

A statistical study of esophageal lesions in Egypt when correlated with Ki-67 activity in GERD, Barrett's esophagus, and carcinoma of the esophagus

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Aim of the work The objective of this study was to identify the most common esophageal lesion among all archival biopsies received in the pathology departments of both faculty of medicine (Cairo University) and Theodor Bilharz Research Institute, Egypt, over 5 years (2004–2008). The pathologic features of all studied cases were studied. Additional work undertaken included immunohistochemical study of one of the most common proliferative markers, Ki-67 (MIB1), in cases of gastroesophageal reflux disease (GERD), Barrett's esophagus, and esophageal carcinoma.

Materials and methods The study included 210 archival esophageal biopsies, all of which were recut from paraffin blocks and stained with hematoxylin and eosin. Patient consent was not taken because of difficulty in contacting them. Cases of GERD, Barrett's esophagus, esophageal squamous cell carcinoma (SqCC), and esophageal adenocarcinoma were evaluated with respect to their proliferative activity using Ki-67 immunohistochemistry.

Results Of the 210 esophageal specimens, 54.76% were GERD, 17.61% were Barrett's esophagus, 3.81% were benign lesions, 18.6% were malignant lesions (10.48% SqCC, 6.19% adenocarcinoma, and 1.9% undifferentiated carcinoma), and 5.24% represented others. There were no statistically significant differences with respect to age among the 210 esophageal specimens. Patients with cancer were predominantly men. The Ki-67 index averaged

9.4 (± 3.7)% in GERD specimens ($n=115$), 23.6 (± 7.0)% in Barrett's esophagus ($n=37$), 35.4 (± 10)% in adenocarcinoma specimens ($n=13$), and 41.5 (± 9.9)% in SqCC specimens ($n=22$). Ki-67 expression was significantly different between the groups. There was a strong linear correlation between Ki-67 expression and the GERD–Barrett's–adenocarcinoma sequence.

Conclusion GERD is the most common pathological lesion encountered in Egypt. The Ki-67 antigen has increased expression along the GERD–metaplasia–adenocarcinoma sequence. There is a strong linear correlation between Ki-67 proliferative activity and Barrett's carcinogenesis. There is also a strong linear correlation between Ki-67 proliferative activity and SqCC of the esophagus. *Egypt J Pathol* 00:000–000 © 2013 Egyptian Journal of Pathology.

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Keywords: adenocarcinoma, Barrett's esophagus, gastroesophageal reflux disease, squamous cell carcinoma

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Introduction

The most common diagnostic problems encountered with esophageal biopsy specimens involve the evaluation of esophagitis and its consequences in persistent or recurrent cases. Esophagitis can be caused by diverse physical, chemical, and biological agents, but by far the most common cause is gastroesophageal reflux (Goldblum and Lee, 2004).

Reflux of gastric contents into the lower esophagus can cause clinical and pathologic manifestations that are described under the general label of gastroesophageal reflux disease (GERD). GERD is the most prevalent clinical condition of the gastrointestinal tract, presenting mainly as heartburn. Epidemiologic studies suggest a 3–4% prevalence of GERD in the general population (Rosai, 2004). Long-standing severe GERD may be complicated by the development of Barrett's esophagus (BE) (Sampliner, 2002).

The prevalence of BE varies across different geographical areas. BE is well recognized as a precursor to the majority

of cases of esophageal adenocarcinoma (EAC). It has been estimated that patients with BE carry a risk for cancer that is 30 times greater than that of an age-matched population (Yousef *et al.*, 2008; Fouad *et al.*, 2009).

Esophageal cancer is the seventh leading cause of cancer-related death worldwide. It occurs most commonly during the sixth and seventh decades of life and is generally more common in men than in women, with a male-to-female ratio of 3–4:1. The disease becomes more common with advancing age; it is about 20 times more common in those older than 65 years than in individuals younger than 65 (Fisichella and Patti, 2009).

BE is currently defined as an endoscopically visible columnar mucosa in the distal esophagus, of any extension, proved to harbor intestinal metaplasia on biopsy, highlighted by the presence of goblet cells (Jenkins *et al.*, 2002). Patients with BE require a rational follow-up in order to allow early identification of

malignant transformation (Cameron *et al.*, 1985; Cameron and Carpenter, 1997; Holscher *et al.*, 1997; Lagergren *et al.*, 1999; Szachnowicz *et al.*, 2005; Rice *et al.*, 2010).

Prognostic molecular markers are used to identify those patients at risk of developing cancer. Barrett's carcinogenesis underlies a series of genetic and epigenetic events, which are revealed phenotypically as a sequence of metaplasia–dysplasia–adenocarcinoma (DeMeester and DeMeester, 2000). Ki-67 has become the marker of choice because of its accuracy and easy feasibility (Skinner *et al.*, 1983; Chandrasoma, 1997; Oberg *et al.*, 1997; DeMeester, 2001; Sikkema *et al.*, 2011).

Numerous studies have suggested that there is elevated Ki-67 expression in the metaplasia–dysplasia–adenocarcinoma sequence in BE. Also, progressive Ki-67 expression has been described through the sequence of normal mucosa to dysplastic tissue in esophageal squamous cell carcinoma (SqCC) (Feith *et al.*, 2004). However, there is variable Ki-67 expression in EAC and inconclusive results for the metaplasia–dysplasia–adenocarcinoma method in BE (Lauwers *et al.*, 1997; Rioux-Leclercq *et al.*, 1999; Xu *et al.*, 2002).

Materials and methods

The study included 210 archival esophageal biopsies, all of which were recut from paraffin blocks and stained with hematoxylin and eosin. Patient consent was not taken because of difficulty in contacting them. Cases of GERD, Barrett's esophagus, esophageal squamous cell carcinoma (SqCC), and esophageal adenocarcinoma were evaluated with respect to their proliferative activity using Ki-67 immunohistochemistry.

Scoring of Ki-67

Cells that displayed a brown nuclear stain, similar to the positive control, were considered to be Ki-67 positive. The final result was assessed by the proliferation index for every case, which is calculated from the average of stained cells in relation to the total analyzed cells, with a minimum count of 500 cells, as described by Binato *et al.* (2008).

Statistical analysis

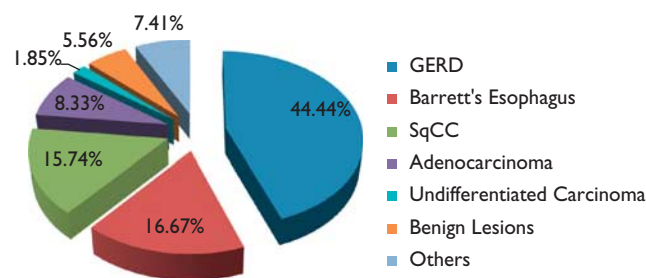
Results were expressed as mean \pm standard deviation (SD) or number and percent (%).

Table 1 Histopathologic distribution of cases

Diagnosis	TBRI [n (%)]	Kasr Al-Ainy [n (%)]	Total [n (%)]
GERD	67 (65.7)	48 (44.4)	115 (54.76)
Barrett's esophagus	19 (18.6)	18 (16.7)	37 (17.61)
Malignant	11 (10.7)	28 (25.9)	39 (18.6)
SqCC	5 (4.9)	17 (15.7)	22 (10.48)
Adenocarcinoma	4 (3.9)	9 (8.3)	13 (6.19)
Undifferentiated carcinoma	2 (1.9)	2 (1.9)	4 (1.9)
Benign lesions	2 (1.9)	6 (5.6)	8 (3.81)
Others	3 (2.9)	8 (7.4)	11 (5.24)
Total	102 (100)	108 (100)	210 (100)

GERD, gastroesophageal reflux disease; SqCC, squamous cell carcinoma; TBRI, Theodor Bilharz Research Institute.

Chart 1



Histopathologic distribution of Kasr Al-Ainy cases.

Table 2 Age of study cases according to pathologic diagnosis

	Kasr Al-Ainy		TBRI		Total	
	Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD	Range
GERD	43.7 \pm 15.9	18–75	42.3 \pm 17.5	17–90	42.8 \pm 16.8	18–90
Barrett's esophagus	44.6 \pm 13.4	17–70	47.2 \pm 16.5	25–67	45.9 \pm 14.9	17–70
Benign lesions	41.8 \pm 13.5	24–55	42.5 \pm 7.8	42–53	43.3 \pm 12.0	24–55
Malignant lesions	54.7 \pm 13.8	28–82	59.8 \pm 11.5	42–70	56.2 \pm 13.2	28–82

GERD, gastroesophageal reflux disease; TBRI, Theodor Bilharz Research Institute.

Results

From 1 January 2004 to 31 December 2008, a total of 210 esophageal specimens were delivered to pathology departments of both Kasr Al-Ainy hospital and Theodor Bilharz Research Institute (TBRI). Out of 34 613 specimens delivered to Kasr Al-Ainy pathology department, 108 were esophageal specimens and represented 0.31% of cases. In TBRI, out of 5193 specimens delivered during the same period, 102 were esophageal specimens and represented 1.96% of cases.

A total of 210 esophageal specimens were included in this study, of which 115 (54.76%) had GERD, 37 (17.61%) had BE, eight (3.81%) had benign lesions, 39 (18.6%) had malignant lesions, 22 (10.48%) had SqCC, 13 (6.19%) had adenocarcinoma, four (1.9%) had undifferentiated carcinoma, and 11 (5.24%) represented others (Table 1).

According to the archived files, GERD was the most prevalent diagnosis among all esophageal specimens in Kasr Al-Ainy, representing 44.4% of all cases (Chart 1).

Similar to cases in Kasr Al-Ainy, GERD was the predominant diagnosis in TBRI but with a relatively higher frequency of 65.75% of all esophageal specimens.

The overall collected cases showed an almost equal male-to-female ratio (M:F ratio = 1.23:1). The male-to-female ratio in Kasr Al-Ainy was 1.2:1 and in TBRI was 1.27:1.

The ages of the patients in this study ranged from 18 to 90 years. However, the mean age steadily increased from GERD to BE and was highest in malignant cases (Table 2).

Table 3 Epithelial changes detected in cases of gastroesophageal reflux disease

Basal cell hyperplasia [n (%)]			Elongated papillae [n (%)]		Surface ulcer/erosion [n (%)]	
Mild	Moderate	Marked	< 2/3 of thickness	> 2/3 of thickness	Present	Absent
57 (49.6)	57 (49.6)	1 (0.8)	85 (73.9)	30 (26.1)	63 (54.7)	52 (45.2)

Table 4 Intraepithelial inflammatory cells detected in gastroesophageal reflux disease

Eosinophils [n (%)]		Neutrophils [n (%)]	Lymphocytes [n (%)]	
<15/HPF	>15/HPF		<10/HPF	>10/HPF
41 (35.6)	0 (0)	6 (5.2)	111 (96.5)	4 (3.5)

In our study, the histopathologic findings in GERD cases included basal cell hyperplasia, elongated papillae, and surface ulcer and erosion (Table 3).

A variety of intraepithelial inflammatory cells could be detected. Special attention was paid to eosinophils, which were detected in 35.6% of cases of GERD, but the maximum number of eosinophils was always lower than 15/HPF – that is, not sufficient to shift the diagnosis from GERD to eosinophilic esophagitis (Table 4).

Features that were present in most cases of GERD in varying degrees were epithelial ballooning (in 93% of cases) and nuclear hyperchromasia with or without increased mitotic figures (in 94% of cases) (Table 5).

The histopathologic findings detected in the studied cases of BE included the presence of villi and goblet cells.

As a cornerstone of diagnosis, the presence of goblet cells was evident in all cases of BE. Villous configuration of mucosa was evident in 83.8% of cases (Table 6).

In this study, most cases of BE showed no dysplastic changes. low grade dysplasia (LGD) dysplasia was evident in 14 cases (representing 37.8%) and no case showed high grade dysplasia (HGD).

Currently, Ki-67 is one of the most studied markers of cell proliferation. Initial studies assessing Ki-67 antigen used flow cytometry. The results obtained using this method did not show significant differences in Ki-67 expression when BE patients with different degrees of dysplasia were compared with adenocarcinoma patients. The disadvantages of flow cytometry include the requirement for frozen sections and sophisticated equipment, tissue architecture compromise, and its labor intensiveness.

The Ki-67 index (percentage of stained cells/total cells) has been used to evaluate the proliferative activity of tumors and requires immunohistochemical analysis in paraffin-embedded tissue. The Ki-67 index is now the method of choice for proliferation studies because of its accuracy and ease of use. Although electronic counting is sometimes used for this method, we did not have access

to the necessary equipment and used the more conventional manual counting method.

Malignant cases included in the study were SqCC (56.4% of malignant cases) and adenocarcinoma (33.3% of malignant cases) with variable degrees of differentiation.

Additional immunohistochemical study for expression of the proliferative marker Ki-67 was performed and the results are expressed in Table 7.

In our study, the cellular proliferation index in malignant cases was higher than that in cases of GERD and BE. The proliferation index in cases of SqCC was higher than that in cases of adenocarcinoma (Figs 1–8).

The Ki-67 antigen has increased expression along the GERD–metaplasia–adenocarcinoma sequence. There is a strong linear correlation between Ki-67 proliferative activity and Barrett's carcinogenesis. There is also a strong linear correlation between Ki-67 proliferative activity and SqCC of the esophagus.

Discussion

In the current study, esophageal specimens accounted for 1.96 and 0.31% of all cases delivered to the Pathology Departments of TBRI and Kasr Al-Aini, Faculty of Medicine, Cairo University, respectively, from the beginning of 2004 until the end of 2008. The relatively higher frequency of cases in TBRI may be attributed to its specialty in gastroenterology, in contrast to Kasr Al-Ainy where the Pathology Department receives more diverse specimens.

In this study, the histopathological diagnosis of GERD represented more than half of all esophageal lesions (54.76%), with a mean age of 42.8 years and an almost equal male-to-female ratio (1.2:1). Although GERD is highly prevalent in western countries, fewer data about it are available in African countries (Ben Chaabane *et al.*, 2012). In a study conducted on 2044 Chinese patients, reflux esophagitis showed a higher male-to-female predominance, reaching 5.6:1 (Chang *et al.*, 1997).

This discrepancy between the results of our study and those conducted by the Chinese may be attributed to various habitual and dietary factors, as well as to lifestyle.

The variation in the frequency and sex predilection of GERD may be related to genetic and environmental factors as well as to different habits and traditions adopted by different populations – for example, smoking and consumption of alcohol, a high-calorie diet, hot drinks, spices, and pickled vegetables (Zarling, 1998).

Table 5 Epithelial ballooning and regenerative changes detected in gastroesophageal reflux disease

	Epithelial ballooning [n (%)]				Regenerative changes [n (%)]		
	Mild	Moderate	Marked	Absent	Hyperchromasia	Hyperchromasia + mitotic figures	Absent
60 (52.2)	41 (35.6)	6 (5.2)	8 (7)	58 (50.5)	50 (43.5)	7 (6.0)	

Table 6 Villi and goblet cells in Barrett's esophagus

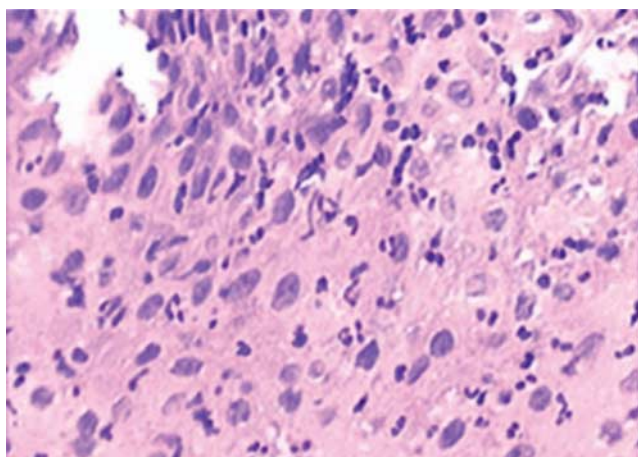
Villi [n (%)]		Goblet cells [n (%)]	
Present	Absent	Present	Absent
31 (83.7)	6 (16.2)	37 (100)	0 (0)

Table 7 Ki-67 labeling index

	Mean (±SD)%	Range (%)
GERD	9.4 (±3.7)	4.2–13
Barrett's esophagus	23.6 (±7.0)	15.2–32.2
SqCC	41.5 (±9.9)	40.2–54.6
Adenocarcinoma	35.4 (±10.0)	24–46.8

GERD, gastroesophageal reflux disease; SqCC, squamous cell carcinoma.

Fig. 1

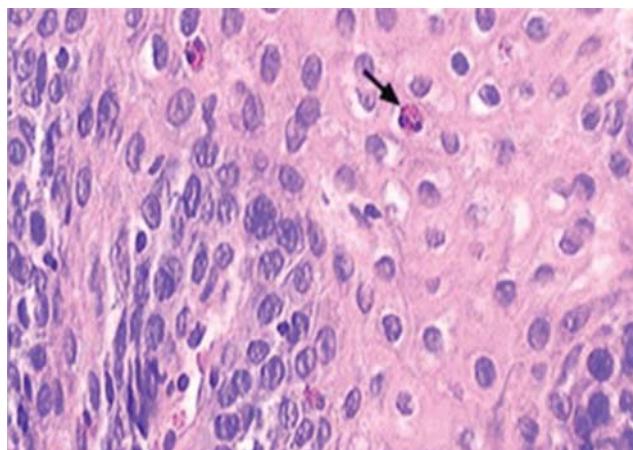


GERD showing collection of intraepithelial neutrophils (H&E, × 200). GERD, gastroesophageal reflux disease.

In our study, the histopathologic changes indicative of GERD were variably detected in the examined cases. Epithelial changes in the form of basal cell hyperplasia, epithelial ballooning, and regenerative changes were found in most cases but in varying degrees. Lamina propria elongation of more than two-thirds of epithelial thickness was found in 26.3% in cases. The presence of intraepithelial eosinophils less than 15/HPF was detected in 20 cases of GERD, representing 35.1% of the examined cases.

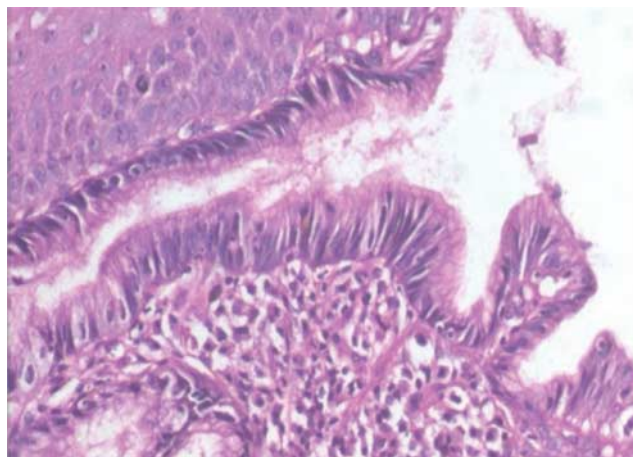
BE shows a striking geographical variation in the prevalence rates, being much more common in the west compared with the east (Shaheen and Richter, 2009). In this work, BE was evident in 17.61% of all esophageal specimens. In a study conducted on 358 patients in the

Fig. 2



GERD showing collection of intraepithelial eosinophils (arrow) (H&E, × 200). GERD, gastroesophageal reflux disease.

Fig. 3

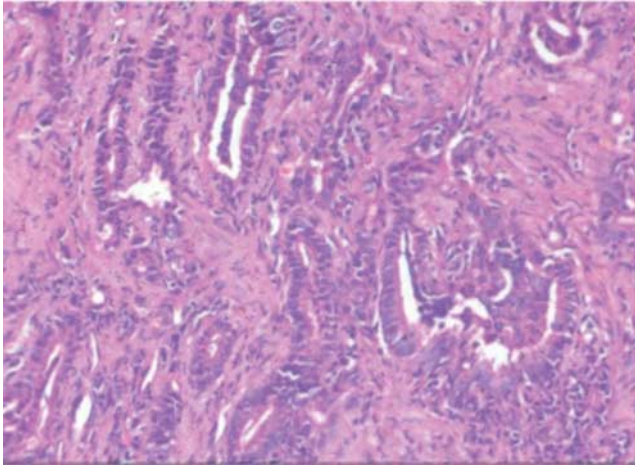


BE with LGD and a focus of villous formation (H&E, × 200). BE, Barrett's esophagus; LGD, low grade dysplasia.

USA, Westhoff *et al.* (2005) reported that the overall prevalence of BE was 13.2%. In another study in Korea, Kim *et al.* (2007) found that, in the general population, the prevalence of BE was less than 1%.

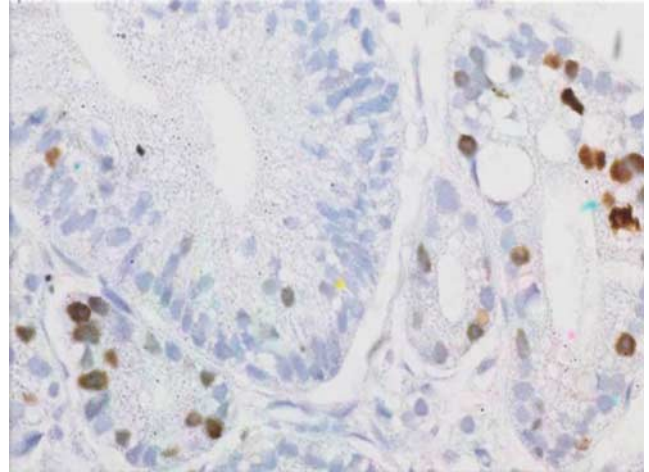
Our study showed that the number of men diagnosed with BE was more than two-fold the number of female patients (ratio of 2.36:1). This nearly agreed with the results of another study conducted in Leeds, UK, in which the male-to-female ratio was 1.96:1 (Cook *et al.*, 2005). Lin *et al.* (2011) studied 543 patients and showed

Fig. 4



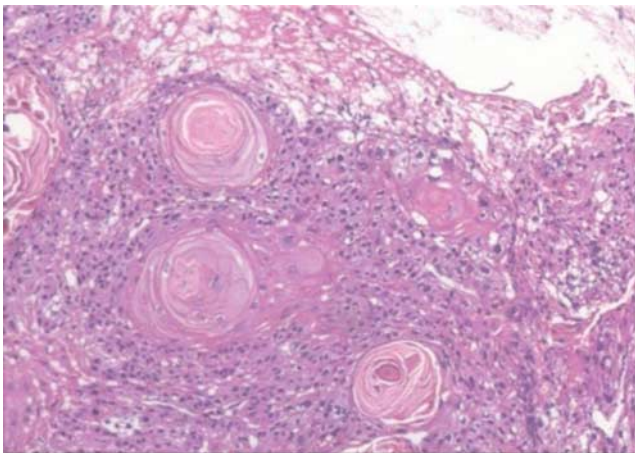
Esophageal adenocarcinoma showing variable-sized malignant acini (H&E, × 200).

Fig. 6



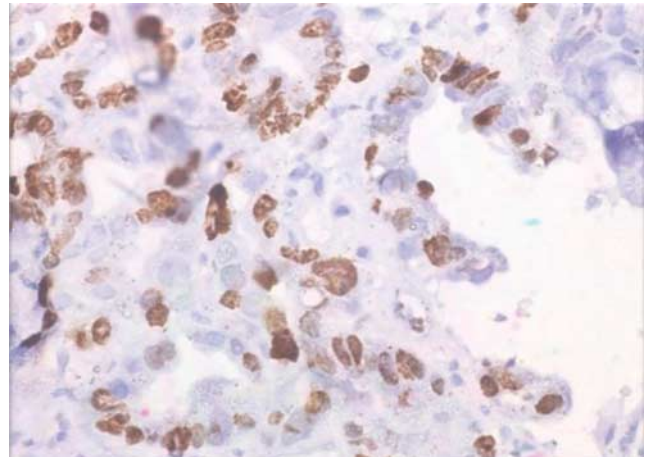
BE showing moderate nuclear brown staining (Ki immunostain, × 400). BE, Barrett's esophagus.

Fig. 5



Esophageal well-differentiated squamous cell carcinoma (H&E, × 200).

Fig. 7



Esophageal adenocarcinoma showing marked nuclear brown staining (Ki immunostain, × 400).

that, although male and female patients demonstrated an equal severity of esophagitis, only 14% of female patients had BE compared with 23% of male patients (M:F ratio = 1.64:1). Meanwhile, Banki *et al.* (2005) have shown that there was an equal prevalence of BE in men and women diagnosed with severe reflux.

Goblet cells were present in all BE cases, and villous configuration was obvious in 84.6% of cases. Most cases of BE (61.5%) showed no dysplastic features, whereas 38.5% of Barrett's cases showed evidence of LGD. However, HGD was not encountered in any of the cases included in this study.

This emphasizes the need for the presence of goblet cells to diagnose BE, whereas villous configuration is not a prerequisite for the diagnosis.

The absence of HGD in any of the esophageal cases could be attributed to the absence of awareness among patients

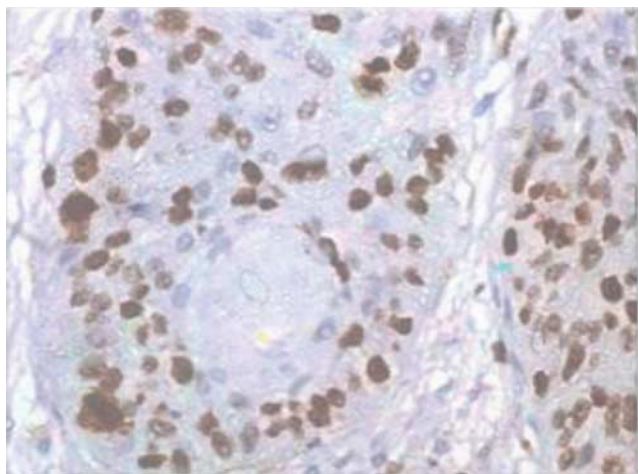
of a low socioeconomic status about the danger of reflux esophagitis as a precursor to malignancy. They become accustomed to symptoms and ignore the manifestations until malignancy occurs.

Benign tumors and tumor-like lesions of the esophagus represented only 4.69% of all specimens included in this study with almost equal sex distribution and a mean age of 43.3 ± 12 years. This indicates that benign lesions of the esophagus are rare.

A diversity of benign tumors and non-neoplastic masses can be seen in the esophagus. However, they are uncommon, usually small, asymptomatic, and appear endoscopically as polyps protruding into the esophageal lumen (Goldblum and Lee, 2004).

In our study, malignant esophageal neoplasms accounted for 18.57% of all esophageal lesions, with women being slightly more affected than men (M:F = 0.95:1). The mean age of

Fig. 8



Esophageal SqCC showing marked nuclear brown staining (Ki immunostain, $\times 400$). SqCC, squamous cell carcinoma.

patients suffering from malignant esophageal neoplasms was 56.2 years. Of all malignant esophageal neoplasms, SqCC was the most common, representing 56.41% of all cases, followed by adenocarcinoma in 33.33%. The remaining 10.25% were diagnosed as undifferentiated carcinomas.

According to Harford (2007), the sex distribution of esophageal cancer in Egypt during 1999–2001 showed a higher male predominance (M:F = 2.4:1), with over 75% of cases affecting individuals above 50 years of age. The incidence of esophageal cancer varies depending on the geographical region. Whereas SqCC accounts for 95% of the pathology of esophageal cancers worldwide, adenocarcinomas are becoming an increasingly common entity in the western world (Lin and Chang, 2010).

This increasing frequency of adenocarcinoma could be explained by changing dietary habits and factors such as alcohol consumption and smoking habits among women.

The immunohistochemical analysis showed that the mean cellular proliferation is highest in SqCC in which about 41.5% of tumor cells are in active phase, followed by adenocarcinoma (35.4%). The mean cellular proliferation in BE cases was 23.6%. In cases of GERD, only 9.4% of cells were in active phase.

In the study by Lin *et al.* (2011) on 46 esophageal specimens, Ki-67 nuclear immunostaining ranged from 19.1 to 21% in cases of esophageal SqCC according to the degree of differentiation. In another study conducted on 200 patients by Binato *et al.* (2009), the Ki-67 index increased according to the severity of histopathological diagnoses. The Ki-67 index was $21.3 \pm 19.5\%$ in normal squamous epithelium, $38.8 \pm 24.9\%$ in GERD, $52.8 \pm 24.6\%$ in BE, and $57.1 \pm 25.1\%$ in EAC.

Hong *et al.* (1995) evaluated the Ki-67 index in 43 patients with BE. Ki-67 expression was 13% in the gastric mucosa (control), 33% in BE without dysplasia, 40% in BE with LGD, and 33% in HGD. There were only five

cases of EAC and Ki-67 expression was 38%. When the glands were stratified, a significant difference was seen in Ki-67 expression between the groups and a superficial proliferating zone was reported in HGD patients.

Rioux-Leclercq *et al.* (1999) assessed Ki-67 expression in 44 esophagectomy specimens in different histological areas. Areas with BE and LGD were positive for Ki-67 in 14% of patients, BE and HGD were positive in 73%, and EAC in 87%. Significant increase was found in the prevalence of Ki-67 in the sequence of dysplasia to adenocarcinoma. Ki-67 staining was not reported in patients with BE without dysplasia or in the control group. Moreover, this study considered Ki-67 to be positive when the index was 10% or more. Such a criterion is somewhat arbitrary as it has not been used in any other publication.

In a recent study conducted by Feith *et al.* (2004) different histological areas were evaluated for Ki-67 in 24 esophagectomy specimens. Ki-67 expression increased significantly in the following sequence: squamous mucosa (20%), BE without dysplasia (35%), BE with dysplasia (45%), and adenocarcinoma (60%). These results differ from those previously reported – that is, up to 45% positivity was seen in BE and 60% in cancer.

These differences may be partly due to small and unrepresentative sample sizes taken from studies based on different histological areas from esophagectomy-resected specimens. In such studies, only one patient may be analyzed using several histological sections. Variations in the immunohistochemical technique may also partly explain the variable results. These may include the types of antibodies, antigenic presentation, and assessment of the marker.

Another recent study by Szachnowicz *et al.* (2005) evaluated Ki-67 in 13 esophagectomy specimens and demonstrated 'moderate' or 'strong' proliferative activity in all cases of BE and EAC. They did not determine the Ki-67 index, but described the staining of Ki-67 in four degrees ('absent', 'weak', 'moderate', and 'strong'). This criterion has, however, not been used before, making comparisons impossible. Statistical analysis was not carried out for Ki-67 expression.

We demonstrated a significant correlation between the Ki-67 index, indicating proliferative activity, and BE to adenocarcinoma progression. The results are concordant with the literature and confirm the progressive nature of this disease relative to the increasing prevalence of this marker.

In the last 10 years, more than 10 studies have noted that adequate gastroesophageal reflux control is associated with histological regression of BE. Antireflux surgery was shown to be an important predictive factor for histological regression, occurring in 36% of patients undergoing surgery. Identification of this subgroup of patients prone to regression is an appropriate field for future research, wherein molecular markers may contribute to treatment decisions for each patient.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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