Malaysian Journal of Microscopy Vol.8 (2012) pg. 139-142

ESOPHAGEAL SURFACE CELLS IN NON- EROSIVE REFLUX DISEASE: A SCANNING ELECTRON MICROSCOPICAL STUDY (SEM)

Y. Y. Myint[1], Y Tun[1], N. Muhamad Amjad[2], M. Z. Mat Hassan[2] and N. L. Rashid[1]

[1] Department of Basic Medical Sciences, Kulliyyah of Medicine , International Islamic University Malaysia, Jalan Istana, Bandar Indera Mahkota, 25200 Kuantan , Pahang Darul Makmur, Malaysia.
[2] Department of Surgery, Kulliyyah of Medicine, International Islamic University Malaysia, Jalan Istana, Bandar Indera Mahkota, 25200 Kuantan , Pahang Darul Makmur, Malaysia.

Dilated spaces at intercellular junctions (DIS) in prickle cell layers are considered as early signs of acid-initiated damage to these esophageal epithelium. In order to enter the mucosal intercellular spaces, acid and pepsin have to penetrate the intercellular adhesion sites of the most superficial epithelial cells. The aim of this study is to explore intercellular communications superficial esophageal cells in non- erosive reflux disease (NERD) patients. The study group included eight (NERD) patients and five normal controls individuals. Specimens were routinely processed for SEM observations. The nature of intercellular attachments and intercellular spaces between each individual superficial cells of the esophageal mucosa were viewed under SEM. Our results showed that cellular attachments in the superficial cells differ significantly (p value 0.007) among the normal controls and patients with NERD (Chi-Square test, $p < 0.05$ was considered to be statistically significant). Our findings seems to support the concept of abnormal tissue resistance in the pathogenesis of NERD patients.

Key words: Non-erosive reflux disease (NERD), dilatation intercellular spaces (DIS), cellular attachment, scanning electron microscopy (SEM)

INTRODUCTION

Non-erosive reflux disease (NERD) is the most common phenotype of gastro-esophageal reflux disease (GERD) and the prevalence is increasing in Asia including Malaysia [1-3].

In a prospective study conducted by Rosaida and Goh (2004), based on either symptoms of heartburn and acid regurgitation and/or findings of reflux esophagitis among 388 patients 254 (65.5%) were diagnosed as having NERD. [1-4].

NERD is defined as troublesome reflux symptoms in the absence of esophageal mucosal damage on endoscopy. The cardinal symptoms of NERD are heart burn and regurgitation and the impact of reflux symptoms on quality of life in NERD patients are similar to those of erosive reflux disease [4-7].

Dilatation of the intercellular space (DIS) of the esophageal epithelium have been identified by both light and electron microscopy as a histopathological marker of early reflux damage in experimental animals and adults with both erosive and non-erosive reflux disease. [8-16].

This project is proposed because there seems to be comparatively few scanning electron microscopic studies of esophageal epithelium in non-erosive reflux disease (NERD) in Malaysia. Thus the aim of this study is to explore cellular attachments in most superficial esophageal cells of NERD patients and in normal controls.

METHODS AND MATERIALS

The study group included 5 normal controls and 8 NERD patients with reflux symptoms, normal mucosa on endoscopy and positive 24-hour pH monitoring. The study was approved by International Islamic University Malaysia Ethics Committee. All the participants gave written informed consent to participate in the study. Then they were interviewed a simple questionnaire, concerning detail about reflux symptoms (heartburn and/or regurgitation) and other associated symptoms as well as their previous treatment. In the preceding two weeks no patient had taken H_2 receptor antagonists, proton-pump inhibitors or non-steroidal anti-inflammatory drugs. All subjects underwent a gastro endoscopy (Olympus GIF-D3 scope, Japan) and the distal part of the esophagus was carefully evaluated to determine the presence of esophageal injury. Endoscopic mucosal biopsy specimens were taken at 5 cm above the Z-line from those participants with normal-appearing mucosa within the esophagus onendoscopy. Then they underwent 24-hour pH monitoring. Patients with GERD symptoms and positive pH monitoring but without esophageal mucosal erosion were diagnosed as NERD.

* *Corresponding author : Tel : 006-09-5716609-2483; Fax: 09 5716-770*
E-mail: yym_iiu@hotmail.com

Scanning Electron Microscopy

One specimen from each participant was processed for scanning electron microscopic study. The specimens were fixed in 4% glutaraldehyde, rinsed and post-fixed in 1% buffered osmium tetroxide. They were then dehydrated through an ascending ethanol series before embedding in epoxy resin. Blocks were trimmed, and ultra-thin sections were post-stained with uranyl acetate and lead citrate.

Morphometric Analysis

The specimens were then examined and photographed in a scanning electron microscope (SEM Carl Zeiss EVO 50, Germany). The intercellular spaces between the most superficially located esophageal epithelial cells were viewed.

Statistical Analysis

Data were analyzed using the Statistical Package for Social Science (SPSS) version 16. The association between DIS and categorical data was accessed by using Chi-Square test. *P* 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

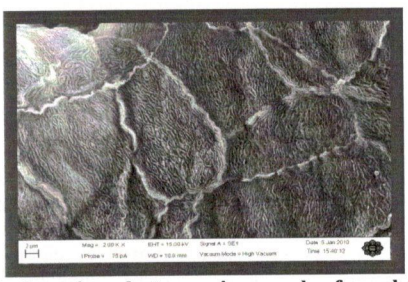

Fig.1. Scanning electron micrograph of esophageal epithelium (Grade1). The superficial cells are closely attached to each other with clear cut cell boundaries.

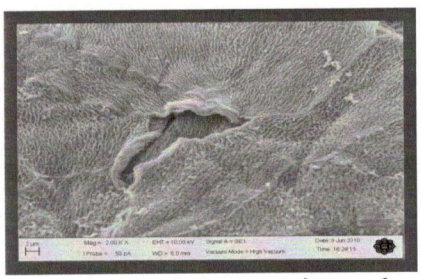

Fig.2. Scanning electron micrograph of esophageal epithelium (Grade 2). Cells closely attached but some areas showed detachment from its intercellular junctions.

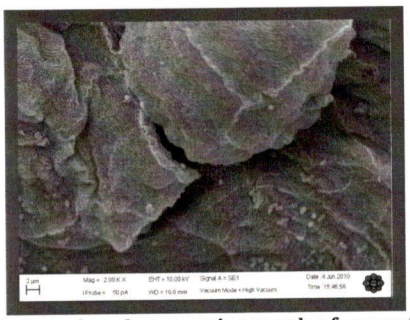

Fig.3. Scanning electron micrograph of superficial esophageal epithelium (Grade 3). Desquamation and less well developed cell boundaries.

Table 1 Cellular attachment in control and NERD.

	Grade 1	Grade 2	Grade 3
NERD (n=8)	0	1 (12.5%)	7 (87.5%)
Control (n=5)	2 (40%)	3 (60%)	0

In this study, SEM was used to examine the cellular attachment of superficially located cells of esophageal epithelium and cellular attachment of superficial cells was divided into 3 grades. Our results showed that cellular attachments in the superficial cells differ significantly (p value 0.007) among the normal controls and patients with NERD.

A unifying hypothesis for symptom generation in NERD based on abnormal tissue resistance. [13]. According to this hypothesis, the presence of abnormal tissue resistance, demonstrated by defects within the intercellular junctional complex between cells of the surface layers of esophageal (stratified squamous) epithelium, is shown to enable the ready diffusion of refluxed gastric acid (H^+) into the intercellular space. Within this space, it encounters and activates chemosensitive nociceptors whose signals are transmitted via the spinal cord to the brain for symptom (heartburn) perception. Activation of the same nociceptors is also capable of initiating a short reflex arc to esophageal (longitudinal) smooth muscle as means of precipitating a sustained esophageal contraction. This hypothesis is supported by the demonstration of dilated intercellular spaces in the distal esophageal epithelium , using electron microscopy, among NERD subjects and by the reversal of these changes by successful acid-suppressive therapy [8-19].

Azumi *et al.*, (2008), reported that the intercellular space between the most superficially located epithelial cells in patients with erosive esophagitis or NERD was not different from that in asymptomatic healthy individuals [20]. The 24-h

esophageal pH monitoring study was not performed in their study and the diagnosis of NERD was made by reflux symptoms and endoscopic findings. They took the esophageal biopsy specimens 2cm from the Z line.

Our result supported the pathophysiology of heart burn which stated as "In order to enter the mucosal intercellular spaces, acid and pepsin have to penetrate the intercellular adhesion sites of the uppermost superficial epithelial cells." [8, 11-13].

A limitation of our study is that the number of subjects investigated was relatively small due to limited time and budget provided.

CONCLUSION

The present study confirms that gastroesophageal reflux provokes intercellular space dilation in esophageal mucosa and supported the pathophysiology of reflux-induced symptoms or mucosal damage in NERD. In conclusion, our findings supported the concept of abnormal tissue resistance in pathogenesis of NERD patients

ACKNOWLEDGEMENTS

A special word of gratitude is dedicated to Research Centre, International Islamic University of Malaysia (IIUM) for their research grant (EDW B 0905-278). Special thanks go to the director and staffs of endoscopy unit, Hospital Tengku Ampuan Afzan (HTAA), staffs of Gastrointestinal Laboratory Unit and Electron Microscopy Unit, IIUM.

REFERENCES

[1] El-Serag HB. (2008) Epidemiology of Non-Erosive Reflux Disease. *Digestion,* **78**(suppl 1):6–10.

[2] Rosaida MS, Goh KL. (2004). Gastro-oesophageal reflux disease, reflux oesophagitis and non-erosive reflux disease in multiracial Asian population: a prospective, endoscopy based study. *Eur J Gastroenterol Hepatol* , **16**:495–501.

[3] Goh KL, Wong CH. (2006) Gastroesophageal Reflux Disease: An Emerging Disease in Asia. *Gastroenterology & Hepatology*, Volume **2**, Issue 2: 118-123.

[4] Hershcovici T, Fass R. (2010). Nonerosive Reflux Disease (NERD) — An Update. *J Neurogastroenterol Motil*, Vol. **16** No. 1: 8–21..

[5] Modlin IM, Hunt RH, Malfertheiner P, Moayyedi P, Quigley EM, Tytgat GN, Tack J, Holtmann G, Moss SF. (2008). Non-erosive reflux disease—defining the entity and delineating the management. *Digestion*. **78** Suppl 1:1-5. Epub Oct 2.

[6] Tack J , Fass R. (2004) Review article: Approaches to endoscopic-negative reflux disease: part of the GERD spectrum or a unique acid-related disorder? *Aliment Pharmacol Ther,* **19** (Suppl. 1): 28–34.

[7] Hershcovici T, Fass R. (2010). Nonerosive Reflux Disease (NERD) - An Update. *J Neurogastroenterol Motil*, Vol. **16** No. 1 16:8-21

[8] Tobey NA, Carson JL, Alkiek RA, Orlando RC (1996) Dilated intracellular spaces: a morphological feature of acid reflux-damaged human esophageal epithelium. *Gastroenterology.* **111**: 1200–1205.

[9] Calabrese C, Fabbri A, Bortolotti M, , Cenacchi G, Areni A, Scialpi C, Miglioli M, Di Febo G. (2003). Dilated intercellular spaces as a marker of esophageal damage: comparative results in gastroesophageal reflux disease with or without bile reflux. *Aliment Pharmacol Ther.***18**:525–32.

[10] Tobey N A, Hosseini S S, Argote C M, Dobrucali A M,Awayda M S, Orlando R C. (2004) Dilated intercellular spaces and shunt permeability in nonerosive acid-damaged esophageal epithelium. *Am J Gastroentero*; **99**: 13–22.

[11] Tobey N. A., Gambling T. M., Vanegas X. C., Carson J. L., Orlando R. C. (2008). Physicochemical basis for dilated intercellular spaces in non-erosive acid-damaged rabbit esophageal epithelium. *Diseases of the Esophagus.* **21**, 757–764

[12] Caviglia R, Ribolsi M, Maggiano N, Gabbrielli AM, Emerenziani S, Guarino MP, Carotti S, Habib FI, Rabitti C, Cicala M. (2005). Dilated intercellular spaces of esophageal epithelium in non-erosive reflux disease patients with physiological esophageal acid exposure. *Am J Gastroentero*; **100**: 543–548.

[13] Barlow WJ, Orlando RC. (2005). The pathogenesis of heartburn in non-erosive reflux disease: a unifying hypothesis. *Gastroenterology.* **128**:771–8.

[14] Ravelli AM, Villanacci V, Ruzzenenti N, Grigolato P, Tobanelli P, Klersy C, Rindi G. (2006). Dilated intercellular spaces: a major morphological feature of esophagitis. *J Pediatric Gastroenterol Nutr.* **42**(5):510-5.

[15] Dent J. Microscopic esophageal mucosal injury in nonerosive reflux disease. (2007) *Clin Gastroenterol Hepatol*; **5**: 4–16.

[16] Alvaro-Villegas JC, Sobrino-Cossío S, Hernández-Guerrero A, Alonso-Lárraga JO, de-la-Mora-Levy JG, Molina-Cruz A, Domínguez-Malagón HR, Herrera-Esquivel JJ.(2010).

Dilated intercellular spaces in subtypes of gastroesophagic reflux disease. *Rev Esp Enferm Dig*; **102**:302–307.

[17] Xue Y, Zhou L Y, Lin S R. (2008). Dilated intercellular spaces in gastroesophageal reflux disease patients and the changes of intercellular spaces after omeprazole treatment. *Chinese Medical Journal.* **121**(14): 1297-1301.

[18] Scialpi C, Miglioli M, Di Febo G. (2005). Reversibility of GERD ultrastructural alterations and relief of symptoms after omprazole treatment. *Am J Gastroenterol*; **100**: 537-542

[19] Calabrese C, Fabbri A, Bortolotti M, Cenacchi G, Carlo S (2005) Zahlane D, Miglioli M, Di Febo G. Effect of omeprazole on symptoms and ultrastructural esophageal damage in acid bile reflux. *World J Gastroenterol* 2005; **11**: 1876-1880

[20] Azumi, T., Adachi, A., Furuta, K., Nakata, S., Ohara, S., Koshino, K., Miki,M., Morita,T., Tanimura,T., Ashizawa,N., and Kinoshita,Y. (2008). Esophageal epithelial surface in patients with gastroesophageal reflux disease: An electron microscopic study. *Journal of Gastroenterology, **14***(37), 5712-5716.