic GERD patients show normal mucosa or only mild hyperemia at endoscopy. Furthermore, histologic GERD may appear normal endoscopically; conversely, hyperemia does not necessarily indicate the presence of histologic features of GERD. Because of these endoscopic and pathologic discrepancies, biopsies are always warranted in symptomatic patients to document the presence of tissue injury. The diagnostic biopsies should generally be obtained more than 2.0 cm above the SCJ, because the lower 1–2 cm of the esophagus, often reveal evidence of mild squamous hyperplasia even in asymptomatic subjects.[11,18‑21] Hence we took biopsies from 3 cm above SCJ for the diagnosis of histologic GERD.
In a prospective study by Zentilin et al., histologic evidence of reflux esophagitis was seen in 76% of cases of NERD patients. Kasap et al. showed that in 40 NERD cases, 57.5% patients had abnormal histology. Zuberi et al. studied 109 NERD patients, of whom 64.2% showed abnormal histologic features. The present study showed that 70.4% of NERD patients had histologic features of GERD. The histologic GERD found in our study was higher than previous studies and also highly specific, because we took biopsies from 3 cm above SCJ in contrast to those in Kasap et al.’s and Zuberi et al.’s studies in which biopsies were taken from 2 cm above SCJ and at SCJ, respectively, as it often appears as mild squamous hyperplasia even in asymptomatic individuals.

Kasap et al. investigated 40 NERD patients by NBI endoscopy. Among the 40 cases, 28 (70%) had normal NBI endoscopy (NERD) and remaining 12 (30%) had mucosal brownish areas, which were considered as acid-related mucosal change in ERD. In this study, authors considered only mucosal brownish area as a feature of ERD, not of NERD. They did not study the other features of NERD on NBI such as microerosions, increased vascularity above and below SCJ, mucosal pit patterns, and mucosal islands. Authors found that NBI is more sensitive than WLE in the detection of esophageal lesions in GERD patients. However, histopathologic evaluation is the most sensitive method to diagnose NERD.

Lee et al. investigated 230 patients with GERD symptoms by both WLE and NBI endoscopy. WLE was normal (NERD) in 65.6% of patients, whereas NBI endoscopy was normal in 59.1% of patients. Up to 6% of patients were reclassified from NERD to grade A esophagitis by the utilization of NBI.

Tseng et al. assessed 62 NERD patients by NBI endoscopy. Out of the 62 NERD patients, only 18 (29%) showed normal NBI endoscopic findings, and the remaining cases 71% were reclassified as ERD. In our study only 9 (12.7%) patients showed microerosions.

Among the NERD (n = 71) cases, NBI endoscopic findings of first 15 and last 15 cases were analyzed to find out learning effect by correlating them with histologic features of GERD. There was no significant apparent learning effect in the use of NBI for diagnosis of histologic GERD as shown in Tables 4 and 5. Our study suggests that it required tremendous familiarity and a long learning curve for interpretation of NBI endoscopic findings in the diagnosis of NERD.

Table 3: Comparison of NBI findings of present study with other studies

| NBI features                  | Fock et al.[9] | Sharma et al.[10] | Present study (NERD) |
|-------------------------------|----------------|-------------------|---------------------|
| NERD n=36 (%)                 | NERD n=20 (%)  | NERD n=30 (%)     | n=71 (%)            |
| Microerosions                 | 52.8           | 23.3              | 12.7                |
| Increased vascularity at SCJ  | 91.7           | 25                | 46.47               |
| Round pit pattern             | 5.6            | -                 | 43.7                |
| Straight pit pattern          | 19.4           | -                 | 56.3                |
| Tubular pit pattern           | 52.8           | -                 | 0                   |
| Villous/ridge pit pattern     | 22.2           | 15                | 0                   |
| Mucosal brownish areas        | -              | -                 | 21.1                |
| Mucosal/columnar islands      | 38.9           | 50                | 25.4                |

NBI: Narrow band imaging; SCJ: Squamocolumnar junction; NERD: Nonerosive reflux disease

Table 4: Learning curve effect in the use of NBI endoscopy in the diagnosis of histologic GERD (1-15 cases of NERD)

| NBI findings                  | Histologic GERD (%) | P* value         |
|-------------------------------|---------------------|-----------------|
|                               | Present | Absent |                  |
| Microerosions                 |         |        |                  |
| Present                       | 4 (57.1) | 3 (42.9) | 0.782 |
| Absent                        | 4 (50) | 4 (50) |                  |
| Increased vascularity above SCJ |        |        |                  |
| Present                       | 4 (80) | 1 (20) | 0.146 |
| Absent                        | 4 (40) | 6 (60) |                  |
| Increased vascularity below SCJ |        |        |                  |
| Present                       | 6 (54.6) | 5 (45.4) | 0.876 |
| Absent                        | 2 (50) | 2 (50) |                  |
| Round pit pattern             |         |        |                  |
| Present                       | 1 (50) | 1 (50) | 0.919 |
| Absent                        | 7 (53.8) | 6 (46.2) |                  |
| Straight pit pattern          |         |        |                  |
| Present                       | 7 (53.8) | 6 (46.2) | 0.919 |
| Absent                        | 1 (50) | 1 (50) |                  |
| Mucosal brownish areas        |         |        |                  |
| Present                       | 1 (25) | 3 (75) | 0.185 |
| Absent                        | 7 (63.7) | 4 (36.3) |                  |
| Mucosal islands               |         |        |                  |
| Present                       | 2 (40) | 3 (60) | 0.464 |
| Absent                        | 6 (60) | 4 (40) |                  |

NBI: Narrow band imaging; NERD: Nonerosive reflux disease; GERD: Gastroesophageal reflux disease. *P value <0.05 was considered statistically significant
Limitations of our study

Failure of correlation of NBI endoscopic findings with NERD and histologic features of GERD may be due to the following

- The present study was performed by using gastrointestinal videoscope (Olympus Evis Exera II GIF-H 180), which had NBI with 1.5× digital magnification. Hence we could not characterize the individual morphology of IPCLs
- It required tremendous familiarity and a long learning curve for interpretation of NBI endoscopic findings
- Lack of enrollment of control group was another important limitation, because histologic features of GERD can be seen in asymptomatic individuals and also for comparison of NBI endoscopic findings between NERD and controls
- To date, the research work concerning the use of NBI endoscopy in GERD is relatively scanty, and there is still a need for large scale studies to validate the endoscopic findings.

CONCLUSION

Our study concluded that biopsy from distal esophageal mucosa has promising diagnostic utility over NBI endoscopy in NERD patients who suffer from symptoms of GERD. Use of newly introduced NBI technique requires tremendous familiarity for the detection of cases of NERD, which show histologic features of GERD.

REFERENCES

1. Kulig M, Nocon M, Vieth M, Leodolter A, Jaspersen D, Labenz J, et al. Risk factors of gastroesophageal reflux disease: Methodology and first epidemiological results of the ProGERD study. J Clin Epidemiol 2004;57:580-9.
2. Liu JY, Woloshin S, Laycock WS, Rothstein RJ, Finlayson SR, Schwartz LM. Symptoms and treatment burden of gastroesophageal reflux disease: Validating the GERD assessment scales. Arch Intern Med 2004;164:2058-64.
3. Heading RC. Prevalence of upper gastrointestinal symptoms in the general population: A systematic review. Scand J Gastroenterol Suppl 1999;231:3-8.
4. Bhatia SJ, Reddy DN, Ghoshal UC, Jayanthi V, Abraham P, Choudhuri G, et al. Epidemiology and symptom profile of gastroesophageal reflux in the Indian population: Report of the Indian Society of Gastroenterology Task Force. Indian J Gastroenterol 2011;30:118-27.
5. Vakil N, van Zanten SV, Kahrilas P, Graham D, Jones R, Global Consensus Group. The Montreal definition and classification of gastroesophageal reflux disease: A global evidence-based consensus. Am J Gastroenterol 2006;101:1900-20; quiz 1943.
6. Assirati FS, Hashimoto CL, Dib RA, Fontes LH, Navarro-Rodriguez T. High definition endoscopy and "narrow band imaging" in the diagnosis of gastroesophageal reflux disease. Arq Bras Cir Dig 2014;27:59-65.
7. Sano Y, Kobayashi M, Hamamoto Y. New diagnostic method based on color imaging using narrow band imaging (NBI) system for gastrointestinal tract. Gastrointest Endosc 2001;53:AB125.
8. Lambert R, Kuznetsov K, Rey JF. Narrow band imaging in digestive endoscopy. Scientific World Journal 2007;7:449-65.
9. Fock KM, Teo EK, Ang TL, Tan JY, Law NM. The utility of narrow band imaging in improving the endoscopic diagnosis of gastroesophageal reflux disease. Clin Gastroenterol Hepatol 2009;7:54-9.
10. Sharma P, Wani S, Bansal A, Hall S, Puli S, Mathur S, et al. A feasibility trial of narrow band imaging endoscopy in patients with gastroesophageal reflux disease. Gastroenterology 2007;133:454-64; quiz 674.
11. Allende DS, Yerian LM. Diagnosing gastroesophageal reflux disease: The pathologist’s perspective. Adv Anat Pathol 2009;16:161-5.
12. Frierson HF Jr. Histology in the diagnosis of gastroesophageal reflux disease. Gastroenterology 1996;110:217-24.
13. Schindlbeck NE, Wiebecke B, Klauser AG, Voderholzer WA, Müller-Lissner SA. Diagnostic value of histology in non-erosive gastro-oesophageal reflux disease. Gut 1996;39:151-4.
14. Velanovich V, Vallance SR, Gusz JR, Tapia FV, Harakabas MA. Quality of life scale for gastroesophageal reflux disease. J Am Coll Surg 1996;183:217-24.
15. Abedi-Ardekani B, Sotoudeh M, Aghcheli K, Semnani S, Shakeri R, Taghavi N, et al. Esophagitis may not be a major precursor lesion for esophageal squamous cell carcinoma in a high incidence area in North-Eastern Iran. Middle East J Dig Dis 2011;3:28-34.
16. Lundell LR, Dent J, Bennett JR, Blum AL, Armstrong D, Galmiche JP, et al. Endoscopic assessment of esophagitis: Clinical and functional correlates and further validation of the Los Angeles classification. Gut 1999;45:172-80.
17. Bytzer P, Havelund T, Hansen JM. Interobserver variation in the endoscopic diagnosis of reflux esophagitis. Scand J Gastroenterol 1993;28:119-25.
18. Yerian LM, Goldblum JR. Esophagus. In: Mills SE, Stacey E, editors. Sternberg’s Diagnostic Surgical Pathology. 5th ed. Philadelphia: Lippincott Williams and Wilkins; 2010. p. 1250-3.
19. Bennet AE, Goldblum JR, Odze RD. Inflammatory disorders of the esophagus. In: Odze RD, Goldblum JR, editors. Surgical Pathology of the GI Tract, Liver, Biliary Tract, and Pancreas. 2nd ed. Philadelphia: Saunders Elsevier; 2009. p. 231-42.
20. Fiocca R, Mastrocchi L, Milione M, Parente P, Savarino V; Gruppo Italiano Patologi Apparato Digerente (GIPAD); Società Italiana di Anatomia Patologica e Citopatologia Diagnostica/International Academy of Pathology, Italian division (SIAPEC/IAP). Microscopic esophagitis and Barrett’s esophagus: The histology report. Dig Liver Dis 2009;43(Suppl 4):S319-30.
21. Zentilin P, Savarino V, Mastrocchi L, Spaggiari P, Dulbecco P, Ceppa P, et al. Reassessment of the diagnostic value of histology in patients with GERD, using multiple biopsy sites and an appropriate control group. Am J Gastroenterol 2005;100:2299-306.
22. Kasap E, Zeybel M, Aşık G, Ayhan S, Yüceyar H. Correlation among standard endoscopy, narrow band imaging, and histopathological findings in the diagnosis of nonerosive reflux disease. J Gastrointest Liver Dis 2011;20:127-30.
23. Zuberi BF, Faisal N, Quraishy MS, Afsar S, Kazi LA, Kazim E. Correlation between clinical endoscopic and histological findings at esophago-gastric junction in patients of gastroesophageal reflux disease. J Coll Physicians Surg Pak 2005;15:774-7.
24. Lee YC, Lin JT, Chiu HM, Liao WC, Chen CC, Tu CH, et al. Intraobserver and interobserver consistency for grading esophagitis with narrow-band imaging. Gastrointestinal Endosc 2007;66:230-6.
25. Tseng PH, Chen CC, Chiu HM, Liao WC, Wu MS, Lin JT, et al. Performance of narrow band imaging and magnification endoscopy in the prediction of therapeutic response in patients with gastroesophageal reflux disease. J Clin Gastroenterol 2011;45:501-6.

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