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RESEARCH ARTICLE

ROLE OF IMMUNOHISTOCHEMISTRY IN EARLY DIAGNOSIS OF ESOPHAGEAL BIOPSIES – A REVIEW

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ABSTRACT

Background: Gastro-Intestinal (GI) cancer is a term for the group of cancers that affect the digestive system which includes cancers of the oesophagus, gallbladder, liver, pancreas, stomach, small intestine, bowel (large intestine or colon and rectum), and anus.

Objective: In this study we show the advantages of immunohistochemistry which includes not only its remarkable sensitivity and specificity but also its applicability to routinely processed formaline fixed material.

Method: The present study had been conducted in the Department of Pathology, B.R.D. Medical college, Gorakhpur, U.P on both IPD & OPD patients in whom endoscopic biopsies were conducted for esophageal lesions .Further all these biopsies were studied by IHC.

Result: The present study included 25 cases of esophageal endoscopic biopsy. Benign lesions were 10 and malignancies were 15 .The significant findings in this study showed p53 and ki67 positivity in 0 and 20% cases of benign esophagealtumors. However it was interesting to note that malignant esophagealtumors were more positive for p53(53.3%) and ki67 had a high positive index(66.6%).

Conclusion: Most gastrointestinal tumors can be differentiated by their unique immunohistochemical profile.

In present study, the sensitivity and specificity of p53 in esophageal cancer was 53% and 100% respectively. Whereas sensitivity and specificity of ki67 in esophageal cancer was 66.6% and 80% respectively.

INTRODUCTION

Esophageal cancer is the eighth most commonly diagnosed cancer type and sixth leading cause of cancer deaths worldwide.[1]

In less developed parts of the world, esophageal squamous cell carcinoma (ESCC) has long been the predominant subtype of this cancer, with rates up to 100 per 100,000 in parts of China, India and central Asia (the so-called, 'esophageal cancer belt'). However, esophageal adenocarcinoma (EAC) incidence is increasing rapidly in Western countries, while ESCC incidence is generally stable or declining.[2]

For different types of esophageal cancer, the risk increases with age, with a mean age at diagnosis of 67years.[3]

This disease is 3 to 4 times more common among men than among women.[4]

Combining smoking and drinking alcohol raises the risk of esophageal cancer much more than using either alone.[4]

Compared to adenocarcinoma of the esophagus which is the more common tumor in the United States, SCC is much more common in Asian countries, where up to 40% have been linked to HPV infection.[5]Although SCC can develop in any part of the esophagus but are more commonly found in the middle and lower third portions of the esophagus.[6]

Immunohistochemical staining for p53 is used as a surrogate for mutational analysis in the diagnostic workup of carcinomas of multiple sites including ovarian cancers. Strong and diffuse immunoexpression of p53 is generally interpreted as likely indicating a *TP53* gene mutation. [7]

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The Ki-67 index had wide distribution of 1-80%.Patients with high Ki-67 were significantly associated with a high grade of malignancy, with poorer prognosis and with increased rate of recurrence. [8]

MATERIAL AND METHOD

The present study had been conducted in the Department of Pathology, B.R.D. Medical college, Gorakhpur, on the patients attending surgery indoor and OPD, in Nehru Chikitsalaya during a period ranging from August 2012 to October 2013.

Freshly biopsied specimens were subjected to overnight fixation and were processed routinely in the histopathology laboratory and retrospective study has also been performed on preserved blocks.

All the paraffin blocks were preserved for section cutting .Thin sections of 4-5µ have been cut after dewaxing, then were stained by hematoxylin and eosin stain. Histopathological diagnosis was made and then freshly cut sections were also used for immunostaining.

Sections from 2 representative paraffin blocks of each case were immunostained with p53 (mouse monoclonal antibody,clone DO-1) and Ki-67 (mouse monoclonal antibody, clone MIB1,)

Sections were mounted on silanized slides, deparaffinized, and rehydrated through graded alcohol to water. Next, slides were microwaved at 95°C for 6 cycles of 5 minutes each in a 10-mmol/L concentration of sodium citrate buffer (pH 6.0) for Ki-67 or for 7 cycles of 5 minutes each for p53. Then the slides were allowed to cool for approximately 1 hour at room temperature to enhance antigen retrieval.

Then specimens were treated with 10% normal rabbit serum for 10 minutes at room temperature in a cover plate. Primary antibodies were incubated with tissue sections for 18 hours at 4°C. After washing with a 0.01-mol/L concentration of phosphate-buffered saline, they were incubated with biotin-conjugated antimouse immunoglobulin for 10 minutes at room temperature and then incubated with peroxidase-conjugated streptavidin for 5 minutes at room temperature using a Histofine kit . Demonstration of binding sites with the peroxidase reaction was achieved with 3.3'-diaminobenzidine tetrahydrochloride (0.25 mg dissolved in 1 mL of 0.02% hydrogen peroxide). Faint nuclear staining, sufficient to aid in orientation but not enough to influence the judgment of positivity, was performed with Mayer hematoxylin solution.

The p53 label was determined as positive or negative by calculating the number of positive nuclei per 500 gastric epithelial cells and cancer cells in 1 representative section. The count was performed under low magnification (×100) using a double-headed light microscope. The staining intensity was arbitrarily graded on a scale of four grades: 0, no staining of cancer cells; 1, weak staining; 2, moderate staining; 3, strong staining. The percentage of staining area was also graded on a scale with four grades: 0, none; 1, <10%; 2, 10-50%; 3, >50%. Theoretically, the overall scores could range from 0 to 8. The specimens with a score of more than 4 were regarded as positive expression, and those with a score ≤4 as negative expression.

The intensity of positivity for ki67 was scored as follows: 0 negative; 1 weak; 2 moderate; 3 strong. The extent of positivity was scored according to the percentage of cells showing positive staining: 0, < 5%; 1, > 5-25%; 2, > 25-50%; 3, > 50-75%; 4, >75% of the cells in the respective lesions. The final score was determined by multiplying the intensity of positivity and the extent of positivity scores, yielding a range from 0 to 12. Any score that was above 4 was interpreted as positive for ki67.

Stain positivenuclei - Brown
Counterstained areas- Blue

All esophageal biopsies were processed for IHC by p53 and Ki67.They were processed along with positive and negative controls. The positive controls were previously positive p53 squamous cell carcinomas with high ki67 labelling index.

RESULTS

Our study included 25 cases of esophageal endoscopic biopsy. Benign lesions were 10(40%) and malignancies were 15(60%). The relative incidence of esophageal tumors came out to be 14.04%.

It was seen in our study that most common age group of esophageal cancer was 66-75years.According to our study 4 cases(26.6%) of squamous cell carcinomaand 1 case(6.6%) of adenocarcinoma lie in this group.In age group of 76 to 85 years ,3 cases(20%) have been found which were squamous cell carcinoma.2cases[1(6.6%) squamous cell carcinoma & 1(6.6%) adenocarcinoma] have been seen in age group between 55 to 65 years. 2 cases of adenocarcinoma were seen beyond 85 years and only 2 cases [1Undifferentiated carcinoma and 1 small cell carcinoma] were seen <55 years.Mean age for esophageal cancer came out to be 67.5 years.

Most common benign esophagealtumor found in our study was GISTi.e 6/10 cases(60%) which was later confirmed by CD117.p53 was positive innone of the 10 benign cases.ki67 score in 2(20%) cases of GIST was 4.

Our study included 15 cases of malignancy in the esophagus. The most common malignancy was squamous cell carcinoma[9/15cases(60%)], followed by adenocarcinoma [4/15 cases(26.6%)]. Two other malignancies were seen in our study, which included one case of small cell carcinoma and one case of undifferentiated carcinoma.

5 out of 9 cases of squamous cell carcinoma were positive for p53 which amounted to 55.5 %.3 cases out of 4adenocarcinoma were positive for p53, which amounted to 75%.Four cases of squamous cell carcinoma and one case of adenocarcinoma were negative for p53.Small cell carcinoma and undifferentiated carcinoma were also negative for p53.

10 cases showed a high ki67 labelling index. A mean score of 8 was obtained for squamous cell carcinoma.8/9 cases of squamous cell carcinoma (88.8%) showed a high ki67 score which was scored as 7-9. And 2/4 (50%) cases of adenocarcinoma showed high ki67 labelling index. Two cases showed negative result which were small cell carcinoma and undifferentiated carcinoma.

Among 25esophagealcases, 68% were male and 32% were females. Male: Female ratio came out to be 2.12:1. In the present study it was found that esophageal cancers are more prevalent inhindus (64%) of urban places(72%) as compared to rural places (28%).

The most common mode of presentation of esophageal tumor was dysphagia (86%) which was seen in 21 cases. Among these 21 cases 9 (42%) cases were of squamous cell carcinoma, 4(20%) cases were of adenocarcinoma, 6 cases(28%)were GIST and two cases(10%) included squamous cell papilloma. Rest 4 cases showed symptoms like epigastric mass and pain, vomiting etc.

Most common location of esophagealtumors were found to be middle one-third(60.4%) which included 15cases.Out of these 15 cases 9 were squamous cell carcinoma(60%),4 were adenocarcinoma(27%) and two cases were of GIST i.e13 %.Next in sequence comes lower one-third which includes 9 cases i.e4(44.4%) cases of GIST, 2(22.2%) cases of lipoma , 2(22.2%)cases of squamous cell papilloma and one case(11.1%) of small cell carcinoma. Least common location was found to be upper one-third which included one case of undifferentiated carcinoma.

The significant findings in this study showed p53and ki67 positivity in 0 and 20% cases of benign esophageal tumors**(Figure 2)**.However it was interesting to note that malignant esophageal tumors were more positive for p53(53.3%) and ki67 had a high positive index(66.6%)**[Table 1&Figure1]**.

Table 1 Comparative evaluation of p53 and ki 67 in esophageal tumors

Distribution	p53 + cases	%	ki 67 + cases	%
Benign	0	0	2	20.00
Malignant	8	53.3	10	66.6

Statistically in case of p53, Yate's chi square is 5.584 and P value came out to be 0.018i.e significant ,whereas ki67 showed chi square to be 6.75 and p value to be 0.0009 which is also significant**[Table 2]**.

Table 2 Benign vs malignant esophageal tumors

Histology	Markers	p value	Significance
Benignvs malignant esophageal tumors	p53	<0.05 (0.018)	Significant
Benign vs Malignant esophageal tumors	ki67	<0.05 (0.009)	Significant

In present study, Sensitivity and specificity of p53 is 53 % and 100% respectively where as sensitivity and specificity of ki67 in esophageal cancer is 66.6% and 80 % respectively**[TABLE 3].**

Table 3 Sensitivity and specificity of p53 and ki67 in esophageal cancer

	p53	ki67
Sensitivity	53%	66. 6%
specificity	100%	80%

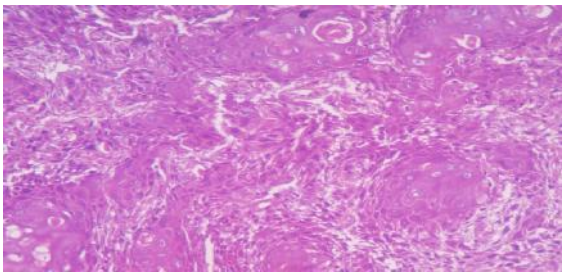
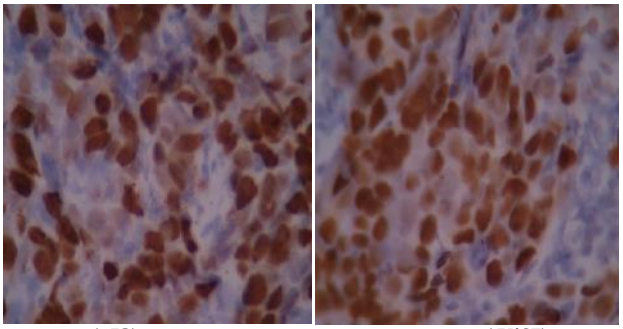


Figure1 Well Differentiated Squamous Cell Carcinoma (H&E X400)



(p53) **(Ki67)**

Well differentiated squamous cell carcinoma tumor cells show nuclear positivity for p53 & ki67(h&e x 400)

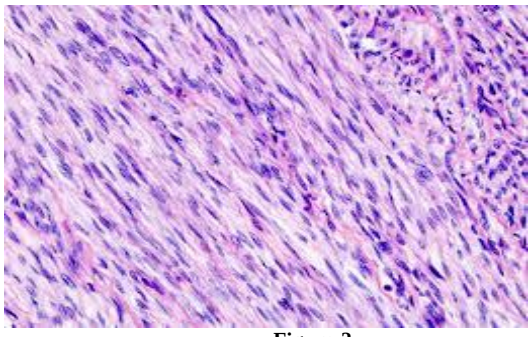
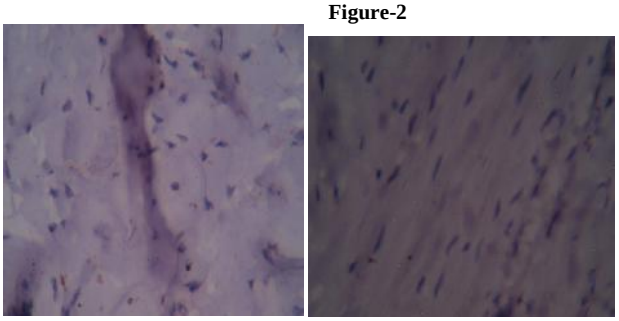


Figure-2



(p53) **(Ki67)**

Leiomyoma (Benign Lesion) Of Esophagus , Shows No Positivity For P53 & Ki67

DISCUSSION

Oesophageal cancer incidence rates have increased overall. The observed trends may be associated with changing exposure to risk factors, which include tobacco use, insufficient intake of fruit and vegetables, overweight, obesity and alcohol consumption.[9]

In the present study, the relative incidence of esophageal tumors(among all gastrointestinal lesions) received in our department was found to be 14.04% .SfoortiGoswami *et al* 2013reported incidence of 15.62% in case of esophageal tumors in his study conducted on 64 cases.[10]
Out of 25 cases of esophagealtumors, benign cases were 40% and malignant cases were 60%.

GIST was found to be most common benign esophageal tumor whereas squamous cell carcinoma as the most common malignant esophageal tumor. Our study was in accordance with the study of Pedeutour *et al* 2000 and DurraniAA *et al*2009 who also found similar results for benign and malignant esophageal tumor respectively.[11,12]

In our study maximum number of cases were in their6th and 7thdecade of life. Mean age for esophageal cancer is 67.5.

Out of 25 cases of esophagealtumors 68% were male and 32%were females. Male: Female ratio came out to be 2.12:1.Ashis Kumar Saha *et al* 2012and M S Khuroo *et al* 1982also described malepreponderance in esophagealtumors with M:F ratio of 2.6:1 and2.2:1 respectively.[13,14]

Esophagealtumors are more prevelant in urban areas(72%) as compared to rural areas(28%) respectively. However different result was found by Wen-RuiTangin his study.[15]He found incidence rate in rural areas being 2.7 times than that in the city.

Inesophagealtumors out of total 25 cases,64% were Hindus and 36% were Muslims. Ashis Kumar Saha *et al* 2012 foundsimilar results in his study.

In case of esophageal cancers the most common mode of presentation is dysphagia(86%) ,followed by pain in the epigastrium (7%) and mass in epigastrium (4 %). Other mode of presentation are vomiting, hematemesis and anemia with frequency of 2% and 1 % respectively.

The most common anatomic site of esophageal tumor was found to be middle third 9 cases (60.4 %) followed by lower third and upper third which are 5 cases (37.8%) and 1 case(1.8%) respectively. M S Khuroo *et al* found similar results in their study.

In the present study IHC revealed that in case of benign esophageal tumors p53 showed no positivity whereas ki67 was positive in 2/10 cases i.e 20 %.

Malignant esophageal tumors showed p53 positivity in 8/15 cases i.e 53.3 % and ki67 showed positivity in 10/15 cases i.e 66.6 %.

Kazuya Tokita *et al* 2013 in their study showed that out of 44 cases of esophageal cancer 46. 6% were p53 positive and 73.3% were ki67 positive.[16]

As stated by Min Xu *et al* 2002 their study showed that out of 169 cases of esophageal cancer 56. 8% were p53 positive and 62.1%were ki67 positive.[17]

According to *Zohreh Sanaat et al 2013* out of 100 cases of esophageal cancer 35% were p53 positive and 53% were ki67 positive.[18]
Present study showed that p53 and ki67 show much more positivit in case of malignant esophageal tumors as compared to benign ones. In case of p53, Yate's chi square is 5.584 and P value came out to be 0.018 i.esignificant, whereas ki67 showed chi square to be 6.75 and p value to be 0.0009 which is also significant.

According to present study sensitivity and specificity of p53 in esophageal cancer was found to be 53% and 100% respectively whereas sensitivity and specificity of ki67 was 66. 6% and 80 % respectively.

Kazuya Tokita *et al* found similar results in his study that showed sensitivity and specificity of p53 in esophageal cancer to be 43% and 98 % respectively and sensitivity and specificity of ki67 to be 68% and100% respectively.

CONCLUSION

Immunohisto chemistry (IHC) visualises tumor markers such as enzymes, oncogenes, tumor-specific antigens, tumor suppressor genes and tumor proliferation markers, doctors can efficiently predict oncogenesis and diagnose a cancer as benign or malignant, determine the stage and the grade of a cancer. It is clear from the present study that increased p53 andki67expression as shown on immunostaining is associated with a higher risk of histologic progression. Hence it is reasonable to recommend more intensive screening in patients who are p53 and ki67 positive.

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