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Electron Microscopic Changes In Esophageal Epithelium In Reflux Esophagitis

Samia I. El-Naggar*, Suhair A. Abdel Hafeez*, Hany Abdel Karium**
Department of pathology*, and surgery **
Faculty of medicine, Assiut University.

Abestract

Dilated intercellular spaces are a sign of epithelial damage in acid-perfused rabbit esophagus, a change best identified by transmission electron microscope. The aim of this work was to study the epithelial changes in reflux esophagitis by transmission electron microscopy, and to determine if this change is also a feature of acid damage to human esophagus. Endoscopic esophageal biopsy specimens from 25 patients with recurrent heartburn were taken. Fifteen out of 25 patients had erosive esophagitis and 10 had normal appearing mucosa on endoscopy. Ultra-thin section was stained with toluidine blue and examined with light microscopy and showed feature of epithelial injury and repair in higher percentage in erosive esophagitis. Transmission electron microscopy examination revealed dilated intercellular spaces in 92% of biopsy specimens irrespective of whether the patients had erosive or non-erosive disease. We concluded that dilated intercellular spaces are a feature of reflux damage to human esophageal epithelium.

Key words: reflux esophagitis, TEM

Introduction

Gastro-esophageal reflux is the involuntary movement of gastric content from the stomach to esophagus (Roy and Orland 1997). Although reflux episodes occur in normal individuals, abnormal gastro-esophageal reflux can cause clinical and pathologic manifestation (Pace *et al*., 1991) Exactly, how physiologic reflux becomes pathologic process is incompletely understood, but numerous complex factors are likely involved including the volume and potency of the refluxate, the duration of esophageal exposure, and the efficiency of esophageal defense mechanisms (Pace *et al*., 1991). There is different approach to establishing the diagnosis. Consequent, there is no completely sensitive and specific gold standard for the diagnosis of gastroesophgeal reflux diseases GERD. Endoscopy with biopsy is an important diagnostic procedure in patients with gastroesophgeal reflux diseases GERD. Moreover, up to 50 % of patients with documented symptom - matic GERD can display normal or only minimal abnormal endoscopic findings (Knuffte *et al*., 1984). Esophageal mucosal biopsy is theoretically useful in patients with symptoms consistent with GERD who have normal appearing esophageal mucosa during endoscopy (Lembo *et al*., 1999). The histological abnormalities of GERD encompass a range of features denoting epithelial injury and repair. Collins *et al* (1985), found that vascular dilatation, epithelial hyperplasia, and papillary elongation can be detected most reliably, but their diagnostic accuracy is limited. Kahrilas

Refree : Prof ; *Dr. Samy El-hakeem*

and Hogan, (1993) found that the degree of damage does not completely correlate with the amount of refluxed material because patients with similar gastroesophgeal reflux have either no damage or different grades of mucosal lesions. Other studies have shown that acid damages esophageal epithelial cells by inducing cell edema, cell acidification, and necrosis after affecting the lipid bilayers of the apical cell. Notably, Camey et al., (1981) found that before the onset of cell necrosis, another morphologic change is identified within the acid perfused rabbit esophageal epithelium, i.e., the presence of dilated intercellular spaces within the esophageal epithelium. This changes is poorly visible by light microscopy but readily identifiable by transmission electron microscopy TEM. In this work, we study the feature of damage to esophageal mucosa on endoscopic biopsy specimens (erosive and non erosive), from patients with heartburn using transmission electron microcopy.

Material And Methods:

This study who dealt with 25 patients selected from the outpatient clinic of surgery Assiut University Hospital, presented with gastroesop - hgeal reflux symptoms. Out of them 15 were males (60%) and 10 were females (40%). Their ages ranged from 20 to 66 year

All patients were subjected to:

1. Clinical history: taking symptoms of GERD, relation of symptoms to meal and posture.
2. General and abdominal examination: to exclude any systemic diseases that may affect gastroesophgeal motility.
3. Upper gastro-intestinal endoscopy: All selected patients were prepared by fasting for 10-12 hours and sedated by diazepam 10 mg I V. and xylocain 10% spray solution. Examination of esophagus was done using esophago-gastroscope pentox video scope E G 2730. Endoscopic results were graded according to Savary – Millar criteria.
4. Esophageal biopsy: biopsy specimens were obtained within the lower 5 cm of esophagus from areas of macroscopically intact (noneroded) esophagealmucosa. The specimens were fixed in 4 % glutaraldehyde solution, rinsed, post fixed in 1 % Osmium tetroxide, solution, rinsed again, dehydrated in a graded series of ethanol, immersed in propylene oxide, embedded in epoxy resin and sectioned in an ultramicrotome. Semi-thin sections were stained with Toluidine blue and examined by light microscopy. From representative areas, thin sections were double-stained with uranyl acetate and lead citrate and then examined by Philips 400 T transmission electron microscope at 80 kV.

Results

Endoscopic biopsy specimens were obtained from 25 patients with GERD. Fifteen out of 25patients with GERD had erosive esophagitis (grades I-IV) according to classification of Savary – Miller, in which grade 1 : is single erosion or erosion on a single fold, grade II: is multiple erosions on more than one fold, grade III :is circumferential erosion, and grade IV: is erosions plus stricture formation. Ten patients had symptomatic reflux with completely normal –appearing esophageal mucosa on endoscopy. Out of 15 patients with erosive esophagitis 5 had esophagitis grade I, 5 grades II, 4 grades III, and 1 grade IV table (1). There was no significant relation between patient's age, or sex, and endoscopic finding.

TABLE (1) Histologic grade of erosive esophagitis

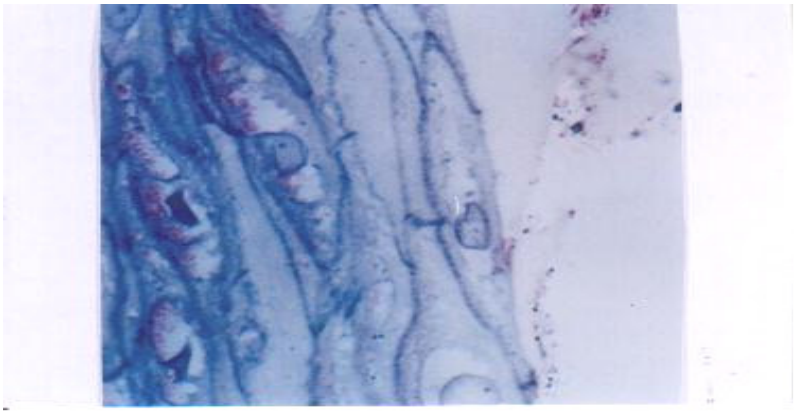
Grade	NO.	%
I	5	33.3
II	5	33.3
III	4	26.4
IV	1	07

TABLE (2) The percentage of histopathologic finding in non-erosive and erosive esophagitis

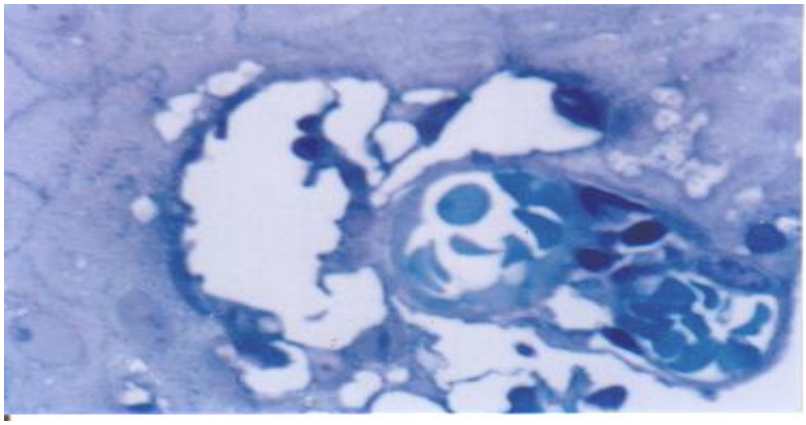
Microscopic findings	Non-erosive esophagitis		Erosive esophagitis	
	NO.	%	NO.	%
Erosion/ulcer	0	0	2	*13.3*
Basal cell hyperplasia	4	40	8	*53.3*
Ballooned sq cell	6	60	7	*46.6*
Intraepithelial inflammation	3	30	12	*80*
Intraepithelial vascularization	5	50	10	*66.6*
Cytoplasmic vaculation	4	40	9	*60*
Perinuclear vaculation	6	60	11	*73.3*
Parakeratosis	*1*	*10*	*10*	*66.6*

Histopathologic finding

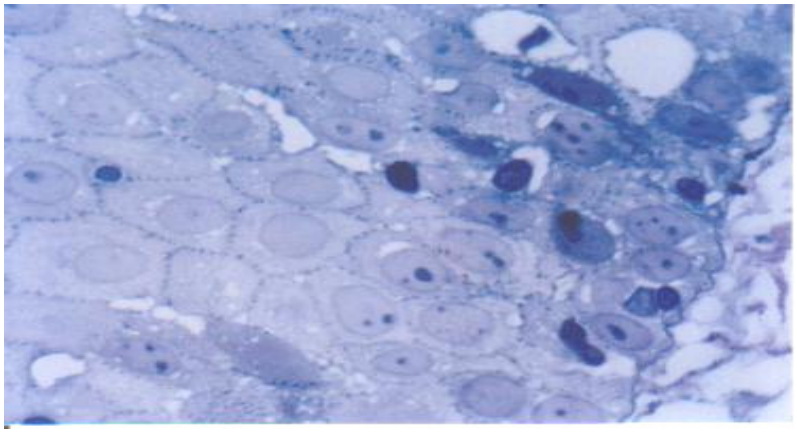
The changes found in the inflammed esophageal epithelium were either related to the intercellular spaces or were within the epithelial cells themselves or both. In the inflammed esophageal mucosa, the intercellular spaces were widened in 20/25sections. This was most marked in the prickle cell layer (Fig 3) but also occurred to some extent in the basal cell layer. Intraepithelial inflammatory cell were regularly found (Fig 1, 3), lympho -cytes were the most abundant of these 19/25, although some polymorph nuclear leukocytes were encountered in 3/15 of erosive esophagitis. Some of the epithelial changes were related to inflammatory cells. The most remarkable of these was increase in the distance of desmosomes on epithelial cells, (Fig 3). Intraepithelial vascularization was found in 18/25 specimens, with extravagation of blood (Fig 2). Perinuclear vaculation, cytoplasmic vaculation were detected in 17/25 &13/25 specimens (Fig 1, 3). Parakeratosis was detected in severely inflammed biopsies 11/25 (Fig I)



Fig(1) section from the superficial layer showing Parakeratosis, cytoplasmic vaculation (X1000)



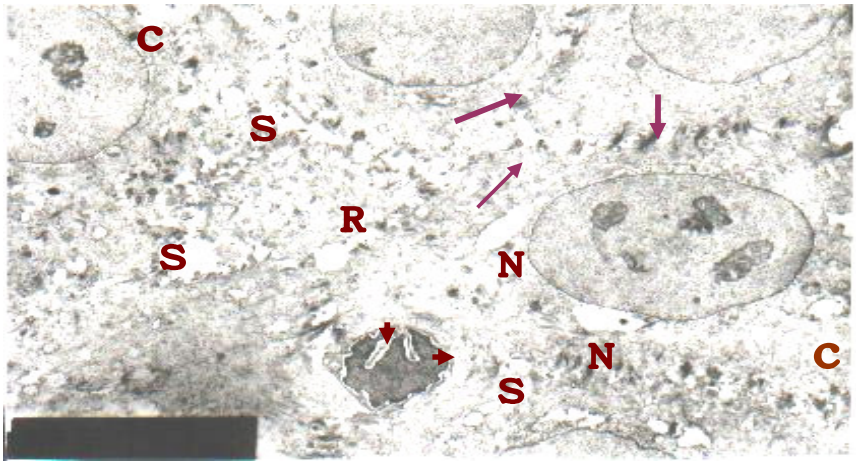
Fig(2) section from prickle cell layer showing intraepithelial blood vessel (X1000)



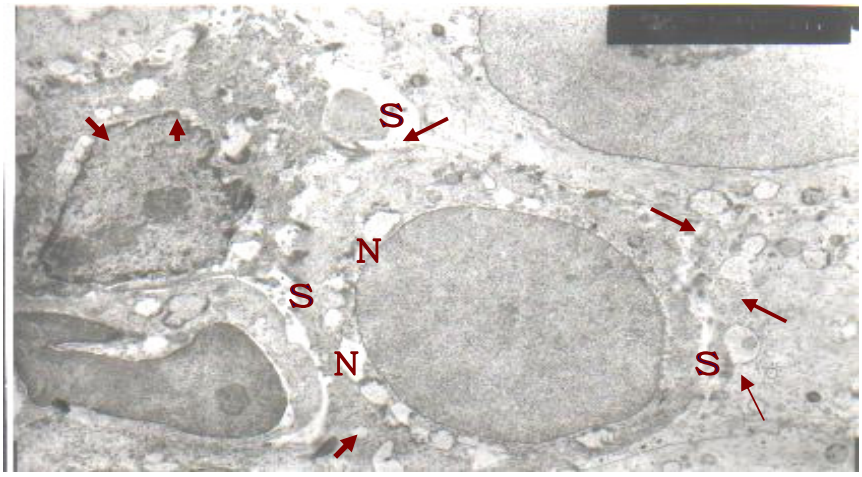
Fig(3) section from the basal layer showing spaces between cells, lymphocyte, cytoplasmic vaculation,perinucleal vaculation,lack of desmosomes beside the inflammatory cell. (X1000)

Electron microscopy finding

Esophageal biopsy specimens from patients with erosive and non-erosive esophagitis showed dilated intercellular spaces 20/25(fig 4, 5), There were various feature in the epithelial cell cytoplasm resulting from damage due to inflammation, mitochondrial damage was found in 10/25 ranging from slight to almost complete destruction (fig 5), peri nuclear vaculation were detected in 8/25. Another feature encountered was the appearance of intraepithelial inflammatory cells (fig 4, 5)



Fig(4) TEM section ,the intercellular space between the prickle cell is considered widened(S), the prickle cell show irregular distribution of desmosomes(arrow) ,ruffling of the cell membrane(R) ,perinuclear (N) and cytoplasmic vaculation (C) ,and inflammatory cell arrow head Note lake of desmosomes at epithelial inflammatory site compared with other side (12,000)



Fig(5) TEM secion from prickle cell layer showing, widened intercellular space (S), Perinuclear (N) ,cytoplasmic (C) vaculation, inflammatory cell (arrow head) and damaged mitochondria (arrow) (20,000)

Discussion

The esophageal epithelium of patients with reflux disease when examined by electron microscope shows feature of classic light microscopic changes and others revealed only by higher magnification .In the present study, ultra thin sections stained with Toluidine blue showed most of the features of esophagitis have been reported by Ismail –Beigi et al., (1974), and Kobashi and Kasiga, (1974). Basal cell hyperplasia was more apparent in 10|15 erosive biopsy and 2|10 non-erosive biopsies. Black et al., (1990). stated that there is increased epithelial proliferation and turn over of the basal cells with reflux. Intra epithelial inflammatory cells were found within the epithelium of the present biopsies. Most of these cells were lymphocytes, which were present in 17/25 of all biopsies while polymorphs present in 3/15 cases of erosive esophagitis .The nature and role of intra epithelial lymphocytes in the gut has been discussed by Riddell, (1996), In the bowel, their function may be related to the antigens, both food and parasitic, within the lumen. In the human esophagus, food passes rapidly down the lumen and only a few bacteria have been encountered in other previous ultra structural studies, Hopwood et al., (1978). Other cells met within the epithelium of GERD include mast cells and eosinophiles (Robert et al., 1995) but these were not encounted in the present study. Mucosal vascularization was present in 13/25 of erosive and non-erosive biopsies. Sarbati et al., (1994) studied the mucosal micro vessels in reflux esophagitis by TEM and demonstrated that microangiopathy is associated with epithelial damage. The epithelial membrane remained intact although the intrusive cells caused stretching or rearrangement, judged by the distribution of desmosomes. Basteson et al.,(1981), studied the effect of incubation of esophageal biopsy from endoscopic normal patients with duodenal juice and the enzymes (trypsin, pepsin, lipase). They found that these enzymes caused lysis and internalization of desmosomes and peripheral cytoplasmic vaculation. Bile acids split desmosomes and induced micovesicu -lation of cell membrane. Also, all media except hydrochloric acid eventually produced organelle damage and leaky and disrupted cells. We found that the esophageal epithelial cells became parakeratotic in the severely inflammed biopsies. This in agreement with Hopwood et al., (1978). Transmission electron microscopy of endoscopically obtained esophageal biopsy specimens showed dilated intercellular space. Further, the dilated intercellular spaces were not only observed within the normal-appearing mucosa of patients with erosive esophagitis but were present in the normal- appearing mucosa of patients with symptomatic (non-erosive) reflux disease. This in agreement with that found by Hopwood et al., (1979)& Salmo et al., (1990)&Tobey et al., (1996). Because this feature in the acid –damaged rabbit esophagus correlates with an increase in (Para cellular) permeability across the acid–damaged esophageal epithelium, its presence may explain why patient with endoscopically normal mucosa readily develop heartburn on exposure to an acidic refluxate (Tobey et al., (1996). Support for this is found in studies showing that sensory neurons within the esophagus terminate within the intercellular space only a few layers from the lumen (Rodrigo et al., (1975) .We concluded that the presence of

dilated intercellular space occurs before the onset of gross morphological damage, suggesting that it may be an earlier lesion (than cell necrosis) in the acid –damage sequence.

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دراسة التغيرات الحادثة لطلائي المرئ في حالات الارتجاع المرئ بواسطة الميكروسكوب الإلكتروني

ساميه إبراهيم النجار*- سهير عبد الحفيظ*- هانى عبد الكريم **
من أقسام الباثولوجي*والجراحة- كليه الطب- جامعه أسيوط**

أجرى هدا البحث على 25 حاله اختيرت من العيادة الخارجية لمستشفى أسيوط الجامعي يعانون من أعرا ض ارتجاع المرئ (حموضة معوية) بعد أن أجريت لهم التحليل الطبية لاستبعاد الأسباب الأخرى
وقد تم أخذ عينات من المرئ بواسطة المنظار الداخلى. وتم تثبيتها فى محلول الجلوترالدهيد . وتقطيعها قطاعات رقيقه جدا وصبغها بمادة التوليديين بلو ودراستها بالميكروسكوب الضوى. وأخذ قطاعات ارق من الأجزاء المعبرة وصبغها ودراستها بالميكروسكوب الإلكتروني .
اثبت الدراسة بالميكروسكوب الضوئي وجود تغيرات هستوباثولوجيه في الغشاء المبطن للمرئْ بنسب أعلى في التهاب المرئ ا لتاكلى ولكنها غير معبر.
كما أكد ت الدراسة بالميكروسكوب الإلكتروني وجود توسعات فراغيه خلوية في الغشاء المبطن للمرئْ بنسبه 92 % بصرف النظر عن سواء المرضى كان عندهم أتجاع مرئْ تآكلي أو غير تأكلى.
استنتجنا أن تلك الفراغات الخلوية المتوسعة هي ميزه للار تجاع المرئْ وتحدث قبل ظهور التغيرات الهستوباثولوجيه الأحرى وتفسر حدوث الحموضة المعوية في الحالات التي يظهر فيهل طلائي المئرئ سليم بالنظار الضوئي الداخلي