

# Cell proliferation of esophageal squamous epithelium in erosive and nonerosive gastroesophageal reflux disease

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## Background/aim

Gastroesophageal reflux disease (GERD) is a major public health problem that may cause erosive or nonerosive esophagitis in symptomatic patients. The severity of esophagitis in GERD seems to be correlated not only to the amount of reflux and altered motor activity but also to the ability of the mucosa to resist injury and repair the damage. This study aimed to evaluate the cell proliferation status of esophageal epithelium in both normal individuals and patients with GERD with or without erosions and its correlation with the degree of inflammation of the esophagus.

## Participants and methods

This study was carried out on 33 individuals; their ages ranged between 17 and 74 years. All participants were subjected to a clinical assessment and an endoscopic evaluation. Four biopsies were taken using an endoscope at 5 cm from the Z-line; histological esophagitis was identified and graded. Cell proliferation was evaluated by Ki-67 immunostaining.

## Results

The prevalence of GERD was the highest in the 15–29 years age group (46.43%) and decreased with age. Cell proliferation [estimated by the Ki-67-labeling index (Ki-67 LI)] was reduced in esophageal epithelium in erosive (13.44%) and nonerosive (36.83%) reflux disease in relation to normal individuals (68%). There was a statistically significant positive correlation between cell proliferation (Ki-67 LI) and the endoscopic grade of esophagitis among patients with erosive disease. However, there was no significant correlation between cell proliferation (Ki-67 LI) and the histological grade of esophagitis in both erosive and nonerosive reflux disease.

## Conclusion

The ability of the mucosa to resist injury and to repair the damage should be considered a key factor in the development of GERD. Esophageal mucosa exposed to chronic acid insult show reduced cell replication, estimated by the Ki-67 LI. Erosive esophagitis in GERD seems to be related to a low cell proliferation rate of esophageal epithelium rather than the amount of reflux.

## Keywords:

cell proliferation, esophageal squamous epithelium, gastroesophageal reflux

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## Introduction

Gastroesophageal reflux disease (GERD) is a major public health problem, with 10–20% of adults experiencing reflux symptoms [1]. The prevalence of GERD has increased considerably in the past few decades. Therefore, GERD is one of the most frequent gastrointestinal diseases in western countries [2].

Most of the patients with GERD fall into one of two categories: those with nonerosive reflux disease (NERD) or those with erosive reflux disease (ERD). Although the differentiation of ERD and NERD at a clinical level is difficult, there are clearly physiological, pathophysiological, anatomical, and even histological characteristics that are unique to NERD [3]. Natural course studies have shown that most NERD patients do not progress over

time to ERD or even Barrett's esophagus [3]. NERD patients compared with those with ERD show a highly variable and unpredictable symptomatic response rate to antireflux treatment [4]. The reason for this heterogeneous impact of GERD on esophageal mucosa is unknown [5].

The relation between the morphological appearance and the severity of GERD seems to be correlated not only to the amount of reflux and altered motor activity but also to the ability of the mucosa to resist injury and repair the damage [5]. Cell replication of basal layers is hypothesized to be one of the causes implicated in the resistance of the mucosa and structural epithelial defense [6].

The Ki-67 antigen is a proliferation-associated nuclear antigen that is expressed in all phases of the cell cycle,

except in the resting (G0) stage [7,8]. Determination of the Ki-67-labeling index (Ki-67 LI) is widely used in surgical pathology as a marker of cell proliferation, and it has been found to correlate with the clinical course of many diseases [9].

### Aim

To evaluate the cell proliferation status of esophageal epithelium in both healthy normal individuals and patients with GERD with or without erosions and to detect the correlation between Ki-67 LI and the degree of inflammation of the esophagus.

### Participants and methods

This study was carried out on 33 patients who attended the Internal medicine and Tropical Medicine outpatient clinics, Sohag University Hospitals. Twenty eight of these patients presented with typical gastroesophageal reflux symptoms (heartburn/regurgitation), whereas the remaining five were healthy controls.

Patients with coagulopathy, patients on anticoagulants, radiotherapy, or chemotherapy, patients with gastroesophageal varices, Barrett's esophagus, gastrointestinal bleeding, use of acid suppressor therapy over the last 60 days, esophageal neoplasia, or those who had undergone surgery were excluded from the study.

Patients were divided into three groups:

- (1) Group 1: 16 patients with gastroesophageal reflux symptoms with erosions.
- (2) Group 2: 12 patients with gastroesophageal reflux symptoms without erosions.
- (3) Group 3: five healthy controls.

All patients signed a written consent and then subjected to the following.

### Clinical data

Data obtained included age, sex, typical GERD symptoms (heartburn and acid regurgitation), atypical manifestations of GERD (pulmonary manifestations such as asthma or chronic cough, ENT manifestations such as laryngitis, hoarseness, sore throat, or globus, and cardiac manifestations such as chest pain), and risk factors for GERD (smoking, consumption of tea and coffee, spicy foods, and drugs).

### Endoscopic evaluation

All patients were subjected to upper GIT endoscopy (videoscope Olympus GIF-XQ 260; Olympus company, Tokyo, Japan) after sedation by intravenous midazolam 5 mg to assess the presence or absence of erosive esophagitis. The Los Angeles (LA) classification system was used to grade esophagitis [10]. In each patient, four specimens were taken using standardized biopsy forceps (Olympus KW2415S; Olympus company, Tokyo, Japan), from each of the four quadrants 5 cm above the

squamocolumnar junction, from macroscopically intact (noneroded) esophageal mucosa.

All the specimens were 10% formalin fixed and paraffin embedded. Then, they were prepared for hematoxylin-eosin and immunohistochemical staining.

### Histological evaluation

Five micrometer tissue sections were obtained from the paraffin-embedded tissue blocks, deparaffinized in xylol, and hydrated. The sections were then washed in distilled water (2 min) and stained with hematoxylin (for 2–3 min). The sections were washed in running tap water and stained with eosin (for 1–3 min). Then, they were washed in distilled water, and dehydration was carried out in 70, 80, 90, 95%, and absolute alcohol (2–5 min each), followed by clearing in xylol. The sections were mounted by covering them with a cover slip using a drop of Canada balsam. The hematoxylin and eosin -stained sections were examined and full comment was done.

Esophagitis was identified and graded according to the Ismail-Beigi *et al.* classification [11]:

- (1) The degree of basal cell hyperplasia was expressed as a percentage of epithelial thickness: none (0–15%), mild (16–33%), moderate (34–67%), and severe (> 67%).
- (2) The presence or absence of papillary zone elongation, determined by calculating papillary length as a percentage of epithelial thickness: absent (0–67%) and present (> 67%).
- (3) The density of neutrophil and eosinophil infiltration: none [0/high-power field (HPF)], mild (1–2/HPF), moderate (3–10/HPF), and severe (> 10/HPF).

The area of one HPF was 0.229 mm<sup>2</sup>.

### Immunohistochemical staining

Formalin-fixed paraffin-embedded sections were immunostained using the peroxidase-labeled streptavidin-biotin technique to detect Ki-67 immunoreactivity. The following primary monoclonal antibody was used: rabbit polyclonal antibody against the human Ki-67 gene product [Catalogue # RB-9043-P0 (0.1 ml); Lab Vision Corporation; Thermo Fisher Scientific Inc., Kalamazoo, Michigan].

### Staining procedure

Immunostaining was carried out in according to the procedures of the previous groups [12]. Briefly, formalin-fixed, paraffin-embedded sections were cut using a microtome into 5 µm thickness and mounted on silane-coated glass slides. Sections were deparaffinized and rehydrated through graded alcohols to distilled water. Endogenous peroxidase activity was blocked with 0.6% hydrogen peroxide for 10 min using peroxidase blocking reagent (Catalogue # TP-012-HD; Lab Vision Corporation). Antigen retrieval was carried out by immersing the slides in sufficient amounts of antigen retrieval solution (10 mmol sodium citrate buffer solution, pH 6.0) and boiling them in a microwave oven at a power 750 W for about 15 min.

Slides were then placed in PBS at pH 6 for 5 min. Sufficient amounts of the primary antibody (Ki-67) were added to the tissue sections at a dilution of 1/150. Then the slides were incubated for 1.5 h in a humid chamber at room temperature. The resulting immunocomplex was detected using a universal staining kit (Catalogue # TP-012-HD; Lab Vision Corporation). First, tissue sections were treated with biotinylated goat antipolyvalent for 10–15 min at room temperature. Second, peroxidase-labeled streptavidin was applied for 10–15 min at room temperature. Then, the slides were incubated with 14-diaminobenzidine and 0.06% H<sub>2</sub>O<sub>2</sub> for 5 min, washed in distilled water, and counterstained using hematoxylin. The tissue sections were washed in tap water, dehydrated in alcohol, and cleared in xylene. Slides were left to dry in air, then mounted in Canada balsam, and cover slipped.

#### Positive controls

Positive control slides were used to confirm that both the staining systems were working properly and positive signals were specific. The positive control slides were prepared from tissue sections obtained from high-grade osteosarcoma.

#### Negative controls

Negative control slides were used to confirm that the negative staining was valid. Additional sections of the tissues were stained in parallel, but with exclusion of the primary antibody [12].

#### Evaluation of immunostaining

For evaluation of Ki-67 immunoreactivity, the entire section was examined histologically using a bright-field microscope at low-power magnification. Then, higher power magnification ( $\times 400$ ) was used for the quantitative evaluation of Ki-67 immunoreactivity. Quantitative evaluation was carried out on portions of epithelium in between vertically sectioned stromal papillae. The number of Ki-67-positive nuclei in relation to the total number of epithelial cells was counted in three different HPFs, and the average was calculated. The Ki-67 LI was defined as the ratio of Ki-67-positive nuclei to the total number of epithelial cells, and was expressed as a percentage.

#### Statistical analysis

The data were processed and analyzed using the statistical package for the social sciences version 17 (IBM company, USA). The frequency, percentage, mean, and SD were calculated.  $\chi^2$ -test was used to compare the percentages.

A *t*-test was used to compare the means. The *P* value was significant at a less than 0.05 level and highly significant at a less than 0.001 level.

## Results

### Demographic characteristics of the studied population

The study included 33 patients, five of whom were healthy controls (mean age  $48 \pm 14.3$  years) as defined by three parameters: absence of typical or atypical manifestations of GERD, normal endoscopic features, and normal histological features.

Twenty eight patients had GERD (11 men and 17 women) as defined by typical symptoms (heartburn and regurgitation). Of these patients, 12 had an apparently normal esophageal mucosa at endoscopy (mean age  $37.75 \pm 18$  years), whereas 16 had erosive esophagitis (mean age  $36.06 \pm 13.89$  years).

In terms of the risk factors for GERD, 33.3% of NERD patients were smokers compared with 31.25% of the ERD patients, 41.6% of NERD patients consumed tea and coffee compared with 75% of the ERD patients, 41.6% of NERD patients consumed spicy foods compared with 37.5% of ERD patients, and 50 and 31% of patients in both groups, respectively, consumed drugs. Table 1 shows the demographic characteristics of the studied population.

GERD patients were divided into four age groups; the prevalence of GERD was the highest in the 15–29 years age group (46.43%) and decreased with age, being the lowest in the age group 60 years or more (14.29%), as shown in Table 2.

### Endoscopic evaluation of the studied population

In this study, five patients were healthy voluntary controls with normal esophageal mucosa at endoscopy. Among 28 patients with GERD, 12 had an apparently normal esophageal mucosa at endoscopy (i.e. NERD), whereas 16 had erosive esophagitis (i.e. ERD).

According to the LA classification [10], patients with erosive esophagitis were classified as follows: grade A (nine patients), grade B (three patients), grade C (two patients), and grade D (two patients) (Table 3).

**Table 1 Demographic characteristics of the studied population**

| Parameters          | ERD (n=16)        | NERD (n=12)    | Control (n=5) | Total (n=33)      | P value |
|---------------------|-------------------|----------------|---------------|-------------------|---------|
| Age (mean $\pm$ SD) | 36.06 $\pm$ 13.89 | 37.75 $\pm$ 18 | 48 $\pm$ 14.3 | 38.48 $\pm$ 15.64 | 0.333   |
| Sex                 |                   |                |               |                   | 0.208   |
| Males               | 7 (43.75%)        | 4 (33.33%)     | 4 (80%)       | 15 (45.45%)       |         |
| Females             | 9 (56.25%)        | 8 (66.67%)     | 1 (20%)       | 18 (54.54%)       |         |
| Smoking             | 5 (31.25%)        | 4 (33.33%)     | 3 (60%)       | 12 (36.36%)       | 0.488   |
| Tea and Coffee      | 12 (75%)          | 5 (41.67%)     | 3 (60%)       | 20 (60.61%)       | 0.203   |
| Spicy foods         | 6 (37.5%)         | 5 (41.67%)     | 2 (40%)       | 13 (39.39%)       | 0.975   |
| Drugs               | 5 (31.25%)        | 6 (50%)        | 2 (40%)       | 13 (39.39%)       | 0.603   |

ERD, erosive reflux disease; NERD, nonerosive reflux disease.

**Table 2 Prevalence of GERD among different age groups**

| Age groups (years) | Number of GERD patients (%) |
|--------------------|-----------------------------|
| 15–29              | 13 (46.43%)                 |
| 30–44              | 6 (21.43%)                  |
| 45–59              | 5 (17.86%)                  |
| ≥ 60               | 4 (14.29%)                  |

GERD, gastroesophageal reflux disease.

**Table 3 LA classification of ERD patients**

| LA | ERD (n=16) | Ki-67 LI (mean ± SD) | P value |
|----|------------|----------------------|---------|
| A  | 9 (56.25%) | 10.889 ± 2.028       | 0.000   |
| B  | 3 (18.75%) | 15 ± 1               |         |
| C  | 2 (12.5%)  | 17.5 ± 2.121         |         |
| D  | 2 (12.5%)  | 18.5 ± 0.707         |         |

ERD, erosive reflux disease; Ki-67 LI, Ki-67-labeling index; LA, Los Angeles.

### Histopathological evaluation of the esophageal biopsies

At histology, among 12 patients with NERD, six patients (50%) had a normal histological pattern and six patients (50%) had mild esophagitis.

Among patients with ERD, eight patients (50%) had a normal pattern, seven patients (44%) had mild esophagitis, and only one patient (6%) had moderate esophagitis.

The normal histological pattern of esophageal mucosa and the various changes that occurred in reflux esophagitis in the studied population are shown in Fig. 1.

### Immunohistological evaluation (Ki-67 LI) of the esophageal biopsies

The cell proliferation index (Ki-67 LI) ranged from 8 to 75% among all patients, with a mean ± SD of 30.21 ± 20.01. The mean Ki-67 LI value of the healthy voluntary controls was 68 ± 5.148. In GERD patients (NERD and ERD), the mean values of Ki-67 LI were 36.833 ± 5.59 and 13.438 ± 3.559, respectively, with highly significant differences among these groups ( $P < 0.001$ ) (Table 4, Figs 2 and 3).

### Correlation of Ki-67 LI with the LA classification grade

In ERD patients, the Ki-67 LI tended to increase with the LA classification grade, being the highest in grade D. The mean Ki-67 LI values were 10.889 ± 2.028 in patients with grade A, 15 ± 1 in patients with grade B, 17.5 ± 2.121 in patients with grade C, and 18.5 ± 0.707 in patients with grade D, with highly significant differences in the Ki-67 LI values among the endoscopic grades ( $P = 0.000$ ) (Table 5, Fig. 4).

### Correlation of Ki-67 LI with the degree of esophagitis

Among patients with NERD, the mean Ki-67 LI value was 38.167 ± 5.672 in patients with normal epithelium and 35.5 ± 5.683 in patients with mild esophagitis (Table 6). However, among ERD patients, the mean Ki-67 LI value was 15.375 ± 2.326 in patients with normal epithelium, 11.429 ± 3.91 in patients with mild esophagitis, and 12 in patients with moderate esophagitis (Table 7). In both ERD and NERD patients, the

differences in the Ki-67 LI values among the histological grades were statistically insignificant ( $P = 0.435$  and 0.083, respectively).

## Discussion

The reason of the present study derives from the existing doubts regarding the present concept of pathophysiology of GERD, in which damaging factors reach the esophageal mucosa mostly because of a failure of antireflux mechanisms. This physiologic view appears to be an incomplete approach to the wide spectrum of presentations of GERD. Several phenomena such as NERD cannot be fully explained on the basis of this sequence [13]. Pathophysiological features linked to the esophageal mucosa and to its resistance and structural defense have been supported recently [14,15]. In this context, cell proliferation of basal layers has been hypothesized to be one of the causes implicated in the epithelial defense mechanisms [13,16].

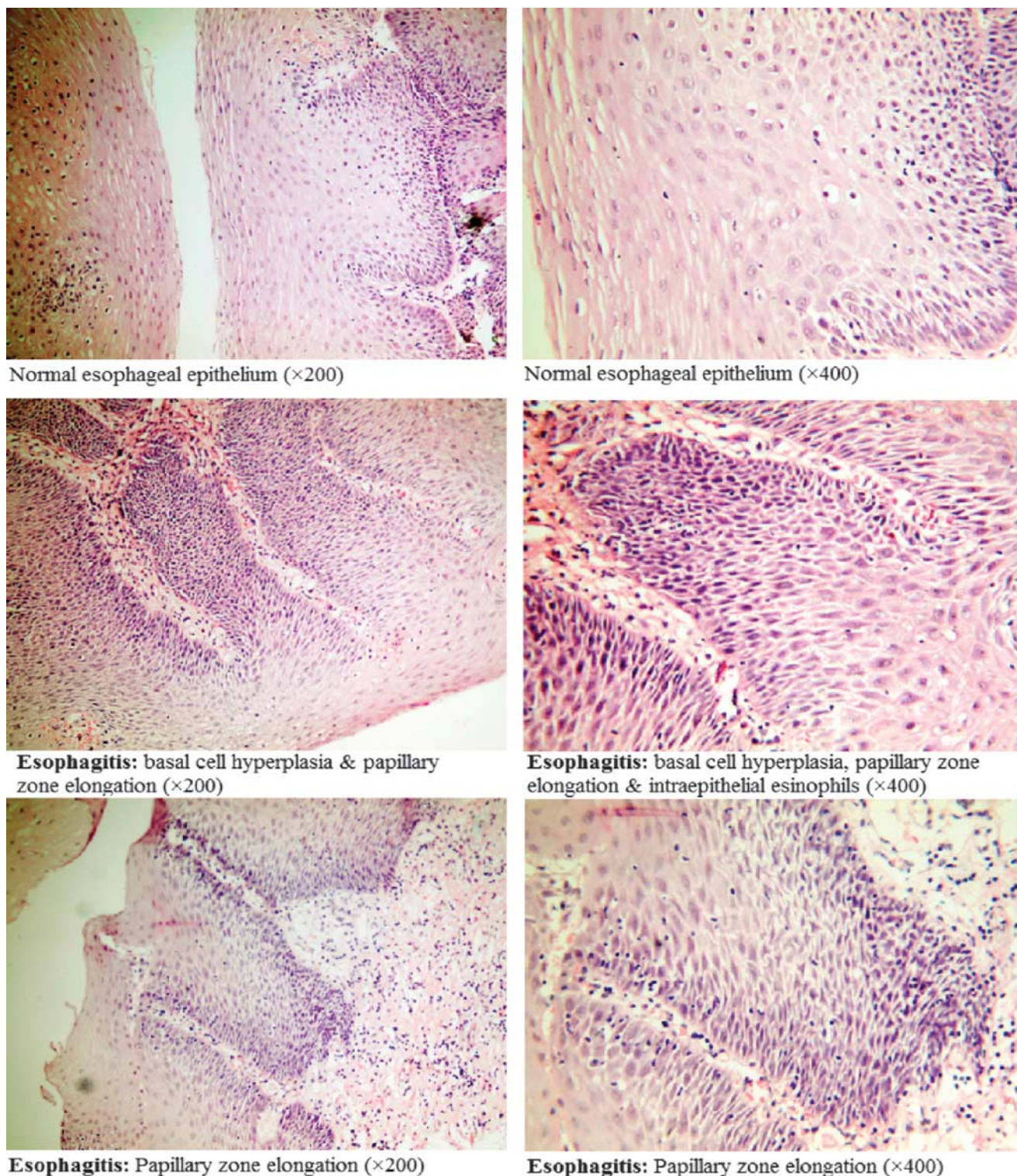
Evidence is accumulating that ERD results from the disequilibrium between aggressive factors and protective mechanisms including cell proliferation [17].

In this study, we evaluated several esophageal biopsies to define the proliferative activity of the epithelium in normal individuals and in patients with ERD and NERD. The Ki-67 antigen has become the most common marker for the evaluation of cell proliferation because of its enhanced accuracy and technical feasibility compared with other methods [18].

Our study showed that the prevalence of GERD was the highest in the 15–29 year age group (46.43%) and decreased with age, being the lowest in the age group 60 years or more (14.29%). Watanabe *et al.* [19], reported almost similar results, that is, the prevalence of GERD was the highest in the 20–29 year age group and decreased with age. However, Fujiwara *et al.* [20] and Ghweil *et al.* [21] reported that the prevalence of GERD was the highest in the 40–49 year age group and decreased with age. Moreover, it has been reported in Singapore [22], China [23], Argentina [24], Japan [20], USA [25], and Germany [26] that middle-aged individuals have GERD symptoms most frequently. Other studies have reported a slight but significant association between the prevalence of GERD and increasing age [27–29]. However, others have reported no significant relation between age and the prevalence of GERD [30,31]. This variability in the prevalence of GERD among different age groups may be attributed to a heterogeneous symptom pattern where the clinical features of GERD in old patients are quite different from those of younger adult patients, with the elderly presenting less frequently with the typical symptoms of heartburn, acid regurgitation, and pain [32,33]. Thus, the relationship between age and GERD is unclear and difficult to establish [1].

In our study, there was no significant relation between sex and the prevalence of GERD ( $P = 0.208$ ), which has also been reported by many other authors [23,27,30,34]. This could be attributed to the fact that both men and

Figure 1



Microphotography showing normally appearing esophageal mucosa and various changes that occur in the mucosa of gastroesophageal reflux disease patients.

women are exposed to the same risk factors as they live in the same communities. However, some studies have reported female predominance [20,35], whereas others have reported male predominance [22,33].

Among 28 GERD patients, nine (32.14%) were smokers, 17 (60.71%) consumed excessive amounts of tea and cof-

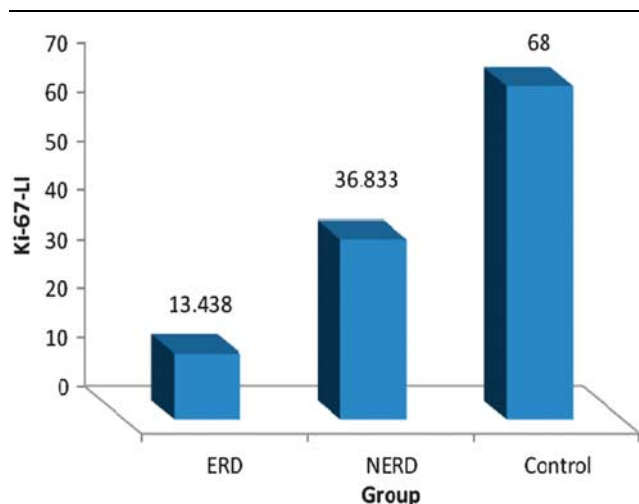
fee, 11 (39.29%) consumed spicy foods, and 11 (39.29%) were on drugs including NSAIDs.

Several studies have shown that smoking is a risk factor for GERD and that it is dose-dependent by increasing intra-abdominal pressure, reducing lower esophageal sphincter (LES) pressure, and decreasing bicarbonate

**Table 4 Ki-67 LI among different groups**

| Groups            | Number of patients | Ki-67 LI (mean $\pm$ SD) | P value |
|-------------------|--------------------|--------------------------|---------|
| Group 1 (ERD)     | 16 (48.48%)        | 13.438 $\pm$ 3.559       | 0.000   |
| Group 2 (NERD)    | 12 (36.36%)        | 36.833 $\pm$ 5.59        |         |
| Group 3 (control) | 5 (15.15%)         | 68 $\pm$ 5.148           |         |

ERD, erosive reflux disease; Ki-67 LI, Ki-67-labeling index; NERD, nonerosive reflux disease.

**Figure 2**

Ki-67 LI among different groups. ERD, erosive reflux disease; Ki-67 LI, Ki-67-labeling index; NERD, nonerosive reflux disease.

secretion in the saliva, thereby reducing saliva's pH-neutralizing effect [26,27,30,34]. Our results showed that there is no significant relation between smoking and GERD ( $P = 0.488$ ), which has also been reported by Saberi-Firoozi *et al.* [36] and Ghweil *et al.* [21].

Drinks such as tea and coffee have also been reported to be linked to GERD, but this is controversial [36]. Although tea has been shown to increase gastric acid secretion, it does not appear to contribute toward GERD [37]. Coffee decreases the LES pressure [38]. According to Johnston *et al.* [39], the role of coffee as a promoter of GERD is consistent. Ghweil *et al.* [21] have reported that consumption of excessive tea and coffee increases the prevalence of GERD. In our study, we found no significant effect of consumption of excessive tea and coffee on GERD symptoms ( $P = 0.203$ ); our results are similar to those of other studies [26,30].

Spicy foods do not cause GERD, although they seem to worsen GERD symptoms in some individuals. Food (in general) can make GERD worse; this is because food fills the stomach and induces more transient relaxations of the LES. In addition, all meals stimulate acid secretion in the stomach to aid digestion and can increase reflux into the esophagus in patients with GERD. The spicy food theory is so compelling, however, that patients with GERD often link their symptoms to a spicy meal [38]. Our results showed that there is no significant relation

between the consumption of spicy foods and GERD ( $P = 0.975$ ). However, Ghweil *et al.* [21] have reported that there is a significant relation between consumption of spicy foods and GERD.

Some studies have observed an association between the use of aspirin or NSAIDs and the presence of GERD [21]. Some authors have reported that the use of NSAIDs is a risk factor for erosive esophagitis because of direct mucosal damage [40], whereas others have not found such an association [30]. In our study, there was no significant relation between the intake of NSAIDs and GERD ( $P = 0.603$ ).

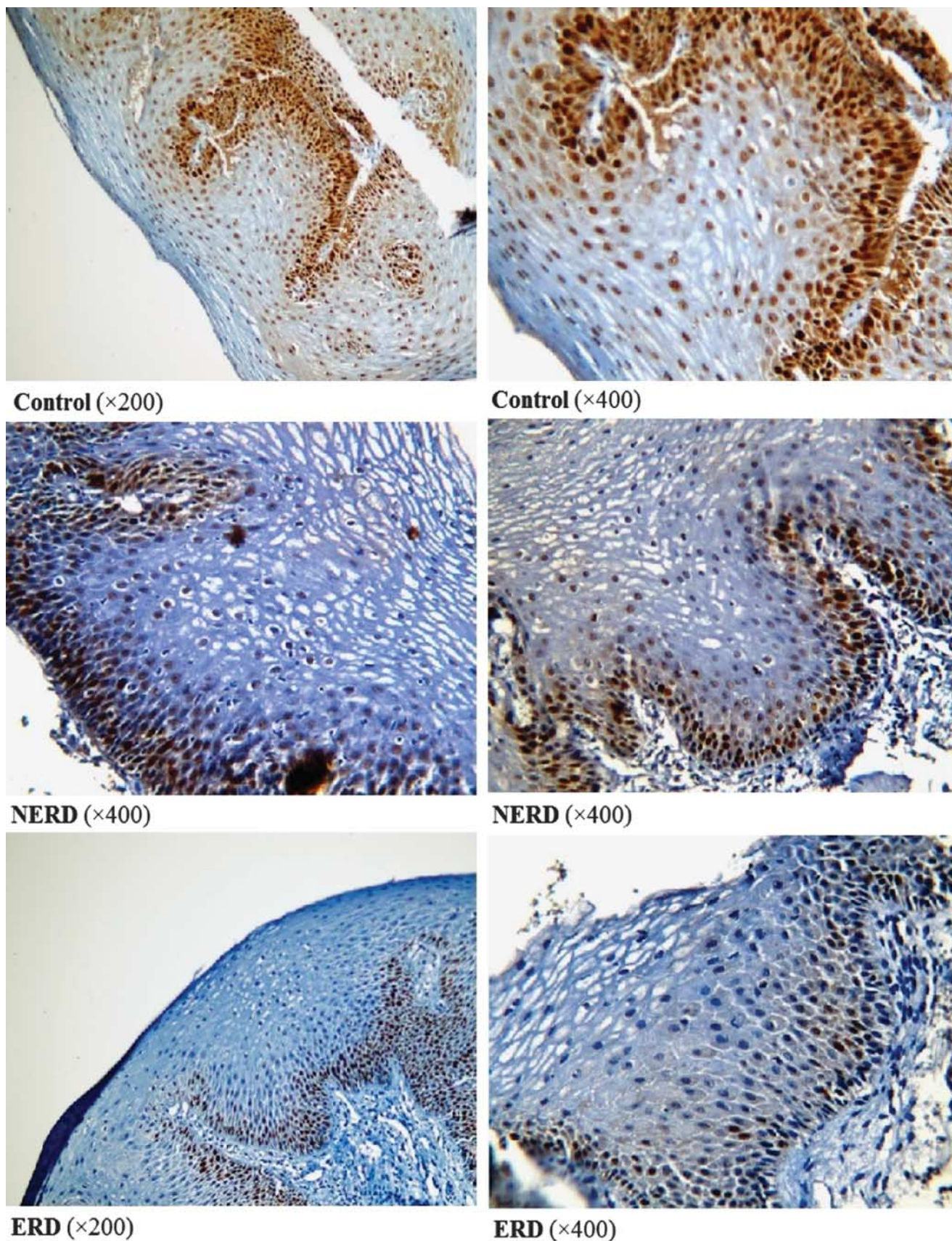
Biopsies were taken from the esophagus only from apparently normal mucosa. In this way, we studied the behavior of the mucosa exposed to chronic acid insult, but far from erosions and, especially, from reparative changes secondary to the lack of the superficial mucosa, where basal cell hyperplasia and elongation of rete pegs have been reported [11]. Also, biopsies were taken at 5 cm above the Z-line and not at the very distal part of the esophagus. It has been argued in other works that histological changes in GERD may occur because of minor degrees of physiological reflux; this phenomenon would limit the applicability of biopsies next to the Z-line, resulting in a decreased sensitivity of the evaluation [41]. Moreover, the proximal part of the esophagus is not taken into consideration. At this level, the overall reflux event is minor compared with the distal esophagus, with the possibility of a decrease in the specificity of data obtained [42].

In our study, biopsies from GERD patients showed a significantly lower number of proliferating cells than in normal individuals, in whom it reduced to 54.17 and 19.76% in NERD and ERD, respectively. Calabrese *et al.* [5] have reported almost similar results, where patients with NERD and ERD showed a decrease in cell proliferation to 47.75 and 26.05%, respectively.

Two reasonable theories could be put forward to explain the reduced epithelial proliferation activity observed in GERD: either the chronic cell damage induced by gastroesophageal reflux leads to a reduction in the proliferation rate of esophageal epithelium or a constitutive lower capacity for cell proliferation leads to a major risk to the damage induced by gastroesophageal reflux [5].

In terms of the first theory, a study by Feagins *et al.* [43] has shown that repeated acid insult exerts antiproliferative effects by delaying cell cycle progression and increasing the expression of p53 in non-neoplastic Barrett's epithelial cells. This study could be a predecessor of a new remarkable way to approach mucosal behavior, that is, repeated acid exposure, as it occurs in chronic reflux episodes, could delay cell cycles and exert antiproliferative effects [5]. In contrast, previous studies on the basis of in-vitro and ex-vivo models indicate that acid exerts proproliferative and antiapoptotic effects in Barrett's esophagus. The application of this finding on the esophageal squamous mucosa still need more research [5].

**Figure 3**

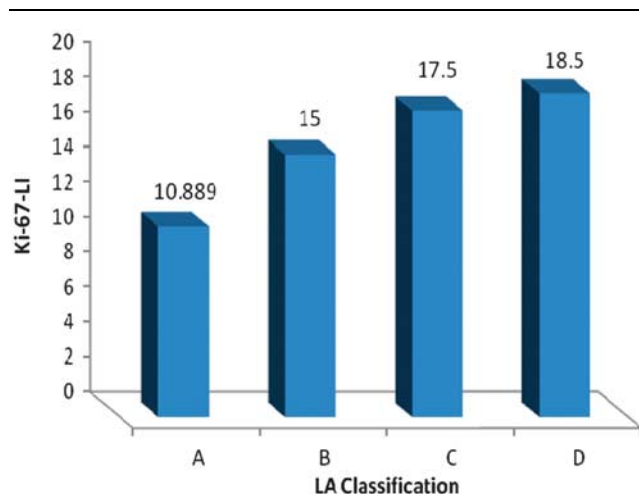


Microphotography of the Ki-67 (MIB-1) antigen immunostaining of histological sections from ERD, NERD, and control patients. ERD, erosive reflux disease; NERD, nonerosive reflux disease.

**Table 5 Correlation between Ki-67 LI and the endoscopic grade of esophagitis (according to LA classification) in ERD patients**

| LA | ERD (n=16) | Ki-67 LI (mean $\pm$ SD) | P value |
|----|------------|--------------------------|---------|
| A  | 9 (56.25%) | 10.889 $\pm$ 2.028       | 0.000   |
| B  | 3 (18.75%) | 15 $\pm$ 1               |         |
| C  | 2 (12.5%)  | 17.5 $\pm$ 2.121         |         |
| D  | 2 (12.5%)  | 18.5 $\pm$ 0.707         |         |

ERD, erosive reflux disease; Ki-67 LI, Ki-67-labeling index; LA, Los Angeles.

**Figure 4**

Correlation between Ki-67 LI and the endoscopic grade of esophagitis [according to the Los Angeles (LA) classification] in erosive reflux disease patients. Ki-67 LI, Ki-67-labeling index.

**Table 6 Correlation between Ki-67 LI and the histological grade of esophagitis in NERD patients**

| Histology | NERD (n=12) | Ki-67-LI (mean $\pm$ SD) | P value |
|-----------|-------------|--------------------------|---------|
| Free      | 6 (50%)     | 38.167 $\pm$ 5.672       | 0.435   |
| Mild      | 6 (50%)     | 35.5 $\pm$ 5.683         |         |

Ki-67 LI, Ki-67 labeling index; NERD, nonerosive reflux disease.

**Table 7 Correlation between Ki-67 LI and the histological grade of esophagitis in ERD patients**

| Histology | ERD (n=16) | Ki-67 LI (mean $\pm$ SD) | P value |
|-----------|------------|--------------------------|---------|
| Free      | 8 (50%)    | 15.375 $\pm$ 2.326       | 0.083   |
| Mild      | 7 (43.75%) | 11.429 $\pm$ 3.91        |         |
| Moderate  | 1 (6.25%)  | 12                       |         |

ERD, erosive reflux disease; Ki-67 LI, Ki-67-labeling index.

The second hypothesis implies the existence of an individual predisposition to a different reaction of the mucosa to acid and pepsin insults. This concept supports the idea that individuals who develop ERD are genetically characterized by a weaker proliferating epithelial cell capacity [5]. However, patients with more efficient epithelial proliferation capacity could have a lower probability of developing macroscopic mucosal lesions when stressed by acid and pepsin. This observation confirms that acid and pepsin insults are necessary to induce damage, but the cell proliferation capacity of the

mucosa could be a key factor that can switch the disease to either one or the other form [27,30].

Our findings contradict the results of a study carried out by Mastracci *et al.* [44] on the cell proliferation of squamous epithelium in GERD. Specimens were taken at 2 and 4 cm above the Z-line. This study found Ki-67% distribution in controls, NERD, and ERD patients and it found a slight percentage predominance of Ki-67-positive cells in patients than in controls. The different outcome of this study may be because of the site from which the biopsies were obtained, which is close to the Z-line, leading to decreased sensitivity of the evaluation [41].

In our study, a strong correlation was found between cell proliferation (Ki-67 LI) and the LA classification grade of esophagitis among ERD patients. The Ki-67 LI tended to increase with the severity of the disease, being the highest in grade D (18.5%) and the lowest in grade A (10.89%). Similar results have been reported by Nishiyama *et al.* [45] and Binato *et al.* [46]; they found a significant correlation between Ki-67 LI and the endoscopic grade of esophagitis.

Our study found no significant correlation between cell proliferation (Ki-67 LI) and the histological grade of esophagitis among both ERD and NERD patients. Other studies have reported different results, that is, a significant correlation between Ki-67 LI and the histological grade of esophagitis, being the highest in severe degrees [45,46]. This difference can be attributed the small number of patients with moderate esophagitis (one case) and the absence of patients with severe esophagitis in our study population.

In conclusion, esophageal cell proliferation should be taken into consideration as one of the factors implicated in the pathogenesis of GERD. The ability of the mucosa to resist injury and to repair the damage should be considered a key factor in the development of GERD. Esophageal mucosa exposed to chronic acid insult show reduced cell replication, estimated by the Ki-67 LI. NERD and ERD show a different pattern of cell proliferation rate of esophageal epithelium. ERD seems to be linked to a low cell proliferation rate of esophageal epithelium rather than the amount of reflux.

Further studies are required to gain a better understanding of the cellular proliferative activity of esophageal mucosa stressed by chronic reflux episodes. Further studies are also required to gain a better understanding of the relation between esophageal cell proliferation and the degree of esophagitis, both endoscopic and histological.

## Acknowledgements

### Conflicts of interest

There are no conflicts of interest.

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