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# Analytical Study of Diagnostic Role of Her2/Neu Expression In Gastroesophageal 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

Esophageal squamous cell carcinoma is one of the common types of malignant lesion and has its highest prevalence round the world. Though it is a common disorder, many of the patients only diagnose it in its advanced stage and these results in poor prognosis of the disease. In later stage, these lesions may severely develop in to lymph node metastasis and has more chances to spread across any of the vital organs. Barrett"s esophagus, herpes simplex esophagitis, candidial esophagitis, leiomyoma, hyperplastic polyps are few of the benign and premalignant type of lesions. The advancement in endoscopy has opened many opportunities and diagnosis of all esophageal lesions became simple and effective. As the surgical procedures associated with the targeted therapy have become promising option for the treatment, many patients are being benefited out of technological advancement. The surplus of malignancies such as esophagus, esophago- gastric junction and stomach have become a significant health problem and threatening the people all over the world. Delayed diagnosis on these disorders particularly resulting in high mortality ratio and warrant for a systematic evaluation in patients. In this context, the present study analyse the association and prognostic significance of HER2/ neu in gastroesophageal lesions with other factors like age, size, histological type and grading and the incidence and distribution of gastroesophageal lesions in patients.

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*Keywords: Esophageal lesions; metastasis; HER2/ neu; squamous.*

## 1. INTRODUCTION

Due to delayed diagnosis, the malignancies related to esophagus, esophago- gastric junction and stomach has become threatening health problem worlwide and resulting in a high mortality [1]. Oesophageal cancer has more prevalent among the people and remained to the 8 $^{th}$ most prevalent cancer type across the world that accounts for approximately 3.2% cancer cases and sixth cause for cancer associated deaths globally. Despite several subtypes, adenocarcinoma and squamous cell carcinoma are usually more prevalent among the patients [2-3]. The variation pattern for its prevalence may vary with the geography, economic, gender and age of the patients. Even a huge difference, nearly 60 fold was observed between the higher and lower incidence regions and Asia, Southern and Eastern Africa and Northern France are usually considered to be higher prevalent regions. A significant gender predominance was also recorded and the disease has its higher spread in men than women (2. 4:1) [ 4].

Stomach cancer accounts for fifth most prevalent malignancy worldwide (6.8% of the total cases) followed by lung, breast, colo rectum and prostate cancers. It is the third most leading cause for cancers associated mortality in men and women (8. 8% of the total cases) [2,5]. Age related association was also found in many parts of the world; the men have about two fold risk than women. The prevalence was also changed with the continents; in Western Africa, the ratio between men and women was 3.3 only but, in Eastern Asia it approached nearly 35.4 fold [2].

Most of the cases were presented with lymph node metastasis in advanced stage and these delayed prognoses frequently make the treatment options in to a difficult one. The prognostic factors play an important role in early diagnosis and use to plan the treatment regime and increase the patient's survivability. The overall survival and prognosis mainly depends on the clinical stage of the disease. Continuous efforts are being taken for identifying the specific biological markers that facilitate the diagnosis methods in early stage for differentiating the malignant and premalignant conditions to improve target therapy. The specific cancer markers such as HER 2/neu, E-cadherin, EGFR, p53 are presently used to assess the stages of gastro esophageal lesions [6–8].

HER2/ neu is a oncogene that encodes 185KD transmembrane tyrosine kinase receptor protein from chromosome 17q21. The HER2/ neu is homologous to epidermal growth factor receptor and regulates many signaling pathways in cell proliferation, differentiation and cellular survival via different biochemical networks including RAS - MAP Kinase and P13 Kinase AKT etc., [9-10]. Recent prognosis developments in Her2/neu expression help to identify many drug candidates for treatment of for gastric carcinoma treatment [11].

In this study, expression pattern of HER/neu in premalignant and malignant gastroesophageal lesions was analysed using the principle of Immunohistochemistry and its prognostic significance and association with other significant factors is being studied.

## 2. MATERIALS AND METHODS

The patients with upper GI symptom enrolled to the department of Gastroenterology and the department of surgery and were studied and further they were evaluated in the department of Pathology, Sree Balaji Medical College and Hospital (SBMCH) from Oct. 2015 till Sep. 2017. 470 cases received were gastroesophageal lesion specimens. Of the total 470 specimens, 455 were biopsy specimens and 15 were gastrectomy specimens. Upper Gastrointestinal endoscopy was performed with C V 170 Olympus video endoscopy system with biopsy forceps. Of these 470 cases, 105 cases were included in the current study, 22 were benign and 83 were malignant cases.

### 2.1 Source of Data

Endoscopic biopsy specimen from the department of gastroenterology as well as resected specimens from the department of surgery of gastroesophageal lesions at SBMCH were received at the department of pathology, SBMCH Chennai during the period of October 2015 till September 2017.

### 2.2 Inclusion and Exclusion Criteria

All malignant patients reported for both endoscopic biopsies and gastroesophageal lesions were included for the study. There was no age or gender based limitation for study

inclusion. All benign lesions were excluded.

### 2.3 Method of Data Collection

Histopathological study was carried out with H&E stained sections and IHC using HER2/neu marker. The specimens were submerged in 10% buffered neutral formalin and dehydrated through the series of graded absolute alcohol (50%, 70%, 80%). They were washed with xylene and embedded using the paraffin. The specimen was cut as 4 micrometer section each after dewaxing. Then they were stained for H&E. They were immune histochemically analysed for HER2/ Neuexpression.

The specimens were analysed using polyexcel HRP/DAB Detection based non - biotin polymeric technology. The sections were transferred onto gelatin coated slides and the antigens were retrieved by heat induction method. Then the antigen was bound with rabbit monoclonal antibody (against HER - 2/Neu proteins) and secondary antibodies conjugated with horse radish peroxidase polymer were added.

### 2.4 Interpretation and Scoring

The immunohistochemically stained slides were observed for the biochemical reaction, cellular localization, percentage of stained cells and stain intensity using the previous method.

### 2.5 Data entry and Statistical analysis

STATA 14 and Microsoft excel (2007) was used for statistical analysis and the results were expressed in percentage and frequency p (>0.05) was taken as cut -off to determine statistical significance.

## 3. RESULTS

### 3.1 Immunohistochemical HER2 analysis in Gastric Lesions

Of the 105 cases included in the study, 36 cases of gastric pathology including adenocarcinoma, neuroendocrine tumour and chronic gastritis with dysplasia were submitted for IHC staining. 13 cases were resected specimens and 23 cases were endoscopic biopsies Table 10, chart 9 ,Fig. 1.

## 4. DISCUSSION

Oesophagus, oesophago gastric junction and stomach related malignancies present a severe health crisis and higher mortality ratio possibly owing to a significant delay in diagnosis. It has relatively low five year survival rate (up to 19%) compared to other cancer types [12]. The majority of the stomach cancer patients were diagnosed in advanced stage because of vague, non-specific signs. The early diagnosis (if in Stage IA) had shown to increase the five year survival ship up to 71% and lateral diagnosis (stage IV disease) resulted in 4% only [4]. The advancements in molecular biology and genomics brought many hallmarks in diagnosis, treatment and clinical outcome. Her2/ neu expression is one of the novel biomarker for the treatment of the gastroesophageal lesions.

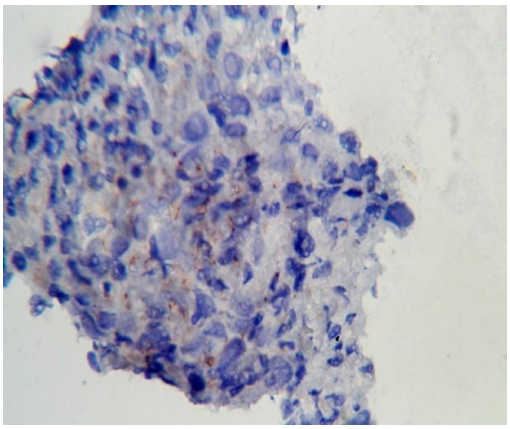
In the current study, a total of 105 cases with gastroesophageal pathology were included out of which 70 random cases were selected for immunohistochemical evaluation for HER2/ neu antibody and various morpholo gical and histopathological factors were correlated.

Indu Rajagopal et al [13] studied 60 cases of gastric adenocarcinoma based on biopsies and resected specimens and found that the HER2 overexpression had occurred in approximately 22.4% cases. Their study showed a statistically significant correlation between the HER 2 overexpression and gender, particularly for men. In addition to, HER2 was also correlated with intestinal type and adenogastric carcinoma differentiation. Dewan et al [14] conducted a study in 100 cases of gastric adenocarcinoma over 2 year period and revealed 17% of patients with well differentiated tumor morphology showed overexpression of HER2/neu proteins. Kim et al [15] conducted a case study among 114 patients and concluded that HER2/neu over expression was seen in 9% cases. In contrast, they did not find any correlation with gender and age of the patients. Meantime, they showed that HER2 expression was associated with moderately differentiated carcinoma cells without any significance. Raziee et al [16] studied 100 cases of gastric carcinoma in Iran and found a significant association of HER - 2/Neu ( 26%) over expression with grade differentiation in Lauren's tumor. Further, in contrast with above mentioned, they had stated that their study did not reveal any association of HER2 expression with age, gender, location of tumor and depth of infiltration in patients. Taghizadeh Kermani et al [17] also showed a direct association oh HER2 expression with tumor grades but, not with gender, age, tumor invasion and location of tumor. Drelich et al [18] showed that 13%

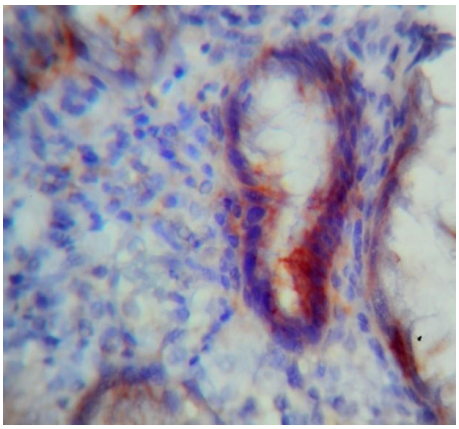
esophageal squamous cell carcinoma and 30% adenocarcinoma esophagus patients had direct association with the over expression of showed over expression HER 2/neu genes. Their study was conducted in 70 cases and concluded that the HER2 overexpression was directly associated with the poor survival rate in esophageal carcinoma patients. Ashwini et al [19] and Ross et al [20] stated that HER2 overexpression could be considered as a strong biomarker in Barretts esophagus patients for survival rate determination. Sato- Kuwabara et al [19] conducted IHC study on HER2 in 185 cases of ESCC and found that 36.8% of patients positive correlation with the tumor grading. 6. 5% cases in their study showed no direct association with clinicopathological data and overall survival rate with HER2 expression. Elisa Rossi et al [21,22,23] concluded that the HER2 overexpression could be used to predict the early transition from dysplasia to adenocarcinoma in Barrets esophagus patients. Further, they showed that 38% studied cases showed the positivity with HER2 overexpression.

**Table 1. Immunohistochemical evaluation**

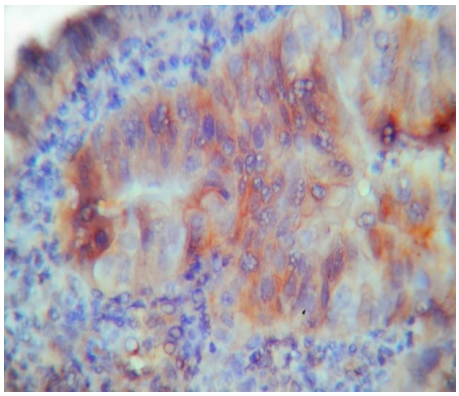
| Antigen | Vendor | Species (clone) | Dilution | Positive Control |
|---|---|---|---|---|
| HER-2/Neu | PathnSitu | Rabbit Monoclonal | Ready to use | Breast ca |



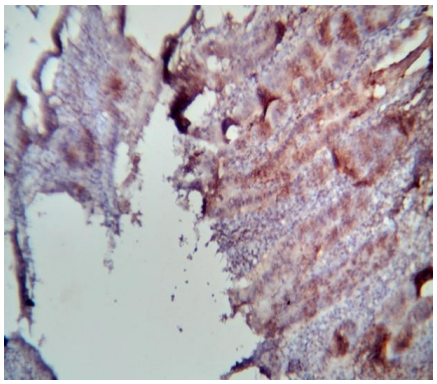
**Grade 0- No staining in all cancer cells**



**Grade 1- The effect of membranous reactivity**



**Grade 2: >10% cancer cells showed weak to moderate membranous reactivity**



**Grade 3-Strong complete, basolateral membranous reactivity was observed >10% cancer cells**

**Fig. 1. HER2/neu IHC grading**

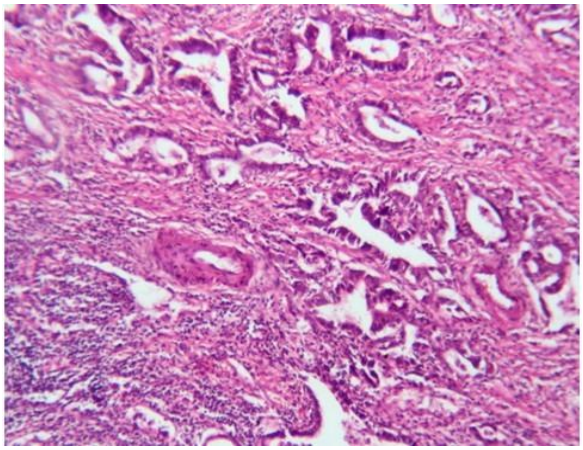


**Fig. 2a. Well differentiated gastric adenocarcinoma**

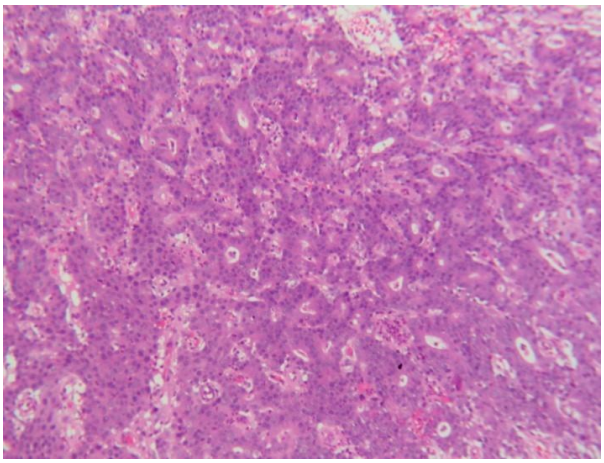


**Fig. 2b. Moderately Differentiated gastric adenocarcinoma**

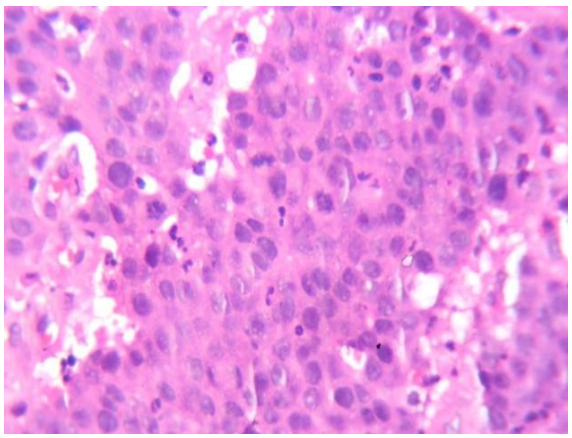


**Fig. 2c. Poorly differentiated gastric adenocarcinoma**

**Fig. 2. astric Adenocarcinoma histological grading**

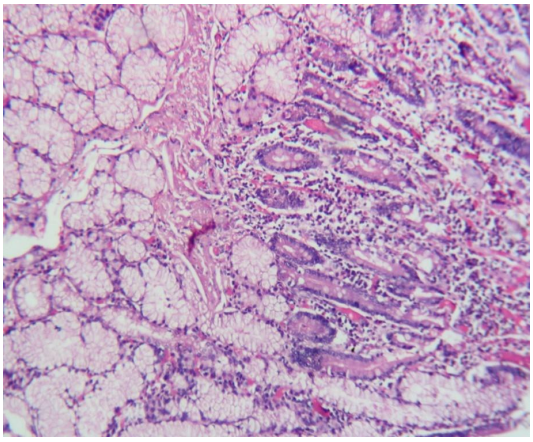


**Fig. 3a. Gastric adenocarcinoma Intestinal**

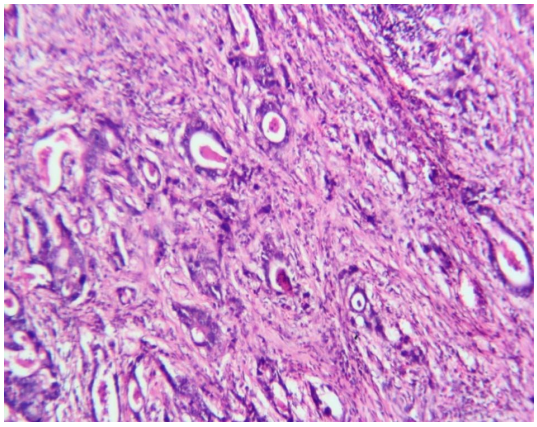


**Fig. 3b. Gastric adenocarcinoma Diffuse type**

**Fig. 3. Gastric adenocarcinoma Lauren's classification**

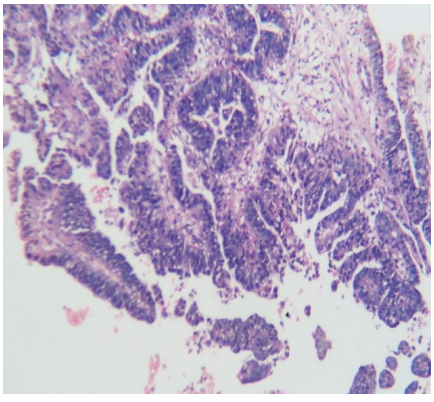


**Fig. 4a. Gastric adenocarcinoma, papillary variant: the anastomosing fibrovascular cores lined by neoplastic cells are characteristic of this type**

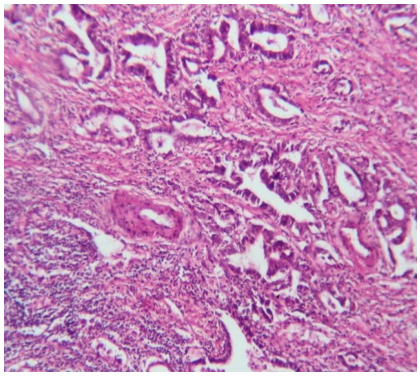


**Fig. 4b. Gastric adenocarcinoma, tubular variant: the neoplasm is composed of irregularly shaped tubules lined by columnar neoplastic cells**

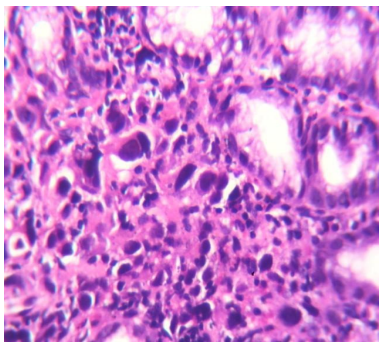


**Fig. 4c. Poorly cohesive gastric carcinoma, cells have a plasmacytoid appearance**

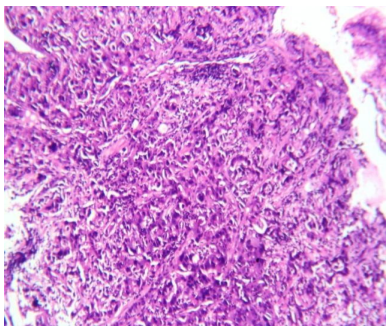


**Fig. 4d. Mixed gastric adenocarcinoma: the neoplasm is composed of tubular and poorly cohesive components**

**Fig. 4. WHO classification gastric carcinoma**

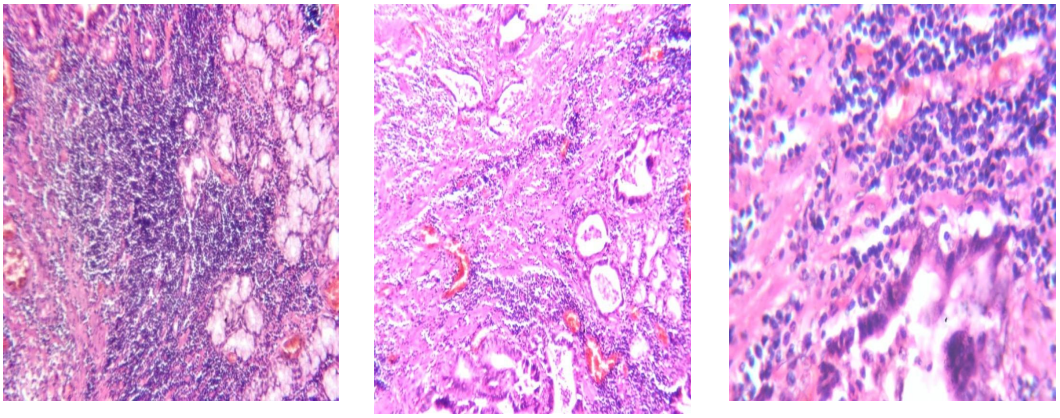


**Fig. 5a, 5b, 5c: Gastric carcinoma with lymphoid stroma: neoplasm is characterized with polygonal epithelial cells (solid entities) and associated with the lymphocytic infiltrate.**

**Fig. 5. Lymphocytic response gastric adenocarcinoma**

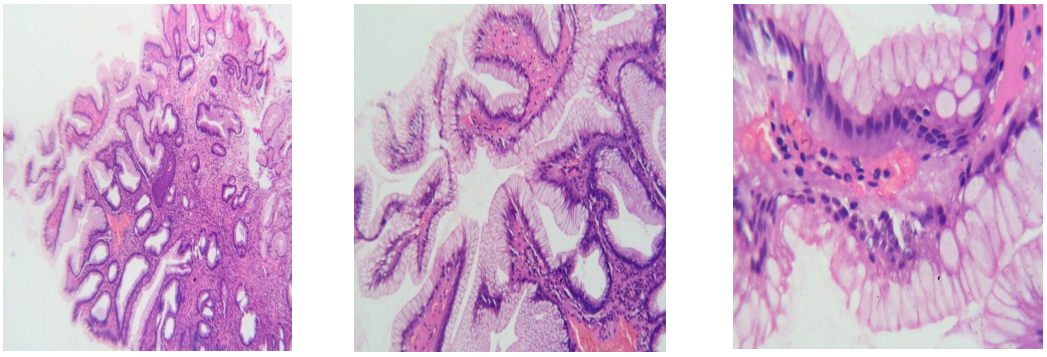


**Fig. 6a, 6b, 6c. Barrett's esophagus,columnar cell lined esophageal mucosa and goblet cells seen**

**Fig. 6. Barrett's esophagus**

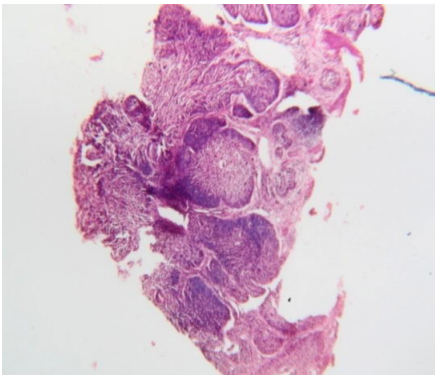


**Fig. 7a. Moderately differentiated SCC, scanner view**

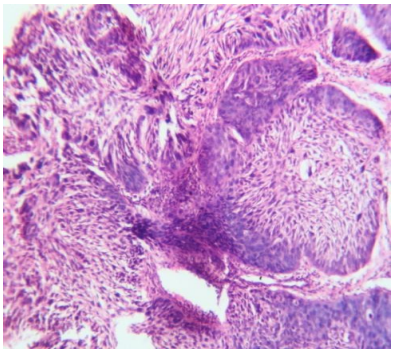


**Fig. 7b. Moderately differentiated SCC, low power view**

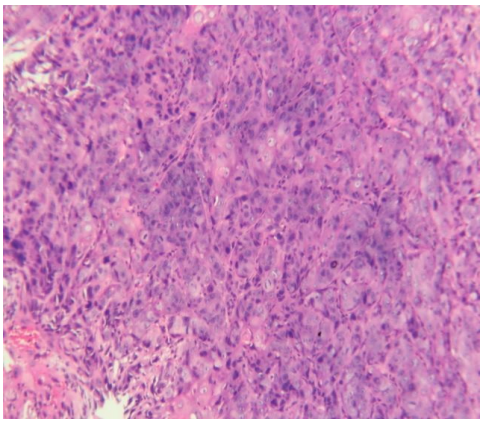


**Fig. 7c. Poorly differentiated SCC,10x view**

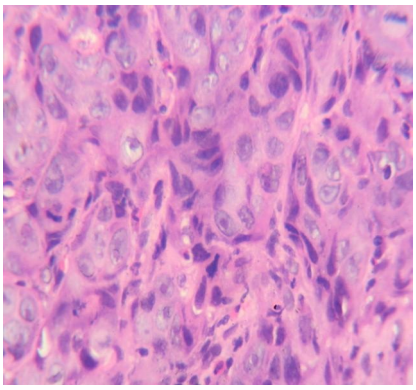
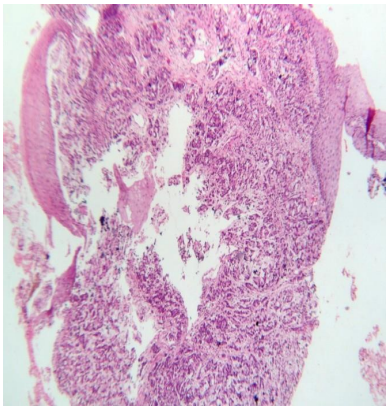


**Fig. 7d. Poorly differentiated SCC,40x view**

**Fig. 7. Squamous cell carcinoma esophagus with histologic grading**



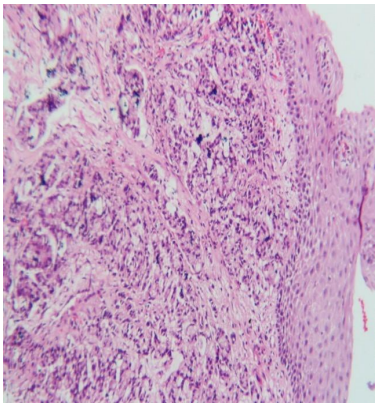


**Fig. 8a & 8b. Infiltrating adenocarcinoma esophagus**

**Fig. 8. Adenocarcinoma Esophagus Adenocarcinoma esophagus**

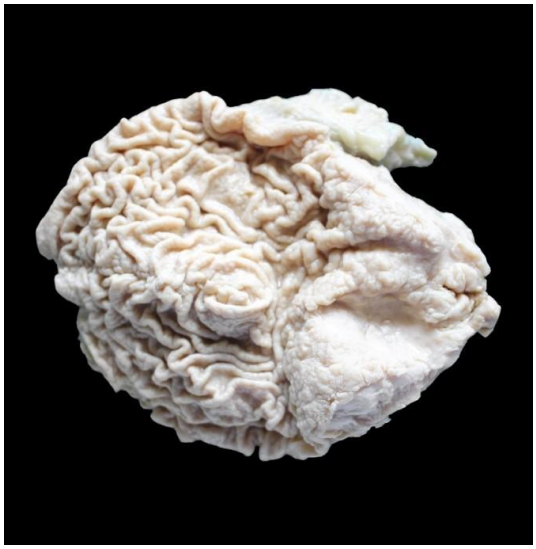


**Fig. 9a. Ulceroproliferative growth gastric carcinoma**

**Fig. 9b. Infiltrative growth gastric carcinoma**

**Fig. 9. Gross images gastric carcinoma**

In the present study, HER 2/ neu over expression was found in 23.3% cases of gastric adenocarcinoma. Our study also showed that the male preponderance nature of the Her2/ neu over expression. In addition, the majority of the cases showed specific expression of HER2/neu over expression at antropyloric region. The HER2 over expression was frequently found in Lauren's intestinal type and moderately differentiated graded tumors. We also found that tumor invasion upto T4 (serosa) showed significant HER2/ neu over expression. In the present there was no statistically significant association between HER2/neu over expression and age, gender, location, gross appearance, size and histological grading.

Current study showed that HER2/ neu over expression in 20% and 13.3% of cases were associated with Barrett's esophagus and esophageal squamous cell carcinoma respectively. HER2/ neu over expression in both

tumor types showed the male preponderance. Majority of the cases were located at lower 1/3 rd of the esophagus. Meanwhile, the sample size is another limitation of the current study, A larger sample size and long term follow up of the patients could be useful in determining the role of HER2/ Neu in the diagnosis and treatment of the patients with gastroesophageal lesions.

## 5. CONCLUSION

The present study showed that Her2/neu overexpression in gastroesophageal lesions could help to determine the tumor stage in diagnosis and to plan a treatment regime in targeted therapy.

## CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

## ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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