



Endoscopic Findings and Histopathological Correlation of Esophageal Lesions

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Incidence of neoplastic and non-neoplastic esophageal lesions. The present study aimed to evaluate the site of occurrence of esophageal carcinomas and to study the various types of esophagitis with histochemical stains. A total of 104 esophageal lesions were received by endoscopic biopsy. A thorough Microscopic evaluation was done in each case. This proves that microscopic examination is the confirmatory diagnostic tool. The microscopic examination along with the accessory histochemical stain, immunohistochemistry for HSV antigen, p53 expression helped to arrive at an accurate diagnosis.

Keywords: Immunohistochemistry; esophagitis; carcinomas and endoscopic biopsy.

1. INTRODUCTION

An esophageal lesion is one of the common disorders affecting people throughout the world. Squamous cell carcinoma is the most common

malignancy of the esophagus in most parts of the world [1]. It is one of the top ten malignant neoplasms affecting the people of Tamil Nadu. Many kinds of benign pathological lesions occur in the esophagus. They include esophageal

atresia, reflux esophagitis, Barrett's esophagus, herpes simplex esophagitis, candidial esophagitis, leiomyoma, hyperplastic polyp. Recent advances in endoscopy have made it possible that all esophageal lesions are biopsied and diagnosed correctly [2].

Esophageal cancer is a malignancy that is well known for its marked variation by geographic area, ethnic group, and sex. The incidence of esophageal cancer fluctuates dramatically throughout various regions of the world. Esophageal cancer is the sixth most common cause of cancer deaths in the world 64.

Approximately 83% of its incidence cases and 86% of deaths occur in developing countries. Barrett's esophagus (BE) is a premalignant condition in which metaplastic columnar epithelium with goblet cells replaces the normal squamous epithelium. Barrett's esophagus is a strong risk factor for esophageal adenocarcinoma [3]. Many adenocarcinomas of the esophagus arise in the setting of Barrett esophagus, and in many cases, areas of dysplastic Barrett mucosa will be found near the carcinoma. The incidence rate of Adenocarcinoma in patients with Barrett's esophagus is increased from 3.6 per million in 1973 to 25.6 per million in 2006 [65, 66]. In the present study, the endoscopic and histopathological feature of Barrett's esophagus is described in detail with the help of special stains. Besides, various benign and malignant lesions of the esophagus are discussed [4-9].

2. MATERIALS AND METHODS

The present study was conducted in the Department of Pathology, Sree Balaji Medical College and Hospital from February 2014 to September 2015 (Ref. No. 002/SBMC/IHEC/2014-19). A total of 104 esophageal lesions were received by endoscopic biopsy from male and female patients irrespective of the ages. A thorough Microscopic evaluation was done in each case. Biopsy specimens were immediately fixed in 10% buffered neutral formalin. Dehydration through graded alcohol of 50%, 70%, 80% absolute alcohol was carried out. 2 steps of clearing with xylene followed by paraffin embedding. Thin sections of 4 micrometers have been cut after dewaxing, then were stained by hematoxylin and eosin stain.

2.1 Hematoxylin and Eosin (H&E) Technique

1. The slides were immersed in the first xylene bath for 3 minutes.
2. Then transferred to second xylene bath for 2 minutes.
3. Immersed in graded alcohol (90%, 80%, and 70%) for 3 minutes in each.
4. Rinsed in running water for 1 minute and then briefly in distilled water.
5. Stained with Harris's hematoxylin for 5 minutes and rinsed in tap water.
6. Differentiated in 1% acid alcohol by dipping 3 times and washed in tap water briefly.
7. Blueing was done with lithium carbonate till sections appeared blue.
8. Rinsed in tap water for 10 minutes.
9. Stained with eosin for 30 seconds.
10. Washed in running tap water for 1 minute.
11. Dehydrated by passing through 3 baths of alcohol (70%, 80%, and 90%).
12. Passed through xylene for 15 seconds.

2.2 Mounted in DPX

The presence of fungal infections was studied with special stains like PAS. In cases like Barrett's esophagus, to see the metaplastic changes, AB/PAS at pH 2.5 is carried out.

The procedure of combined AB PH 2.5 PAS FOR ACID AND NEUTRAL MUCINS 84 acid mucins and neutral mucins are separated by this technique. The rationale is that by first staining all acid mucins with Alcian Blue, those acid mucins which are also PAS positive will not react with the subsequent PAS reaction, neutral mucins alone will take up PAS stain. In this way, a good color distinction can be made between acid and neutral mucins.

2.3 Methods

1. Dewax sections and bring to the water
2. Alcian Blue solution - 5 minutes
3. Wash in distilled water
4. 1% aqueous periodic acid - 5 minutes
5. Rinse well in distilled water
6. Schiff's reagent - 15 minutes
7. Wash in running tap water - 5-10minutes
8. Stain nuclei lightly with Harris Haematoxylin
9. Differentiate as appropriate and blue.
10. Wash in distilled water
11. Rinse in absolute alcohol
12. Clean in Xylene and mount as desired

2.4 Pas Stain 84 Technique

1. Smears were fixed in methanol for 15 minutes.
2. Sections were oxidized with Periodic-Acid-Schiff solution for 5 minutes and rinsed in distilled water.
3. Sections were placed in Schiff's leuco-fuchsin for 15 minutes, followed by running tap water for 10 minutes till pink color is developed.
4. Counterstaining was done with Harris Hematoxylin for 15 minutes and rinsed in tap water.
5. Differentiation was done in acid alcohol and the sections were rinsed in tap water.
6. Dry the slide and mount in DPX. IHC in appropriate cases was used. HSV antigen for confirming Herpes simplex esophagitis, p53 for confirming squamous dysplasia.

2.5 Immunohistochemistry Procedure

1. The 4-micrometer sections were taken in Poly-L-lysine coated glass slides.
2. Slides were incubated for 1 hour on the hot plate.
3. Sections were deparaffinized in xylol, hydrated through graded alcohols, brought to water for 5 minutes.
4. Antigen retrieval was done in a pressure cooker using citrate buffer (pH 6) for 15 minutes.
5. Washed in PBS (Phosphate Buffer Solution) for 2 times, 5 minutes each.
6. A 0.4% casein block was used to block the nonspecific antibody reaction.
7. Washed in PBS for 2 times, 5 minutes each.
8. Incubated with primary antibody (HSV/p53) for 1 hour at room temperature.
9. Washed in PBS for 2 times, 5 minutes each.
10. Biotinylated secondary antibody was applied and kept for 30 min at room temperature.
11. Washed in PBS for 2 times, 5 minutes each.
12. 5 min in DAB substrate solution.
13. Washed in distilled water for 5 minutes, 2 times.
14. Counterstaining was done with Meyer's hematoxylin for 5 minutes.
15. Washed in running tap water for 2 minutes. Sections were dehydrated with graded ethyl alcohols, cleared with xylol, and mounted with DPX (Dextrene Polysterene Xylene).

3. RESULTS

This study covered a total of 104 endoscopic biopsies received from the Gastroenterology Department for Histopathological evaluation. In 104 endoscopic biopsies, 73 were males (70.19%) and 31 were female (29.81%) with age ranging from 22 years to 90 years.

3.1 Age Incidence

The patients biopsied were divided into 7 groups according to their age and sex. (i.e. 20 -30 yrs., 31-40 yrs, 41-50 yrs, 51-60 yrs, 61-70 yrs, 71-80 yrs, 81-90 yrs).

Increased incidence of esophageal lesions was observed in 51-60 years (35.58%) age group followed by 61 - 70 years (22.12%).

Dysphagia (90 cases, 86.54%) is the most common complaint in our study followed by Regurgitation of food 104 cases of the esophageal biopsy were received for histopathological examination and evaluation. Among the 104 cases studied, 65 cases were Squamous cell carcinoma (62.5%), 11 cases were Squamous dysplasia (10.58%), 5 cases were Adenocarcinoma (4.81%), 4 cases were Barrett's esophagus(3.85%), 1 case was Fibrovascular polyp(0.96%), 12 cases were interpreted as esophagitis (18.46%), 4 cases were interpreted as normal stratified squamous epithelium.

As shown in Table 3, Squamous cell carcinoma (SCC) of the esophagus is the common malignant neoplasm in our study comprising 65(62.5%) cases followed by 11 cases of Squamous dysplasia (11 cases, 10.58%). The incidence of Adenocarcinoma is 5 cases (4.81%) whereas Barrett's esophagus is 4 cases (3.85%). Esophageal lesions were common in males, with the Male: Female ratio is 2.68:1.

Among the 104 cases, one case of Fibrovascular polyp (50/M) with characteristic features of Squamous epithelial lining with foci of ulceration and core of mature fibromyxoid tissue with scattered thin-walled blood vessels was observed.

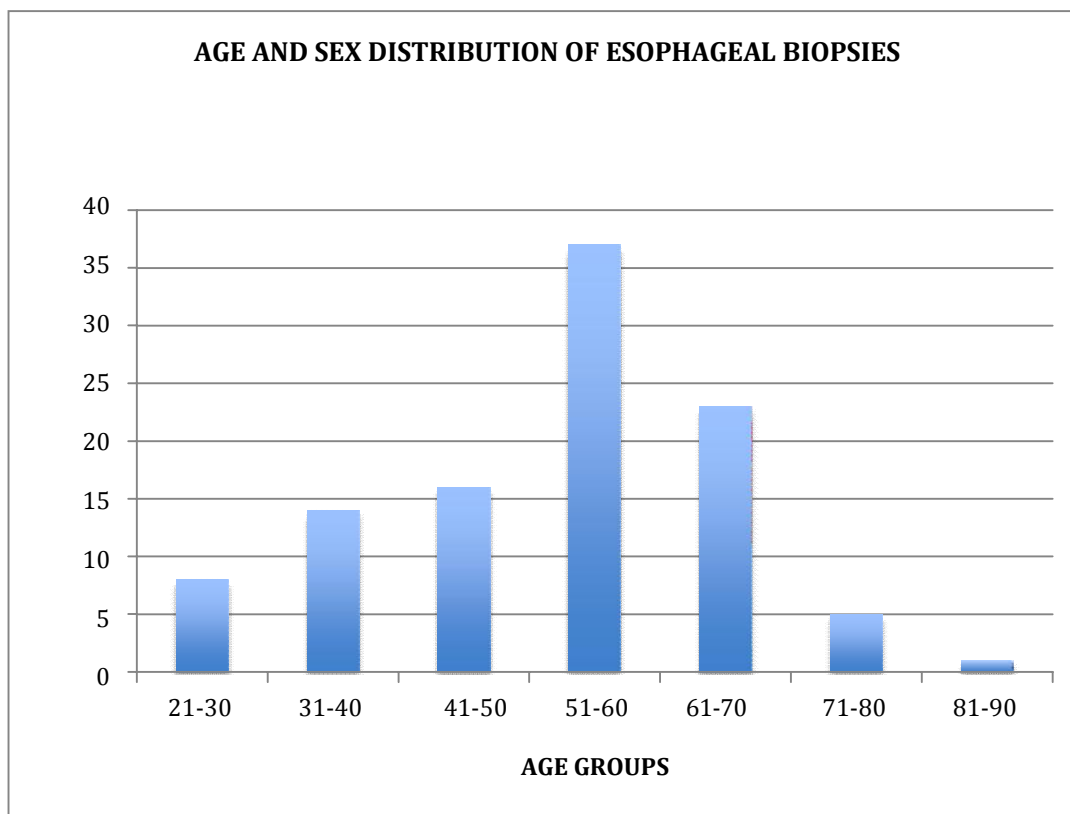


Chart 1. Age and sex distribution of esophageal biopsies

Table 1. Highlights the distribution of cases according to their clinical presentation in endoscopic biopsies

S. No	Clinical features	No of cases	Percentage
1	Dysphagia	90	86.54%
2	Loss of weight	65	62.5%
3	Anorexia	63	60.58%
4	Dyspepsia	34	32.69%
5	Vomiting	40	38.46%
6	Odynophagia	23	22.12%
7	Regurgitation of food	85	81.73%
8	Heart burn	43	41.35%

In our study, 5 cases of adenocarcinoma have been reported. Ulcerating and infiltrative lesions were the commonest endoscopic findings.

Shows distal esophagus is the commonest site of occurrence in Adenocarcinoma (80%) followed by the middle third (20%) of the esophagus. No case was reported in the upper 1/3 rd of the esophagus.

Most of the Adenocarcinoma was Well-differentiated (60%). 1 case each were moderately differentiated (20%) and poorly differentiated (20%) respectively. Well,

differentiated tumors showed more than 90% gland formation, with columnar cells, hyperchromatic and pleomorphic nuclei with eosinophilic cytoplasm infiltrating the muscular are mucosae with areas of metaplastic intestinal-type Barrett epithelium. Moderately differentiated tumors showed more than 50% gland formation with distorted glands lined by dysplastic epithelium in a papillary pattern infiltrating the muscular propriety. Poorly differentiated adenocarcinoma showed less than 50% gland formation with large, bizarre hyperchromatic and pleomorphic nuclei.

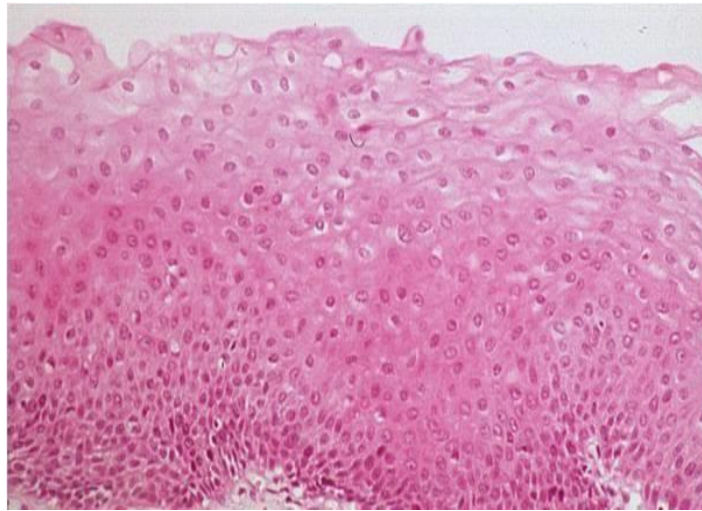


Fig. 1. Stratified squamous epithelium of esophagus (H&E 10 X)



Fig. 2. Endoscopic picture of squamous cell carcinoma showing fungating growth

Table 2. Shows the incidence of lesion and its percentage

S.no	Type of lesions	Male	Female	Total	Percentage
1	Normal	1	3	4	3.85%
2	Inflammatory/Infective Reflux				
	Esophagitis	4	2	6	5.77%
	Eosinophilia Esophagitis	1	0	1	0.96%
	Herpes Simplex Esophagitis Candida	2	0	2	1.92%
	Esophagitis	3	0	3	2.88%
3	Barrett's Esophagus	3	1	4	3.85%
4	Neoplastic –Benign Fibro vascular Polyp				
	Hyperplastic Polyp	0	1	1	0.96%
		1	1	2	1.92%
5	Squamous Dysplasia	7	4	11	10.58%
6	Malignant Neoplasm				
	Squamous Cell Carcinoma	47	18	65	62.50%
	Adenocarcinoma	4	1	5	4.81%

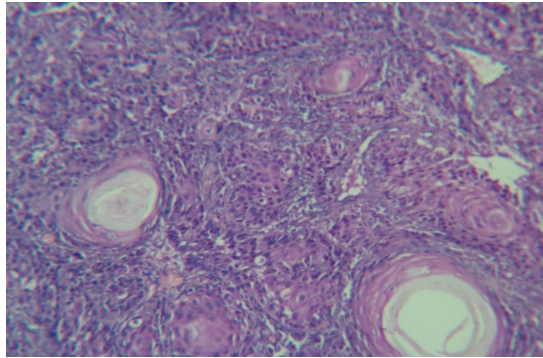


Fig. 3. Squamous cell carcinoma-well differentiated-keratin pearl formation (H&E 10X)

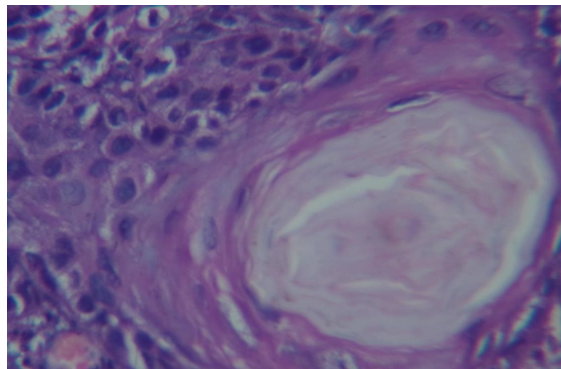


Fig. 4. Squamous cell carcinoma-well differentiated-keratin pearl formation (H&E 40X)

Table 3. Incidence of esophageal lesions in male

S. no	Type of lesion	No of cases	Percentage
1	Barrett's Esophagus	3	2.88%
2	Squamous Dysplasia	7	6.73%
3	ScC	47	45.19%
4	Adenocarcinoma	4	3.85%

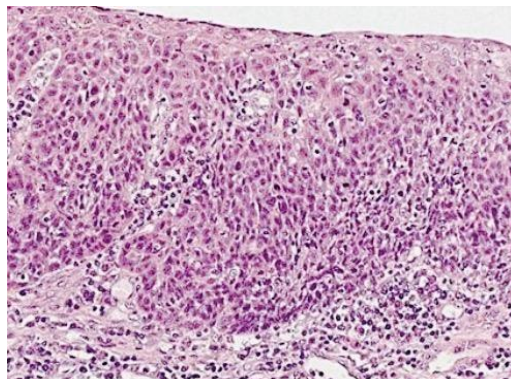


Fig. 5. Squamous dysplasia: High grade dysplasia showing dysplastic cells in more than 50% of the epithelium (H&E 10X)

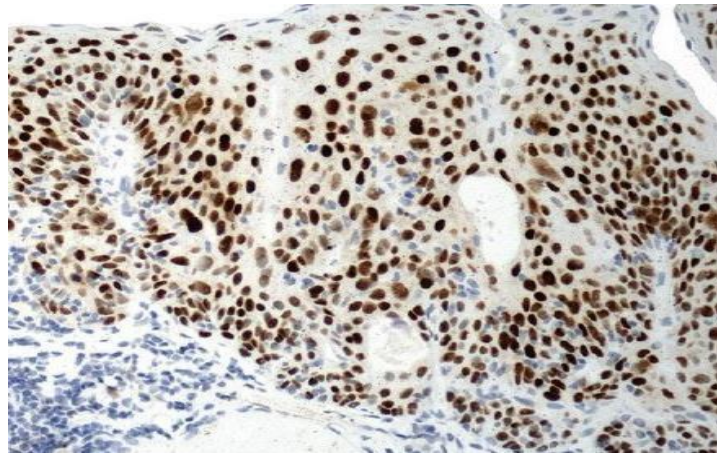


Fig. 6. High grade squamous dysplasia: P53 staining showing nuclear positivity in more than 50% of the epithelium (10X)

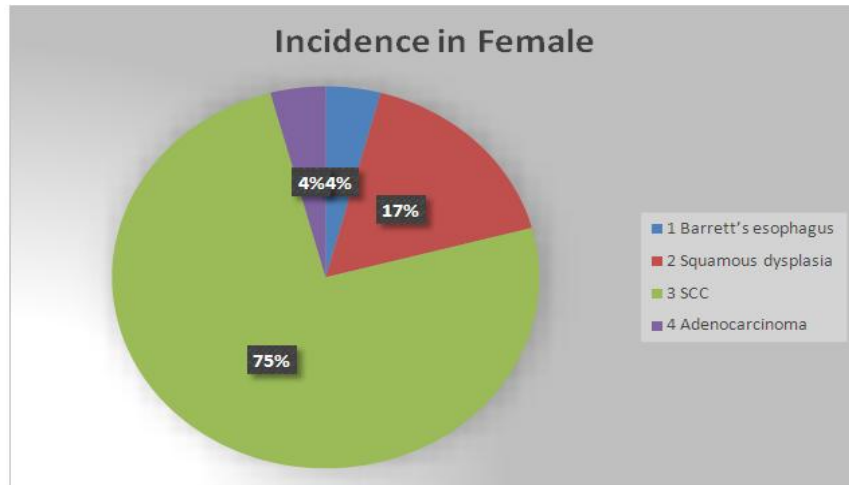


Chart 2. Incidence in female



Fig. 7. Endoscopic picture of Barrett's esophagus showing salmon coloured mucosa

Table 4. Stenosis of the esophageal lumen was reported in two cases. Relative distribution of adenocarcinoma

S. No	Site	No of cases	Percentage
1	Upper 1/3rd	0	-
2	Middle 1/3rd	1	20%
2	Lower 1/3rd	4	80%

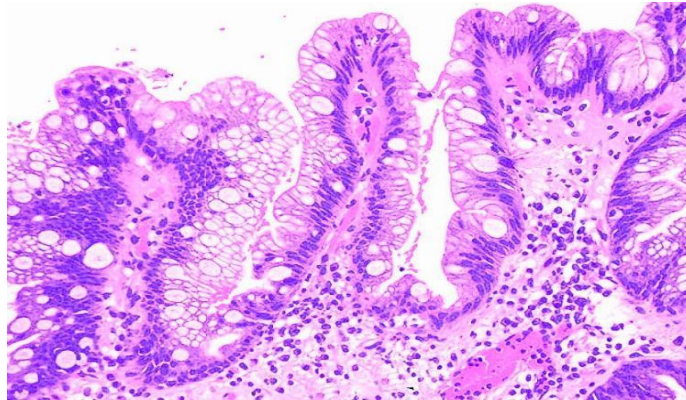


Fig. 8. Barrett's esophagus: Low grade dysplasia (H&E 10X)

Table 5. Shows the relative distribution of adenocarcinoma according to its mode of differentiation

S. no	Degree of differentiation	No of cases	Percentage
1	Well differentiated Adenocarcinoma	3	60%
2	Moderately differentiated Adenocarcinoma	1	20%
3	Poorly differentiated Adenocarcinoma	1	20%

4. DISCUSSION

In the present study, 104 cases of endoscopic esophageal biopsies received at the Department of Pathology, Sree Balaji Medical College and hospital from February 2014-September 2015 were processed routinely and sections were stained by Hematoxylin and Eosin. Special stains such as PAS, AB/PAS at pH 2.5, and HSV antigen and p53 antigen immunohistochemistry were used in relevant cases. Endoscopically, 59 cases were diagnosed as the malignant lesion, 21 cases interpreted as suspicious of malignancy, 7 cases diagnosed as benign lesions, and 13 cases diagnosed as Esophagitis of both inflammatory and infective pathology [10]. 4 cases were diagnosed as Barrett's esophagus. Out of 59 cases diagnosed as malignant lesions, 58 cases (98.31%) correlated with microscopic findings comprising of 56 cases

of Squamous cell carcinoma and 2 cases of Adenocarcinoma. 1 case diagnosed as Candida esophagitis which is confirmed by PAS stain. 13 cases of esophagitis diagnosed endoscopically, out of which 12(92.31%) correlated with microscopic findings, which were reported as Reflux esophagitis (6 cases), Candida esophagitis (3 cases), Herpes simplex esophagitis (2 cases), Eosinophilic esophagitis (1 case). 1 case was diagnosed as Squamous cell carcinoma. So one infective lesion suspected by endoscopy was turned out to be a malignant lesion [11-13].

Out of 21 cases diagnosed endoscopically as suspicious of malignancy, 7 cases (33.33%) diagnosed as Squamous cell carcinoma, 11 cases (52.38%) diagnosed as squamous dysplasia, 3 cases (14.29%) diagnosed as Adenocarcinoma, in microscopic examination.

Out of 7 cases of endoscopically diagnosed benign lesions, 4 cases (57.14%) were interpreted as normal stratified squamous epithelium, 2 cases (28.57%) diagnosed as Hyperplastic polyp and 1 case (14.29%) was diagnosed as Fibrovascular polyp. 4 cases of Barrett's esophagus were confirmed with microscopic examination and were correlated [14].

The present study shows that Squamous cell carcinoma (65 cases, 62.5%) is the most common esophageal neoplasm. Our study was by the study of Durrani AA et al. 77 who also found similar results. In our study, esophageal carcinomas occur more commonly in males, with a male, female ratio of 2.6:1 [15]. Ashis Kumar et al. described male preponderance in esophageal tumors with M: F ratio of 1.8:178. Squamous dysplasia is an epithelial precancerous lesion characterized by nuclear enlargement and hyperchromasia with increased mitotic activity. The incidence of Squamous dysplasia in our study is (11 cases, 10.58%), in correlation with the study conducted by Michael et al. 81 who reported 14% incidence. The male: female ratio in our study was 1.8:1. Squamous cell dysplasia is found preceding or combining with squamous cell carcinoma. In our study, 9 cases were Low-grade dysplasia (81.82%) and 2 cases (18.18%) were high grade dysplasia. The study conducted by Kuwano 80 et al. also had similar results [16].

One case of Fibromuscular polyp with intact overlying mucosa and core of mature fibrous tissue exhibiting foci of myxoid areas and thin-walled vessels is seen. Two cases of Hyperplastic polyp in gastroesophageal junction had been reported in our study. 12 cases of esophagitis have been reported in our study [17]. The male: female ratio for esophagitis was 5:1. In 2002, the incidence of esophagitis lesions was 2.4 per 1,000 people - years in a study conducted by Lassen et al. 75. 6 cases of reflux esophagitis have been reported. 1 case of eosinophilic esophagitis, 2 cases of herpes simplex esophagitis, and 3 cases of Candidial esophagitis. Out of 12 cases, 11 cases were correlating with endoscopic and microscopic findings [18].

Diagnosed as infective esophagitis in endoscopic findings, was turned out to be Squamous cell carcinoma by microscopy. So the microscopic examination is mandatory to arrive at an accurate diagnosis. In our study, Herpes

simplex esophagitis had been diagnosed in an immunocompetent individual, DeGaeta et al. 83 also reported a similar case in an otherwise healthy patient.

5. CONCLUSION

Esophageal cancer is a common malignant tumor in the digestive tract. Esophageal lesions once thought to be rare, are nowadays being one of the common disorders affecting people throughout the world. A diversity of benign tumors and non-neoplastic masses can be seen in the esophagus. They are however mostly uncommon lesions, small and asymptomatic, whose importance lies in their distinction from malignant tumors. The development and popularization of endoscopy become a necessary inspection means of early esophageal diseases. It proved that early detection is necessary to identify malignant lesions and also premalignant conditions such as squamous dysplasia and Barrett's esophagus so that malignant transformation can be identified with proper follow-up and treatment may render. Lesions that appeared to be of infective pathology in endoscopy turned out to be malignant in microscopy examination. This proves that microscopic examination is the confirmatory diagnostic tool. The microscopic examination along with the accessory histochemical stain, immunohistochemistry for HSV antigen, p53 expression helped to arrive at an accurate diagnosis.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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