ORIGINAL RESEARCH

Role of EGFR, HER2 and P53 in the Spectrum of Oesophageal Lesions

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ABSTRACT

Background:Oesophageal cancer is the eighth most common cancer and sixth in cancer related mortality, worldwide. The current study was carried out to see the role of three markers (P53, HER2, EGFR) in various oesophageal lesions, their association in cancer progression and their role as tumour markers.

Materials and Methods: Study was conducted on 100 cases of oesophageal lesions consisting of both neoplastic and non-neoplastic spectrum. Immunohistochemistry was performed on all the specimens for three markers as per the set criteria and expression of these markers was studied and scoring was done accordingly.

Results: Present study was conducted on 100 cases with oesophageal lesions both neoplastic and non-neoplastic. Parameters like age, gender, family history, alcohol intake, cigarette smoking and location of lesion in oesophagus were also studied. Expression of P53, Her2, EGFR were studied using IHC.

Conclusion: P53, HER2 and EGFR show a strong association with EC and premalignant lesions, the absence of their expression in normal oesophageal tissue and non-neoplastic conditions, points out their role in development of EC and tumour progression. Out of the three markers, P53 has the strongest association with EC. HER2 overexpression was more in case of EAC than ESCC, indicating its importance in guiding further treatment. EGFR over- expression was noted in fewer cases.

Keywords: P53, HER2, EGFR, oesophageal cancer, IHC.

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INTRODUCTION

Oesophageal cancer is the eighth most common cancer and is the sixth most common cause of cancer related mortality worldwide. It affects more than 4,50,000 people every year worldwide.[1,2] It is the fourth common cause of cancer related deaths in India with approximately 47,000 new cases reported each year and reported deaths reaching upto 42,000 each year in India.[3] In Punjab, data collected from nineteen districts between 1st January 2012 to 31st December 2014 shows it is the second most common cancer among males and fourth most common among females.[4]

P53, is a tumour suppressor gene, located on short arm of chromosome17. It is one of the most commonly mutated gene in human cancers.[5]It plays an important role in tumorigenesis by controlling cell growth, apoptosis and angiogenesis.[6] P53 exons 5–9 are mutational hotspots as they contain the zinc-finger domain and the trans-activating domain. More than 80% of P53 mutations are clustered in this region.[7]

The epidermal growth factor receptor (EGFR) family of receptors, also called HER family of tyrosine kinases, include four receptors ERBb1 or EGFR, ERBb2 or HER-2, ERBb3 and

ERBb4. The EGFR gene is located on chromosome 17p12. They are encoded by ERB oncogenes and have a role in tumour cell growth and differentiation. When a receptor attaches to its surface, the intracellular tyrosine kinase domain is activated resulting in a cascade of signalling pathways-mRas/Raf/mitogen-activated protein kinase (MAPK)/cyclin-D1, PI3K/AKT pathway and signal transducers and activators of transcription (STAT) signalling pathways, which are involved in cell proliferation and differentiation.[8] The EGFR nuclear signalling also has a role in tumour progression, invasion and metastasis.[9]
The current study was carried out to see the role of these markers (P53, HER2, EGFR) in various oesophageal lesions, their role in oesophageal cancers, cancer progression, tumor grade and their role as tumour markers.

MATERIALS & METHODS

The present study was conducted after approval from the ethics committee of our institute. This is an observational study conducted over a period of two and half years on 100 patients coming to our institute. The patients of either gender or age group who presented with oesophageal complaints and without other coexisting morbidities that could influence the final outcome of study were included. All the patients who had received neoadjuvant chemo/radiotherapy were excluded from study.
Procedure- Tissue blocks were prepared from biopsy specimens that were sent in 10% buffered formalin for both histo-pathological and immuno-histochemical (IHC) examination. Special attention was paid to minimise the cold ischemia time. The tissue was processed within 6-72 hours to avoid the antigen masking or false negativity.
For IHC, expression of three proteins i.e. EGFR, HER2 and P53 were studied on tissue blocks after deparaffinization and following the standard protocols.
The slides were interpreted as follows-
For P53:- Score 0- <10% tumour cells show nuclear positivity; Score1- 10-25% cells show weak nuclear positivity; Score 2- 26-50% cells show moderate nuclear positivity; Score 3- >50% cells show strong nuclear positivity. Score of 2 and 3 was taken as positive.
For HER2:- 0,, negative- no membranous staining or <10% of tumour cells show weak staining; 1+, staining is weak or detected in only one part of membrane in >10% cells; 2+, moderate/ weak complete or basolateral membranous staining in ≥10% cells; 3+, strong complete or basolateral membranous staining in ≥ 10% cells. Score 2+, 3+ was taken as positive.
For EGFR:- 0, negative- no discernible staining or background staining; 1+, definite cytoplasmic staining and/or equivocal discontinuous membrane staining; 2+, unequivocal membrane staining with moderate intensity; 3+, strong and complete plasma membrane staining. Score 2+, 3+ was taken as positive.

RESULTS

Present study was conducted on 100 cases with oesophageal lesions both neoplastic and non-neoplastic. Parameters like age, gender, family history, alcohol intake, cigarette smoking and location of lesion in oesophagus were also studied. Expression of P53, Her2, EGFR were studied using IHC.

Table 1: Age wise distribution of various oesophageal lesions

Age(in years)	Total cases	Percentage	Number of cancer cases	percentage
<20	00	00%	00	00%
21-40	05	05%	03	05%
41-60	48	48%	30	48%
61-80	43	43%	27	42%
>80	04	04%	03	05%

Of the 100 cases studied it was seen that oesophageal lesions mostly presented in higher age groups. No cases were seen below the age of 20. Both neoplastic and non-neoplastic cases were seen more commonly in higher age groups. Cases of oesophageal cancer (EC) were seen more commonly between the age group 41-60 (48% cases) and 61-80 (42% cases). Oesophageal malignancy was less common in young age groups. (Table 1)

Table 2: Gender wise distribution of EC

Gender	Cases	%age
Male	34/63	53.9%
Female	29/63	46%

EC is more common in males as compared to females. In males the percentage is 53.9% while in females 46% positive cases are seen. (Table 2)

Table 3: Spectrum of oesophageal lesions

Diagnosis	Number of cases	%age
Oesophagitis	14	14%
Polyp	01	01%
Dysplasia	14	14%
Barrett without dysplasia	07	07%
Barrett with dysplasia	01	01%
ESCC	55	55%
EAC	08	08%

The various spectrum of lesions seen in oesophagus were oesophagitis (14%), polyp (01%), dysplasia (14%), barrett's oesophagus (BE, 08%), oesophageal squamous cell carcinoma (ESCC, 55%) and oesophageal adenocarcinoma (EAC, 08%). (Table 3)

Table 4: IHC expression of P53 in various oesophageal lesions

Condition	P53 expression	% age
Oesophagitis	0/14	00%
Polyp	0/01	00%
Dysplasia	07/14	50%
Barrett oesophagus	00/08	00%
EC	34/63	53.9%

Table 5: P53 expression according to tumour subtype

Type of tumor	P53 positivity	%age
ESCC	30/55	54.5%
EAC	04/08	50%

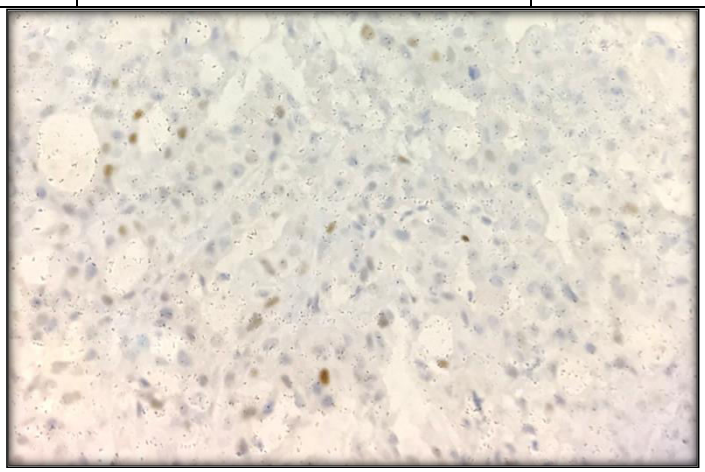

Figure 1: P53 with weak nuclear positivity in <10% tumour cells, score 1 (400x)

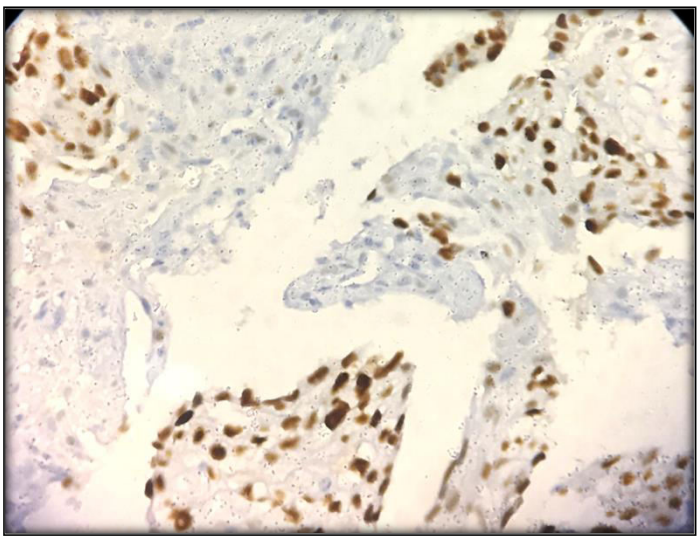

Figure 2: P53 with nuclear positivity in >10% tumour cells, score 2 (400x)

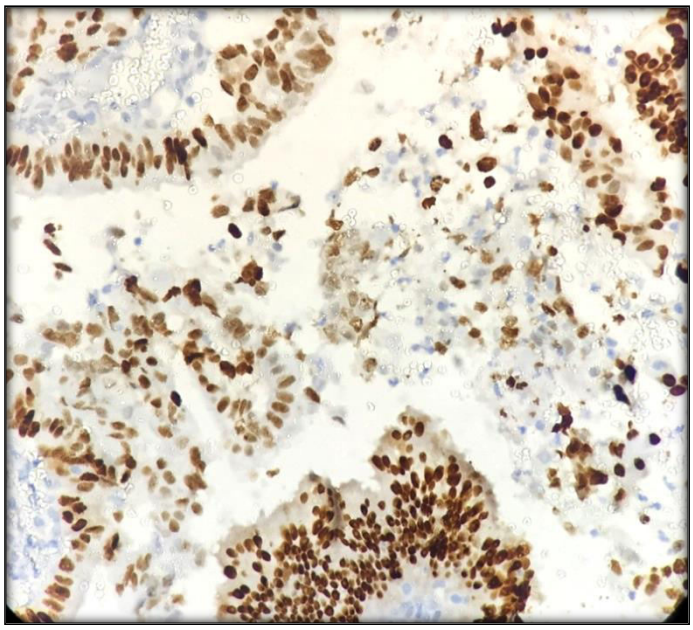

Figure 3: strong nuclear positivity in P53, score 3 (400x)

On IHC, score of 2+, 3+ was taken as positive for P53 and it showed nuclear positivity. The expression of P53 was high in EC (53.9%) and dysplasia (50%), while the expression was absent or weak in case of oesophagitis, polyp and BE. Among EC, expression was slightly more common in ESCC (54.5%) than EAC (50%). (Table 4,5) (Figure 1,2&3)

Table 6: IHC expression of HER2 in various oesophageal lesions

Condition	HER2 expression	%age
Esophagitis	00/14	00%
Polyp	00/01	00%
Dysplasia	03/14	21%
Barrett oesophagus	02/08	25%
EC	17/63	26.9%

Table 7: HER2 expression according to tumour subtype

Type of tumor	Her 2 expession	%age
ESCC	14/55	25.4%
EAC	03/08	37.5%

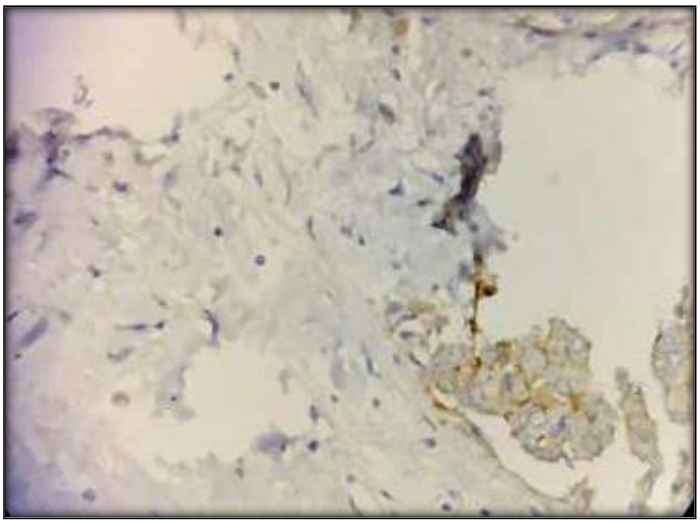

Figure 4: HER2 with weak membranous positivity in <10% tumor cells, score 1 (400x)

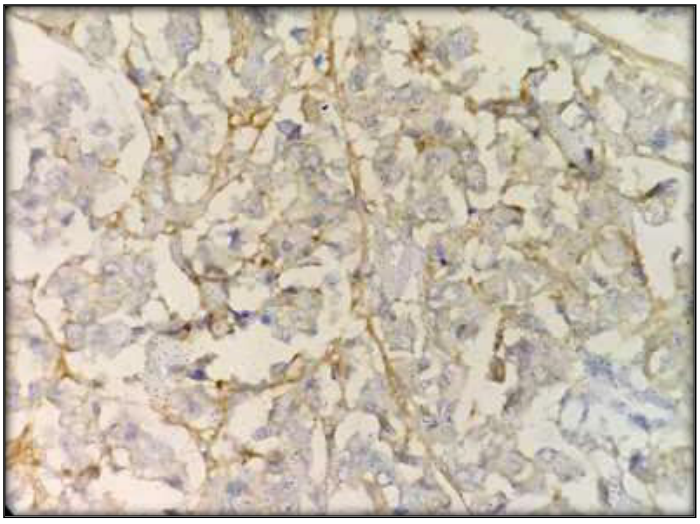

Figure 5: HER2 with weak but complete membranous positivity in >10% tumour cells, score 2 (400x)

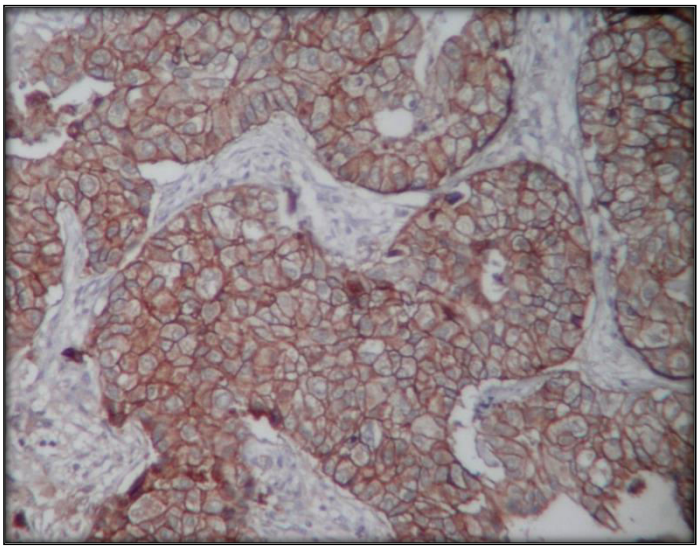

Figure 6: HER2 with strong complete membranous positivity, score 3 (400x)

For HER2, score of 2+, 3+ with membranous staining was taken as positive and scoring was done as already stated. The expression was absent or weak in non-neoplastic conditions like oesophagitis and benign polyp. However, expression was seen in 21% cases of dysplasia, 25% cases of BE and 26.9% cases of EC. Upon subtyping, expression was more common (m.c) in EAC (37.5% cases) than ESCC (25.4% cases). (Table 6,7) (Figure 4,5,6)

Table 8: IHC expression of EGFR in various oesophageal lesions

Condition	EGFR expression	%age
esophagitis	00/14	00%
Polyp	00/01	00%
dysplasia	00/14	00%
Barrett oesophagus	01/08	12.5%
EC	11/63	17.5%

Table 9: EGFR expression according to tumour subtype

Type of tumor	EGFR expression	%age
ESCC	10/55	18%
EAC	01/08	12.5%

Figure 7: EGFR showing only background cytoplasmic positivity, negative (400x)

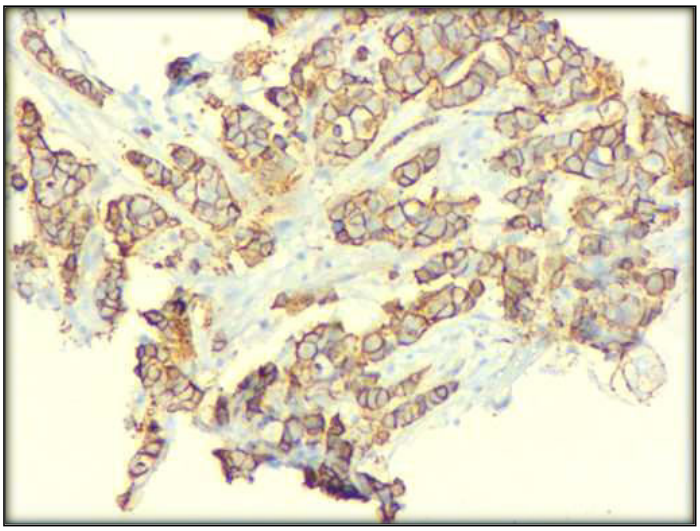

Figure 8: Moderate EGFR membranous positivity, score 2 (400x)

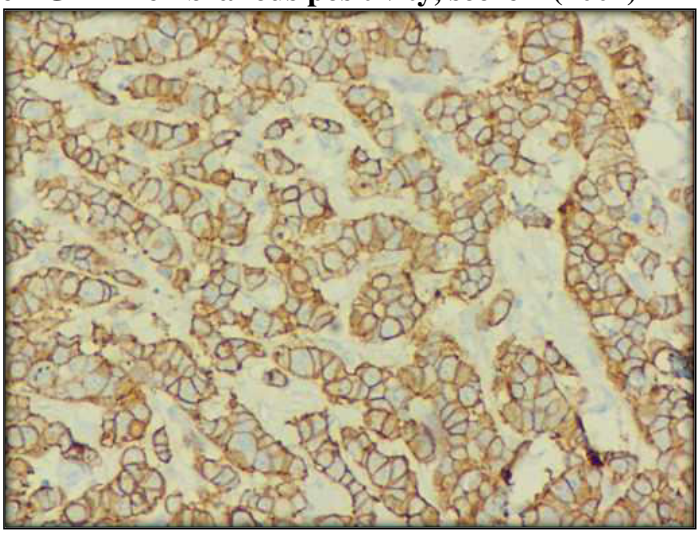

Figure 9: Strong EGFR membranous positivity, score 3 (400x)

For EGFR, again score 2+, 3+ with membranous staining was taken to be positive. In the spectrum of various oesophageal lesions, EGFR positivity was seen in cases of barrett's oesophagus and oesophageal cancer with 12.5% and 17% positivity, respectively. The score was negative or weak in oesophagitis, polyp and dysplasia. (Table 8,9) (Figure 7,8,9).

DISCUSSION

The maximum number of cases of EC were in age group 41-60 years, which is also the most common age group for general presentation of oesophageal lesions. In both terms, overall presentation of oesophageal lesions and EC, the percentage is 48, which is in accordance with studies by Giri et al and Kapoor et al.[10,11] Hence, it shows EC is the disease of the higher age group.

Of the 100 cases studied, the majority weremales i.e.55%, with male: female ratio of 1.2:1. Males are more prone for development of EC due to increased exposure to alcohol and smoking, which is an important risk factor for EC. Similar results are shown by Giri et al, Kapoor et al and Ganga et al.[10,11,12]

Results of present study showed 63% malignant cases and 15% benign. This is in agreement with studies conducted by Ganga et al and Khandelia et al.[12,13] However in study conducted by Qureshi et al showed more number of benign cases in oesophageal biopsies.[14] This could be because in our hospital most of the patients belong to low socio-economic strata and they

present to tertiary care centre only when they have increased difficulty in swallowing and disease has already progressed to malignancy.

P53 IHC- P53 is the most common tumour suppressor gene that undergoes mutation in various tumours. In current study P53 positivity was seen in 53.9% cases of EC. The results are similar to Riberio et al, Casson et al and Melling et al.[15,16,17] There are studies from China and Golestan province of Iran where the P53 expression was as high as 80-90%. This could be due to the very high number of cases of EC and different genetics of the population under study. Increased expression of P53 on IHC signifies either underlying mutation or amplification of genes.

Positivity was seen more in the case of ESCC than EAC. The results were similar to Coggi et al and Bellini et al.[18,19]However, it is in contrast to study published by Melling et al. where the expression of P53 was more in case of EAC as compared to ESCC.[17] This could be due to difference in genetic composition of populations included in study or difference in techniques used for IHC.

In BE, no or weak P53 positivity i.e less than 10% tumour cells, was noted in the current study. This shows alteration in expression of P53 is related to either dysplasia or cancer. The results are similar to Younes et al and Keswani et al.[20,21] The P53 expression in dysplasia of oesophagus was seen in 50% cases. Stronger positivity was seen in cases of high grade dysplasia as compared to low grade dysplasia showing expression of P53 varies with grade of lesion.

HER2 IHC- In case of EC, strong HER2 positivity was seen in 26.9% cases. The expression was more in case of EAC (37.5% cases) as compared to ESCC (25.4% cases). Similar results were noted by Hardwick et al, Gowryshanker et al, Wei et al, Schoppmann et al and Cancer Genome Atlas Research, 2017.[22,23,24,25,26] Over-expression of HER2 on IHC and its increased expression in EC indicates its use as a tumour marker. Also expression of HER2 is stronger in poorly differentiated tumours. Its increased expression in EAC also indicates the use of targeted therapy against HER2 as a possible treatment option.

In case of dysplasia, expression was seen in 21% cases (both low grade and high grade dysplasia included). The results were similar to Almhanna et al and Fassan et al but in contrast to Rossi et al where expression was seen in much higher percentage of cases (54%).[27,28,29] This could be due to much higher number of high grade dysplasia cases included in the referral study.

In case of BE, expression was seen in 25% cases. The results were similar to Gowryshanker et al and Almhanna et al but in contrast to Rossi et al where HER2 expression was absent in BE.[23,27,29] This could be due to difference of population genetics, difference in stage of disease, IHC techniques and interpretation.

EGFR IHC- In study the expression was seen in 17.5% cases of EC. The expression was more in case of ESCC (18%) as compared to EAC (12.5%). The results are in agreement to The Cancer Genome Research, 2017 and Testa et al while in contrast to Wei et al where the expression was quiet high in EC.[26,30,24] This could be due to difference in population genetics,grade of tumour, IHC interpretation technique and difference of antibodies used.

In the current study, EGFR positivity was seen in none of cases of dysplasia and 12.5% cases of BE. The cases included in the study were not differentiated on basis of low grade or high grade dysplasia. The results are similar to study published by Cronin et al and Pretto et al. The study is however in contrast to study published by Rani et al which shows 61% positivity in BE.[31,32,33] This can be due to difference in population genetics, grade of dysplasia and difference in techniques of IHC. Since not many studies were published, any further comparison could not be made.

CONCLUSION

This study examined various markers using IHC in 100 cases of oesophageal lesions covering its various spectrums. It showed that EC is a disease of the middle to elderly age group, sparing the children and young adults, affecting males more than females. The three markers (P53, HER2, EGFR), show a strong association with EC and premalignant lesions, the absence of their expression in normal oesophageal tissue and non-neoplastic conditions, points out their role in development of EC and tumour progression. Out of the three markers, P53 has the strongest association with EC. HER2 over-expression was more in case of EAC than ESCC, indicating its importance in guiding further treatment and use of anti- her2 antibodies. EGFR over- expression was although in fewer cases; but the expression was more in case of higher tumour grade as compared to lower grade.

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