Original Article

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Pathology Section

Significance of HER2/neu Expression in Oesophageal Carcinomas and its Association with the Histopathological Grading

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ABSTRACT

Introduction: Oesophageal carcinomas are one of the most aggressive human malignancies which are associated with a poor prognosis because most of the cases are in stage 2 or 3, at the time of diagnosis with a high frequency of lymph node metastases. It is important to know the prognostic factors can help us on therapeutic decisions and improve the survival of these patients. A member of Epidermal Growth Factor Receptor (EGFR) family, Human Epidermal Growth Factor Receptor 2 (HER-2/neu) is a very useful antigenic marker expressed in oesophageal carcinomas, which has increasing evidence of therapeutic significance.

Aim: To determine the immunological expression of HER2/neu in oesophageal carcinomas and associate it with the histopathological grading.

Materials and Methods: This cross-sectional study was conducted in Department of Pathology at Coimbatore Medical College, Coimbatore, Tamil Nadu, India, from January 2015 to February 2016. Total 30 cases of histologically proven oesophageal carcinomas were subjected for HER2/neu immunoexpression. Membranous staining was considered as positive and the intensity of staining was scored and compared with various histopathological parameters. The p-value <0.05 using a two-tailed test was taken as level of significance for all statistical tests.

Results: Among 30 cases, 24 were squamous cell carcinomas and six were adenocarcinomas. Out of 24 cases of squamous cell carcinomas, there were 20 males and four females. All the six adenocarcinoma cases were males. HER2/neu was positive in 10 cases (41.6%) of squamous cell carcinoma and 4 cases (66.6%) of adenocarcinoma. It was seen in 10% of well-differentiated, 60% of moderately differentiated and 80% of poorly differentiated carcinomas. There was significant correlation with staging and lymph node metastases. Higher grade tumours had higher level of expression of HER2/neu.

Conclusion: The HER2/neu immunoexpression was significantly higher with progression of tumour grade. Hence, such patients with high grade oesophageal carcinomas and with lymph node metastases could be benefitted with targeted therapy.

Keywords: Adenocarcinoma, Human epidermal growth factor receptor 2, Membranous staining, Squamous cell carcinoma

INTRODUCTION

Oesophageal carcinoma is the sixth most common cause of mortality due to cancer in the world. The two major histological types of oesophageal carcinomas are Squamous Cell Carcinoma (SCC) and Adenocarcinoma (ADC). However, it is the squamous cell carcinoma that predominates globally over adenocarcinoma but the frequency of occurrence of oesophageal adenocarcinoma has dramatically increased during the last few decades [1,2]. Owing to the elasticity of the oesophagus and aggressiveness of the tumour growth, oesophageal carcinomas usually proceed to the advanced stage prior to the diagnosis. The mortality rate still remains high, despite the development of advanced therapeutic modalities apart from surgical resection [3].

In this aspect, amplification of Human Epidermal Growth Factor Receptor 2/neu (HER2/neu) gene and the overexpression of the HER2/neu protein has known to occur in oesophageal carcinoma as a part from adenocarcinomas of gastric and gastro-oesophageal junction [4]. With a very dismal survival rate, the study is carried on with an idea that patients with these cancers may stand to benefit the identification of certain possible molecular targets such as HER2/neu for both prognostic and therapeutic purposes [5,6].

There are some reported cases of carcinomas which express HER2/neu having poorer prognosis and fail to respond to the conventional chemotherapy. Such tumours respond well to specific targeted therapy with HER2/neu antibody (trastuzumab), thereby, prolonging the survival of the patients [7]. Hence, the present study has been focused on the importance of expression of HER2/neu protein in the different grades of differentiation of oesophageal carcinomas and thereby, any positive result could be used for further treatment of the patient via anti-HER2/neu monoclonal antibodies (Herceptin) as a molecular targeted therapy, for the increased survival of the patients.

MATERIALS AND METHODS

This cross-sectional study was conducted in Department of Pathology at Coimbatore Medical College, Coimbatore, in Tamil Nadu, India, from January 2015 to February 2016. Ethical clearance was obtained from Government Coimbatore Medical College committee experts (dated: 15/7/2014). Total 30 oesophagectomy specimens received, were appropriately fixed in 10% neutral buffered formalin and processed for routine Haematoxylin and Eosin (H&E) staining. The H&E stained sections were observed under light microscope and the histopathological types and grades were assessed based on American Joint Committee on Cancer Staging (AJCC) manual [8]. According to this AJCC 7th edition, staging for both SCC and ADC, prognosis of the patient depends on level of tumour infiltration in the wall of oesophagus (T), Nodal metastasis (N), metastasis to other organs (M), Grading of tumour (G). But in SCC, location of the tumour in the oesophagus is also considered for staging.

Inclusion criteria: All histopathologically proven epithelial oesophageal malignancies were taken included in the study.

Exclusion criteria: Benign tumours, neuroendocrine tumours and lymphomas are excluded from the study.

The immunohistochemical technique used was a two-step indirect technique based on the antigen detection in the cells and the tissues.

The HER2/neu expression by immunohistochemistry was evaluated qualitatively and quantitatively by intensity of staining and percentage of cells showing positive expression criteria used in the Trastuzumab for GAstric cancer (ToGA) [9] trial 6 for scoring HER2 expression by Immunohistochemistry (IHC) pattern as in [Table/Fig-1] and correlated with the histological grade of the tumour [10,11]. Finally, the percentage of HER2/neu expression in the entire sample was calculated quantitatively.

HER2/neu score by IHC	Pattern of HER2/neu reactivity in surgical specimens
0 (negative)	No/membranous staining in <10% tumour cells.
1+	Faint or barely perceptible membranous reactivity in ≥10% of cancer cells; cells are reactive only in part of their membrane.
2+	Weak to moderate complete, basolateral or lateral membranous reactivity in >10% of tumour cell.
3+	Strong complete, basolateral or lateral membranous reactivity in >10% of tumour cells of cancer cells.

[Table/Fig-1]: HER2/neu expression by Immunohistochemistry (IHC) in oesophageal carcinomas.

STATISTICAL ANALYSIS

The data were reported as the mean and standard deviation or the median, depending on their distribution. The differences in quantitative variables between groups were assessed by means of the unpaired t-test. Comparsion between groups was made by the non parameteric Mann-Whitney U test. A Chi-square test was used to assess the differences in categoric variables between the groups. The p-value <0.05 using a two-tailed test was taken as being of significance for all statistical test. All datas were analysed with a Statistical Package for Social Sciences (SPSS) version 16.0. Data and results were obtained, coded and entered into Microsoft excel spread sheet and were analysed.

RESULTS

A total of 30 cases were studied, that included all oesophageal carcinomas for which surgical resection of oesophagus was done. Among 30 cases, 24 were squamous cell carcinomas and six were adenocarcinomas. Out of 24 cases of squamous cell carcinomas, there were 20 males and four females. All the six adenocarcinoma cases were males. Out of total, 16 cases (53%) had carcinoma in the middle third of the oesophagus/thoracic oesophagus extending from suprasternal notch above to diaphragm and 14 cases (47%) had involvement of lower third of the oesophagus/abdominal oesophagus extending from diaphragm to gastric cardia. The incidence of oesophageal carcinoma was high 13 cases (43%) in the age group of 51-60 years followed by 11 cases (36.6%) in 61-70 years, 3 cases (10%) in <50 years and 10% (three cases) in >70 years of age.

Histological grades assessed are as depicted in [Table/Fig-2]. Maximum number of cases were of grade II morphology. Out of 24 cases of squamous cell carcinomas, 50% of cases were grade II tumours and 13% cases were of grade III morphology. Among 6 adenocarcinoma cases, 50% cases were grade II and 33.3% cases were of grade III tumours. Immunohistochemical assessment was done and results are as depicted in [Table/Fig-3]. Out of total, 10 cases (41.6%) of squamous cell carcinoma and 4 cases (66.6%) of adenocarcinoma showed HER2/neu positivity.

Histopathological grade and HER2/neu positivity were associated and the results were as in [Table/Fig-4]. Out of 30 cases, 10 cases were grade I, 15 cases were grade II and five cases were grade III. Grade I tumours showed 10% HER2/neu positivity. Grade II tumours showed 60% HER2/neu positivity. Grade III tumours showed 80% HER2/neu positivity [Table/Fig-5-10]. The p-value was statistically significant (p-value <0.05). In the present study,

Type	Grade	n (%)
Squamous cell carcinoma (n=24)	Grade I	9 (37%)
	Grade II	12 (50%)
	Grade III	3 (13%)
Adenocarcinoma (n=6)	Grade I	1 (16.6%)
	Grade II	3 (50%)
	Grade III	2 (33.3%)

[Table/Fig-2]: Percentage of different grades of oesophageal carcinoma.

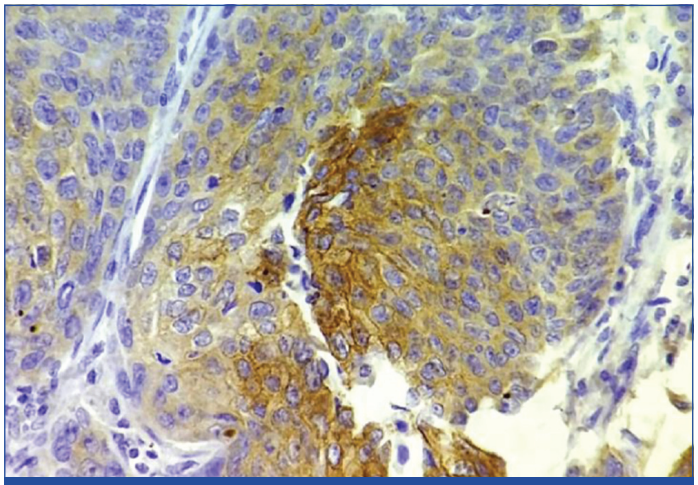
Types of carcinoma	HER2/neu expression				
	Negative (%)	+1	+2	+3	Positive (%)
Squamous cell carcinoma	58.4%	3	3	4	41.6%
Adenocarcinoma	33.4%	1	1	2	66.6%

[Table/Fig-3]: Frequency of HER2/neu expression.

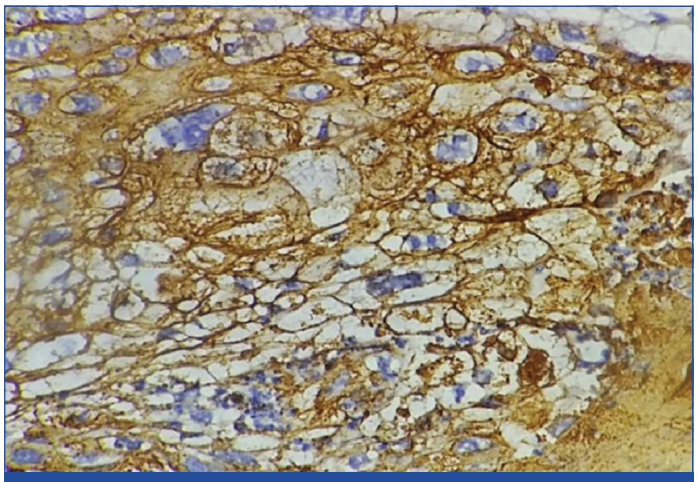
out of 15 cases with stage 2 (advanced disease with lymph node metastasis at the initial time of clinical presentation), only four cases showed HER-2/neu positivity (26.6%). Out of 15 cases with stage T3, 10 cases showed HER-2/neu positivity (66.6%) and it was statistically significant (p-value <0.05). In the present study, 11 out

Grade	HER2/neu				
	Negative (%)	+1	+2	+3	Positive (%)
Grade I (n=10)	90	1	0	0	10
Grade II (n=15)	40	3	3	3	60
Grade III (n=5)	20	0	1	3	80

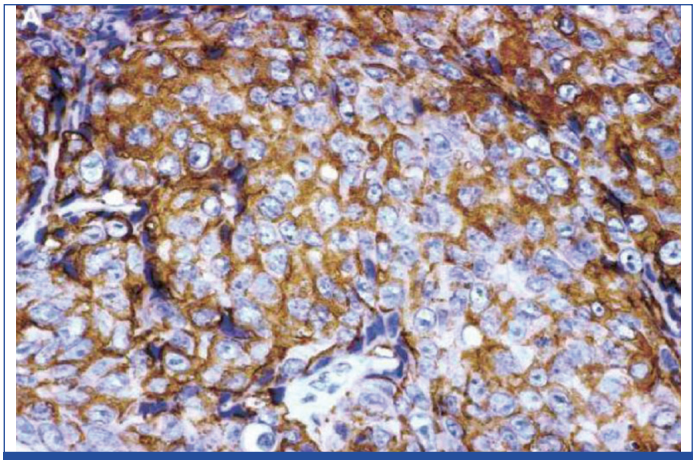
[Table/Fig-4]: Association of histological grade with HER2/neu expression grading in SCC and ADC oesophagus (N=30).



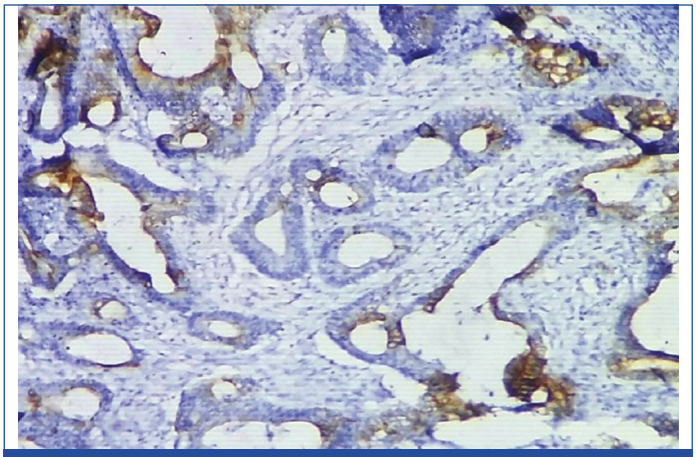
[Table/Fig-5]: Weak focal 1+ membrane positivity of HER2/neu in grade I SCC (40X, IHC).



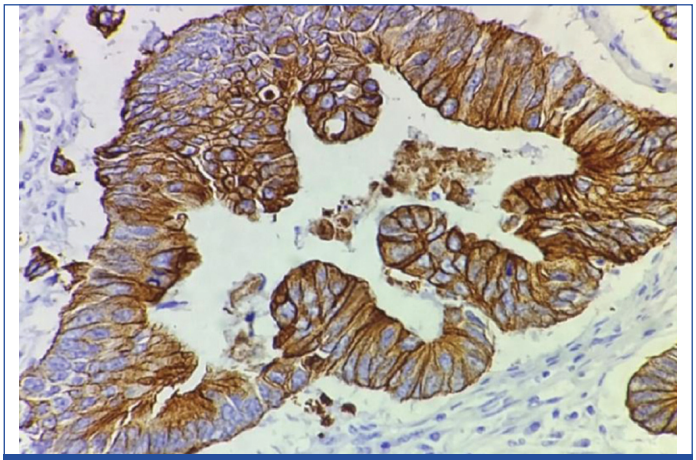
[Table/Fig-6]: Moderate diffuse 2+ HER2/neu positivity in grade II SCC (40X, IHC).



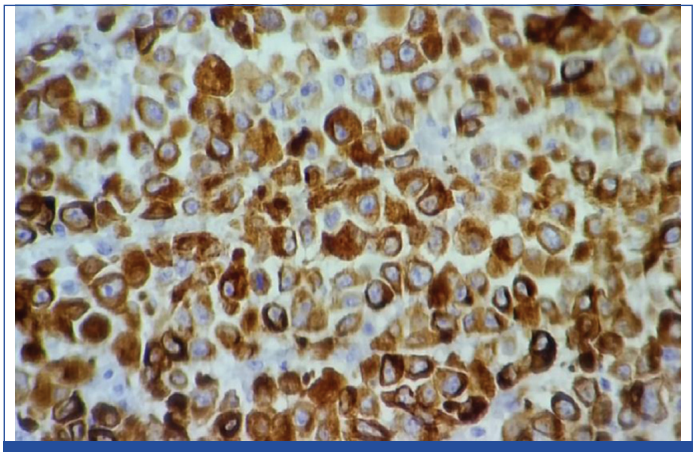
[Table/Fig-7]: Strong 3+ membranous staining in grade III SCC (40X, IHC).



[Table/Fig-8]: Weak focal 1+ HER2/neu positivity in grade I Adenocarcinoma (10X, IHC).



[Table/Fig-9]: Moderate diffuse 2+ membranous positivity of HER2/neu in grade II adenocarcinoma (40X, IHC).



[Table/Fig-10]: Diffuse strong 3+ HER2/neu staining in grade III Adenocarcinoma (40X, IHC).

of 30 cases had lymph node metastasis of which 72.7% cases showed HER2\neu positivity. Variables were statistically significant (p-value <0.05).

DISCUSSION

Oesophageal carcinoma has been rated as the sixth most frequent cause of cancer deaths worldwide. Carcinoma oesophagus is generally associated with a poor prognosis, the reason being that most of these tumours present with stage T2 or T3 with lymphnode metastasis at the initial time of clinical presentation. Thus, it is essential to know the molecular pathogenesis for targeted therapy [12].

Even though the tumour can be assessed by histopathology in terms of tumour grading and staging which are strong prognostic indicators, invention on newer prognostic markers such as expression of various immunological markers have come under lime light nowadays. The expression of these proteins was known to alter the impact of the survival rate in these patient. Regarding this aspect, there are several molecular proteins that regulate the pathogenesis of oesophageal carcinomas. One of them is the HER2/neu family of receptor tyrosine kinases which play an important role in modulating cell proliferation, cell survival and differentiation [13].

Recent ongoing trials have been successful enough to prove that HER2/neu expression has a huge significance in oesophageal carcinomas apart from a variety of human cancers such as breast cancer, colorectal cancer, gastric, lung and prostrate tumours [14]. Many strategies directed against epidermal growth factor receptor and HER2/neu were developed such as monoclonal anti-HER2/neu antibodies and small molecule kinase inhibitors. There is sufficient data from the clinical trials demonstrating the positive effects of applying epidermal growth factor receptor tyrosine kinase inhibitors to oesophageal squamous cell carcinomas and adenocarcinomas. These facts indicate that HER2/neu is a very useful molecular marker with a range of therapeutic implications in the overall survival rates of oesophageal squamous cell carcinoma and adenocarcinomas [15-18].

In the present study, the frequency of HER2/neu expression in oesophagectomy specimens and an analysis of its correlation with the histopathological grading of the tumour was done. Thirty cases of oesophageal carcinomas were included in the study and the age wise distribution was studied. Out of 30 cases, age of the patients ranges from 45 to 72 years with a mean age of 60.5 years. A similar study conducted by Reichelt U et al., showed the mean age of the patients to be 62 years ranging from 34 to 92 years and Sato-Kuwabara Y et al., showed the mean age of occurrence of 54.5 years [19,20].

In the present study, out of 24 cases of squamous cell carcinomas, 20 were males and four were females with a male:female ratio being 5:1 and all the six cases of adenocarcinoma were males. These results were in accordance with a study done by Sato-Kuwabara Y et al., which also showed a male preponderance of 4:1 for squamous cell carcinomas and 100% preponderance for males in adenocarcinomas [20].

Regarding the location of the tumour, 51% cases were in the middle third of the oesophagus and 47% cases were in the lower third of the oesophagus and none in the upper third. According to study done by Mimura K et al., out of 66 cases, 56% were in the lower third of the oesophagus, 25% cases were in the middle third and 20% cases were involving the upper third of the oesophagus [21]. In a similar study by Sato-kuwabara Y et al., 52.3% of the cases were found in the middle third, 25% in the lower third and 17% cases in the upper third of the oesophagus [20].

In the present study, 80% cases were reported as SCC. Remaining 20% cases were as adenocarcinoma. The ratio of SCC to adenocarcinoma was found to be 4:1. Among the 24 cases of

SCC, 9 cases were of grade I morphology (38%), 12 cases were grade II tumours (50%) and 3 cases were grade III tumours (12.5%). Among six cases of adenocarcinoma, one case was grade I, three cases were grade II and two cases were grade III. Similarly, study by Hardwick RH et al., 36% of the ADC cases were grade I tumours, 52% grade II and 12% cases under grade III morphology [22]. The present study was also in consistent with the results obtained from study done by Lam KY et al., where in 26% cases were grade I, 58% of the oesophageal SCC were grade II tumours and 25% belonged to grade III category [23].

The immunohistochemical expression of HER2/neu in both SCC and ADC was evaluated [Table/Fig-4-10]. and graded accordingly by new revised CAP protocol. From the results obtained, out of 24 SCC 10 cases showed HER2/neu positivity (41.6%), three cases with 1+, three cases with 2+ and four cases with 3+ score. Among six adenocarcinoma, 66.6% positivity was shown (one case with 1+, one case with 2+ and two cases with 3+ score) [Table/Fig-3]. This was similar to the study by Reichelt U et al., which showed 37% of SCC and 28% of ADC showed positive HER2/neu immunostaining [19].

In the present study, out of 30 cases grade I tumours showed 10% HER2/neu positivity, grade II tumours had 60% positivity and grade III tumours showed 80% HER2/neu positivity as shown in [Table/Fig-4]. These results were consistent with study done by Dreilich M et al., which showed 45% positivity rate for poorly differentiated oesophageal adenocarcinoma compared to 28% positivity rate with well and moderately differentiated tumours [24]. In the study by Mimura K et al., 4.5% of SCC cases had 3+ staining score, 9% cases showed 2+ scoring and 16.7% cases had 1+intensity of scoring 8% cases had 2+ score and 45% cases showed 3+ score [21]. According to these results, Chi-square analysis was done and it was found to be statistically significant (p-value <0.05). These results were similar to the study done by Mimura K et al., wherein there was 100% HER2/neu positivity in metastatic lymphnodes [21].

In present study, it was observed that 11 out of 30 cases had lymph node metastases of which 72.7% of the cases showed HER2/neu positivity and 31.5% of the cases with reactive follicular hyperplasia showed HER2/neu positivity. From this, it was concluded that the variables were statistically significant. In a second study by Dreilich M et al., HER2/neu expression was found to be correlated with the cases with lymphnode metastases [24].

The HER2/neu expression was analysed with the stage of the tumour. Four out of 15 cases of stage II tumours were positive with HER2/ neu. Whereas, 10 out of 15 cases of stage III tumours showed positive HER2/neu expression. These results were found to be statistically significant (p-value <0.05) by Chi-square analysis. This can be compared with the results from the study by Mori S et al., which showed 49% positivity with HER2/neu in stage III tumours and only 16% positivity in stage II tumours [25].

Limitation(s)

As like any research study, the present study too have some limitations. Present study sample size was 30, which eventually represents only a small population of patients with oesophageal carcinoma. A larger sample size would have given a better overall representation of the parameters of the present study. Manual immunohistochemical method authors used may have some limitations in standardisation, when compared with automated immunohistochemistry technique. Now-a-days, molecular study using fluorescent in-situ hybridisation techniques for HER2/neu expression is available which will give more promising results. Only the expression of HER2/neu in oesophageal resection specimens was seen and graded in present study, but patients were not followed-up.

CONCLUSION(S)

The HER2/neu over-expression indicate the higher grade and stage of the tumour. Such patients could be benefitted with targeted therapy such as anti-HER2/neu monoclonal antibody for a better prognosis. However, due to variability in expression by IHC method, further ancillary studies such as Fluorescent In Situ Hybridisation (FISH) for gene amplification involving larger number of patients should be used to synergise the positivity of HER2/neu expression for a greater sensitivity and to develop targeted therapy in such patients.

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REFERENCES

[1] Lepage C, Rachet B, Jooste V. Continuing rapid increase in esophageal adenocarcinoma in England and Wales. Am J Gastroenterol. 2008;103:2694-99.
[2] Thrift AP, Whiteman DC. The incidence of esophageal adenocarcinoma continues to rise: Analysis of period and birth cohort effects on recent trends. Ann Oncol. 2012;23:3155-62.
[3] Bremholm L, Funch-Jensen P, Eriksen J. Barrett's esophagus. Diagnosis, follow-up and treatment. Dan Med J. 2012;59:C4499.
[4] Wang S, Zheng G, Chen L. Effect of HER-2/neu over-expression on prognosis in gastric cancer: A metaanalysis. Asian Pac J Cancer Prev. 2011;12:1417-23.
[5] Picardo SL, Maher SG, O'Sullivan JN. Barrett's to oesophageal cancer sequence: A model of inflammatorydriven upper gastrointestinal cancer. Dig Surg. 2012;29:251-60.
[6] Abbes Belkhiri, Wael El-Rifai. Advances in targeted therapies and new promising targets in esophageal cancer. Oncotarget. 2015;6(3):1348-58.
[7] Bang YJ, Van Cutsem, E Feyereislova A. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): A phase 3, open-label, randomised controlled trial. Lancet. 2010;376:687-97.
[8] Berry MF. Esophageal cancer: Staging system and guidelines for staging and treatment. J Thorac Dis. 2014;6(Suppl 3):S289-97. Doi: 10.3978/j.issn.2072-1439.2014.03.11.
[9] Cutsem EV, Bang YJ, Feng-Yi F, Xu JM, Lee KW, Jiao SC, et al. HER2 screening data from ToGA: Targeting HER2 in gastric and gastroesophageal junction cancer. Gastric Cancer. 2015;18(3):476-84.
[10] Barrett C, Magee HO, Toole D. Amplification of the HER2 gene in breast cancers testing 2+ weak positive by HercepTest immunohistochemistry: False-positive or false-negative immunohistochemistry? J ClinPathol. 2007;60:690-93.
[11] Bartly AN, Washington MK, Ventura CB, Nofisat Ismaila N, Colasacco C, Al B Benson AB, et al. HER 2 Testing and clinical decision making in gastroesophageal adenocarcinoma: Guideline from the college of American pathologists, American society of clinical pathology, and American society of clinical oncology. Arch Pathol Lab Med. 2016;140(12):1345-63.
[12] Almhanna K, Meredith KL, Hoffe SE, Shridhar R, Coppola D. Targeting the human epidermal growth factor receptor 2 in esophageal cancer. Cancer Control. 2013;20:111-16.
[13] Kasspooles M, Moore JH, Orringer MB, Beer DG. Amplification and over-expression of the EGFR and erbB-2 genes in human esophageal adenocarcinomas. Int J Cancer. 1993;54(2):213-19.
[14] Hong S, Lee HJ, Kim SJ, Hahm KB. Connection between inflammation and carcinogenesis in gastrointestinal tract: Focus on TGF-beta signaling. World J Gastroenterol. 2010;16(17):2080-93.
[15] Miyazono K, Suzuki H, Imamura T. Regulation of TGF-beta signaling and its roles in progression of tumors. Cancer Sci. 2003;94(3):230-34.
[16] Bosset JF, Gignoux M, Triboulet JP. Chemoradiotherapy followed by surgery compared with surgery alone in squamouscell cancer of the esophagus. N Engl J Med. 1997;337:161.
[17] Izbicki JR, Hosch SB, Pichlmeier U, Rehders A, Busch C, Niendorf A, et al. Prognostic value of immunohistochemically identifiable tumor cells in lymph nodes of patients with completely resected esophageal cancer. N Engl J Med. 1997;337:1188-94.
[18] Herskovic A, Martz K, Al-Sarraf M, Leichman L, Brindle J, Vaitkevicius V, et al. Combined chemotherapy and radiotherapy compared with radiotherapy alone in patients with cancer of the esophagus. N Engl J Med. 1992;326:1593-98.
[19] Reichelt U, Duesedau P, Tsourlakis MC, Quaas A, Link BC, Schurr PG, et al. Frequent homogeneous HER-2 amplification in primary and metastatic adenocarcinoma of the esophagus. Mod Pathol. 2007;20(1):120-29.
[20] Sato-Kuwabara Y, Neves JI, Fregnani JHTG, Sallum RA, Soares FA. Evaluation of gene amplification and protein expression of HER-2/neu in esophageal squamous cell carcinoma using Fluorescence in situ Hybridization (FISH) and immunohistochemistry. BMC Cancer. 2009;9:06.
[21] Mimura K, Kono K, Hanawa M, Mitsui F, Sugai H, Miyagawa N, et al. Frequencies of HER-2/neu expression and gene amplification in patients with oesophageal squamous cell carcinoma. Br J Cancer. 2005;92(7):1253-60.

[22] Hardwick RH, Barham CP, Ozua P. Immunohistochemical detection of p53 and c-erbB-2 in oesophageal carcinoma; no correlation with prognosis. Eur J Surg Oncol. 1997;23:30-35.
[23] Lam KY, Tin L, Ma L. C-erbB-2 protein expression in oesophageal squamous epithelium from oesophageal squamous cell carcinomas, with special reference to histological grade of carcinoma and pre-invasive lesions. Eur J Surg Oncol. 1998,24(5):431-35.
[24] Dreilich M, Wanders A, Brattström D, Bergström S, Hesselius P, Wagenius G, et al. HER-2/neu expression (3+) in patients with SCC oesophagus correlates with poorer survival. Dis Esophagus. 2006;19(4):224-31.
[25] Mori S, Akiyama T, Morishita Y, Shimizu S, Sakai K, Sudoh K, et al. Light and electron microscopical demonstration of c-erbB-2 gene product like immunoreactivity in human malignant tumors. Virchows Arch B Cell Pathol Incl Mol Pathol. 1987;54:08-15.

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