CLINICOPATHOLOGICAL SIGNIFICANCE OF HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR- 2 (HER-2/NEU) OVER-EXPRESSION IN GASTRIC AND ESOPHAGEAL CARCINOMAS OF UPPER GASTROINTESTINAL BIOPSIES

By

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Dissertation submitted to the

B.LD.E. University, Vijayapura, Karnataka

In partial fulfillment of the requirements for the award of the degree of

DOCTOR OF MEDICINE

IN

PATHOLOGY

Under the Guidance of

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LIST OF ABBREVATIONS USED

Gastrointestinal tract	GIT
Duodenum part 1	D1
Duodenum part 2	D2
Tyrosine kinase	TK
Epidermal growth factor receptor	EGFR
Human epidermal growth factor receptor 2	HER-2/neu
Gastro-esophageal	GE
Gastroesophageal junction	GEJ
Lower esophageal sphincter	LES
Squamo-columnar junction	SCJ
Confocal laser endo-microscopy	CLE
Fluorescent in situ hybridization	FISH
Esophagogastroduodenoscopy	EGD
Endoscopic ultrasonography	EUS
Endoscopic mucosal resection	EMR
Tumor growth factor	TGF

Immunohistochemistry	IHC
Androgen receptor	AR
Cytokeratin 20	CK20
Cytokeratin 7	CK7
Mucin core peptide cores	MUC
Glycoprotein all together known as receptor tyrosine kinases	RTKS
Flouroscent in situ hybridization	FISH
Chromoatographic in situ hybridization	CISH
Monoclonal antibodies	mAb
Extra cellular domain	ECD
Cyclin dependent kinase	CDK
Antibody-dependent cell-mediated cytotoxicity	ADCC
Trastuzumab for Gastric Cancer	ToGA
Neutral buffered formalin	NBF
Transitional cell carcinoma	TCC
Di-amine-Benzidine	DAB

Squamous cell carcinoma	SCC
Adecocarcinoma	AC

ABSTRACT

INTRODUCTION

HER-2/neu plays a key role in the pathogenesis of gastric and esophageal carcinomas and it's over expression has been documented in 6.8–34% of gastric carcinomas and 10-12.1% of oesophageal adenocarcinoma. Detecting the HER-2/neu status is a prerequisite for monoclonal antibody therapy. In this study, immunohistochemistry was used to detect HER-2/neu over- expression in gastric and esophageal carcinomas.

OBJECTIVE

To associate HER-2/neu over-expression with age, sex, type and grade of gastric and esophageal carcinomas in upper gastrointestinal (UGI) endoscopic biopsies.

MATERIALS AND METHODS

HER-2/neu expression was investigated by immunohistochemistry on 107 esophageal and gastric carcinomas of UGI endoscopic biopsies received at our institution. Association between the expression of HER-2/neu and clinico-pathological parameters was statistically analysed.

RESULTS

The association was not statistically significant between age, sex and grade of the tumour with HER-2/neu over-expression. HER-2/neu over-expression was seen in 14.2% of gastric adenocarcinomas, 20% of esophageal adenocarcinomas and 4% of esophageal squamous cell carcinomas. Predominantly, intestinal type (9.5%) of gastric carcinoma showed HER-2/neu over-expression followed by diffuse type (2.3%) and mixed type (2.3%).

CONCLUSION

In view of increasing trend of UGI tract malignancies and associated poor survival of advanced carcinomas, assessing HER-2/neu over expression in gastric and esophageal carcinomas is helpful to decide the utility of adjuvant targeted chemotherapy.

KEY WORDS

Adenocarcinoma, endoscopic biopsy, HER-2/neu, immunohistochemistry.

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INTRODUCTION

Gastrointestinal tract (GIT) is a very dynamic organ system. It serves the function of a digestive, immunological and endocrine organ system as well. GIT acts as an interface between the outside world and the rest of the body. Hence, GIT is most likely to present a wide spectrum of pathological changes varying from mild infections to non-neoplastic lesions to malignant carcinomas.

Gastric carcinoma is the second leading cause of mortality around the world,[1] whereas there is a quick increase in the incidence of esophageal carcinoma compared to any other malignancy.[2] Despite the trend for decreasing incidence, gastric adenocarcinoma is still the second cause of cancer death worldwide.[3] The 5 year survival of gastric and esophageal carcinomas ranges between 5-10% due to the advanced stage at presentation. The diagnosis of gastric and esophageal carcinoma is based on histopathological confirmation.[4]

With the advent of endoscopes and endoscopic biopsies, upper gastrointestinal endoscopy helps to evaluate various diseases of esophagus, stomach, duodenum part 1 (D1) and duodenum part 2 (D2) helping in early diagnosis and intervention.

Due to advanced stage of the disease during presentation, gastric and esophageal carcinomas exhibit poor prognosis. Hence, the screening of high risk patients with endoscopic evaluation and biopsy as and when required plays a major role in detecting the cancer at an early stage.

Due to the unavailability of effective medical agents, there is a high recurrence rate and ultimately low survival rate with poor prognosis in patients with upper gastrointestinal carcinomas.

Excellent long-term survival is now possible due to advances in diagnosis and treatment; however, poor prognosis still remains the corner stone of advanced cancer.[5]

HER- 2/ neu (E-erbB2), a proto-oncogene, is a member of the EGFR family with intrinsic protein tyrosine kinase (TK) activity. TK receptors control cell survival, invasion, metastasis and angiogenesis.[5]

Current targeted therapy for advanced gastric and esophageal carcinoma depends on the evaluation of target gene status. Human epidermal growth factor receptor 2 (HER-2/neu) plays an important role in activation of HER-2 protein, and its overexpression has been documented in 6.8–34% of gastric carcinomas including gastroesophageal junctional tumors.[6] HER-2/neu positivity status plays a critical role in the development, progression and metastasis of malignancies such as breast cancer & gastric cancer.[2]

Various studies have documented that overexpression of HER- 2/ neu is widely seen in breast carcinomas. The targeted monoclonal antibody – Trastuzumab (Herceptin) has achieved great success in tumor treatment.[7]

Detecting the HER-2/neu status in gastric and esophageal carcinoma is a prerequisite of monoclonal antibody therapy.[1] A combination of monoclonal antibody against HER-2/neu (Trastuzumab) with standard chemotherapy improves significant survival rates in patients with HER-2/neu positive advanced gastric and esophageal cancers.[2]

Hence, overexpression of HER- 2/ neu plays an important role in cancer development and progression. However, this has not been documented extensively in literature. Under the light of this knowledge, the present study aims at investigating the frequency and the clinicopathological significance of overexpression of HER- 2/ neu in gastric and esophageal carcinomas.

OBJECTIVE OF THE STUDY

To correlate HER-2/neu over-expression with the type and grade of esophageal and gastric carcinomas in upper gastrointestinal endoscopic biopsy specimens.

REVIEW OF LITERATURE

Embryology

The formation of primitive gut occurs in the 4th week of gestation. At this time, the embryo folds and incorporates the dorsal part of the yolk sac. The epithelial lining of majority of the digestive tract is derived from the endoderm of the primitive gut, except for the cranial and caudal ends which are ectodermal in origin. The muscular and connective tissue components of the GIT are derived from the splanchnic mesenchyme surrounding the primitive gut. Multiple cell types are acquired during the development and they divide in vertical and horizontal planes. Vertical plane allows to identify the different layers of the gut wall while, horizontal plane develops into the esophagus, the stomach, the small intestine, the colon and the anus. [8]

The epithelial lining gradually develops from simple columnar epithelium to well differentiated adult type of epithelium. Distinct histological features allow specific physiological functions to be carried out by each anatomic region.[9]

For the purpose of endoscopy, the GIT is divided into **(Fig 1)**:

1. Upper gastrointestinal endoscopy (esophagus, stomach and duodenum)
2. Lower gastrointestinal endoscopy (anus, rectum and colon)

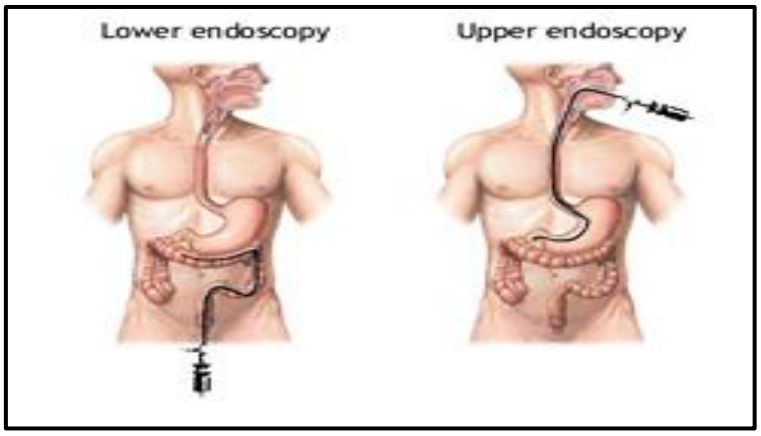

Fig. 1: Schematic representation of lower and upper GI endoscopy

Anatomy and Histology

Esophagus:

The esophagus is a strong muscular tube that conveys food from the oropharynx to the stomach. Swallowing is initiated as a voluntary act involving the skeletal muscles of the oropharynx. This is then followed by a strong peristaltic reflex, conveying the bolus of food or fluid to the stomach.[10]

Esophagus is a hollow, distensible, muscular tube, extending from the cricopharyngeal muscle up to the gastroesophageal junction.[9] In a normal adult, the esophagus measures, approximately 25 – 35 cm long in a normal adult individual. For the endoscopist, the esophagus starts at 15 cm from the incisor teeth and ends at the Gastroesophageal junction (GEJ). At the lower end it forms the lower esophageal sphincter (LES) which helps in preventing the reflux of gastric contents at rest and regulates the passage of food into the stomach.[11]

The wall of esophagus has four layers:[9]

1. Mucosa – it is subdivided into three layers as stratified non-keratinizing squamous lining, lamina propria and muscularis mucosa. Lamina propria is narrow and is made up of loose connective tissue, lymphoid aggregates and distally mucosal glands (cardiac glands).[9, 10] Esophageal muscularis mucosa is comparatively thicker than rest of the gastrointestinal tract.
2. Submucosa – It contains seruminous glands opening into the lumen of esophagus, lymphoid follicles, Meissner's plexus, lymphatics and blood vessels.
3. Muscularis propria – It contains a mixture of striated and smooth muscle in upper quarter and only smooth muscle in rest of the esophagus. Auerbach or myenteric plexus are also seen in this layer. The muscularis propria can be

reduplicated in Barrett esophagus; which is important to know while staging adenocarcinoma arising from Barrett esophagus.

4. Serosa – It is the outermost layer made up of connective tissue. It is absent in most of the esophagus except in the distal most portion.

Gastro-esophageal junction (GEJ)

The most distal portion of LES is the GEJ. Anatomic and endoscopic definition of LES is the 'point of flaring' of tubular esophagus, the proximal limit of the gastric rugal folds, and the site of LES. Hence, true GEJ does not necessarily correspond to the squamo-columnar junction (SCJ) or 'Z' line.[9, 10]

Stomach

The stomach is also called the gaster (Greek *belly)* or venter**.** It is a 'J' shaped organ which extends from the lower end of esophagus (Z line), crosses midline and ends into the duodenum. It is a muscular bag forming the most distensible part of the digestive tube. It is grossly divided into 5 anatomical regions:[9]

- Cardia
- Fundus
- Body (corpus)
- Antrum
- Pylorus

The superior-medial margin forms lesser curvature and inferolateral margin forms greater curvature. Incisura is a notch on the serosal aspect of lesser curvature at the site of the junction of antrum and pylorus. The mucosa is thrown into folds called rugae.

The wall of stomach consists of 4 layers; [12,13]

1. Mucosa- it protects against auto digestion. It has 2 components;
    a. Superficial foveolar component – The mucosal surface is lined by epithelial / foveolar cells.
    b. Deep glandular component – It consists of gastric glands in varying composition and thickness at different anatomic regions.
        - Cardiac mucosa (junctional mucosa) - It consists of branching mucous glands without parietal cells.
        - Fundic mucosa (oxyntic mucosa) - It consists of straight glands. Chief cells, parietal cells, endocrine cells and mucus cells are arranged tightly one next to the other. It has a higher glands: foveolae ratio than that of antral mucosa.
            - Chief / Zymogenic cells: Due to increased number of endoplasmic reticulum, these cells display a deeply basophilic cytoplasm. They release pepsinogen I and II which require low pH for activation into pepsin.
            - Parietal / Oxyntic cells: They have eosinophilic cytoplasm due to abundance of mitochondria. They produce acid via H+/K+ ATPase pump and intrinsic factor.[14]
            - Endocrine cells: They have clear cytoplasm. They produce:
                - Gastrin (G cells)
                - Serotonin (Enterochromaffin cells)
                - Somatostatin (D cells)
            - Mucous cells: They have lightly eosinophilic to clear bubbly cytoplasm. They produce neutral (PAS positive) mucin.
        - Antral mucosa- It consists of branched mucous glands.

2. Submucosa: It is composed of loose areolar tissue which contains Meissner plexus.
3. Muscularis propria: It consist of 3 layers
    a. External longitudinal layer
    b. Middle circular layer
    c. Innermost oblique layer
4. Serosa: It is a glistening peritoneal layer.

Duodenum

Duodenum is the first part of the small intestine and it encloses the head of pancreas in its concavity. It is a 'C' shaped organ, approximately 20-25 cm in length. It is divided into 4 parts.[9, 10]

1. Part I (superior part): It is approximately 5 cm long and lies anterior and superior to the head of pancreas.
2. Part II (descending part): It is approximately 7-8 cm long. On the postero-medial aspect there is the ampulla of Vater into which the pancreatic duct and common bile duct opens.
3. Part III (horizontal part): It is approximately 10 cm long. It arches transversely across aorta and vena cava.
4. Part IV (ascending part): It is approximately 2-3 cm long. It curves up to the left of the duodeno-jejunal flexure. Here it is attached by the ligament of Treitz.

The duodenum is mostly retroperitoneal and fixed, except its two ends where it is suspended by folds of peritoneum, and is, therefore, mobile. Anteriorly, the duodenum is only partly covered by the peritoneum.

Histologically duodenum is composed of 4 layers: [13, 15]

1. Mucosa: It consists of characteristic, leaf-like villi lined by tall columnar epithelium with few goblet cells; crypts of Lieberkuhn, lamina propria and muscularis mucosae.
2. Submucosa: Submucosa of the esophagus contains many extensive coiled Brunner's glands. It consists of connective tissue, blood vessels, lymphatics, Brunner's glands and neural plexus. The ducts of these glands open into the crypts.
3. Muscularis propria: It comprised of two layers:
    a. Inner circular layer
    b. Outer longitudinal layer

   Auerbach's plexus are noted between these two layers of muscularis propria.
4. Serosa: It is covered by a single layer of attenuated mesothelium, separated from the muscularis propria by a normally thin layer of connective tissue.

Endoscopy

Endoscopy provides a unique opportunity to visualize the mucosal surface of the GIT as well as a variety of extra-luminal, extra-intestinal organs and structures.[16]

Kussamal, in 1868 first examined the interior of the bowel with the help of a sword swallower. Then in 1881, Mickulicz-Redecki used an electric incandescent lamp as a source of illumination.[17] Later, Von Hacker in 1904, devised rigid endoscopes. In 1932, a new era of endoscopy began, when the semi flexible gastroscope designed by Rudolf Schindler and manufactured by George Wolf in Berlin was introduced. Hopkins and Kapany, in 1954 produced a clad glass fiber that allowed light to travel from one end of the glass fibre to the other, irrespective of the twisting and coiling of the fibre.[14] Flexible fiber optic upper GI endoscope was first

used in 1968, which proved to be a major break-through in the diagnosis of GI lesions.[18] The first fiberoptic gastroscope was developed by Basil Hirschowitz and Lawrence Curtis at the University of Michigan.[4]

Confocal laser endomicroscopy (CLE) is a new endoscopic optical technique that provides histology - like images of tissues in vivo enabling the endoscopist to take 'targeted biopsies' only if required. Though this helps in reducing the cost and risk of conventional biopsies it has its own pitfalls which limit its practical utility.[19]

Bio-endoscopy is a latest technique now known. It uses fluorescent labelled monoclonal antibodies or fluorescent DNA probes for FISH (fluorescent in situ hybridization). The reporter probe enters the cell and allows to detect in situ molecular changes or chromosomal abnormalities.[20]

Esophagogastroduodenoscopy (EGD) or upper GI endoscopy visualizes the mucosal surfaces of the esophagus, stomach and proximal duodenum. [21]

Endoscopic biopsies:

Upper GI endoscopy in combination with biopsy helps in the early diagnosis of GI malignancies.[20] It allows both diagnostic and therapeutic functions. Its importance is reflected by the increase in number of lesions diagnosed at an early stage. [22]

The ability to diagnose a disease potentially relevant to patient care, defines the efficacy of an endoscopic procedure.[23]

Though there are ongoing advances in the endoscopes and its procedure, utility of endoscopic biopsy to arrive at appropriate diagnosis remains limited to the co-ordination between endoscopist and pathologist. A critical adjunct to endoscopic assessment of the GI tract is histological examination of the biopsy specimen. Along

with the biopsy specimen the endoscopist should provide age, sex, clinical details, site of biopsy and also a copy of endoscopy report.[22]

A few aspects of sampling and processing of the biopsy specimen which should be considered by the endoscopist and the pathologist are: [20]

- Size of the forceps used for sampling should be appropriate. (forceps with a diameter of 2.00-2.5mm permits an adequate sample involving the muscularis propria.
- A point to be considered by the endoscopist and the pathologist is that if the central spike of forceps stays in the mucosa during the procedure, it induces artefactual erosion.
- There should be limited air insufflation during the procedure as over-insufflation may cause stretching of mucosa towards the underlying submucosa resulting in a superficial biopsy.
- Burrowing technique i.e. taking multiple biopsies from the same site should be used to obtain information of deeper lesions.
- Multiple biopsies with the minimum of 2 biopsies from each site are recommended but, with the advancement of newer techniques, targeted biopsies can be taken which reduces the number of biopsies required.
- In case of polypectomy, section margin should be marked and specimen should be cut along the marked area.
- Biopsy sample should be placed on a millipore filter paper in such a manner that the mucosal surface faces upwards and should be immediately immersed in the fixative.
- 10% Neutral buffered formalin (NBF) is a recommended fixative which allows analyzing immunohistochemistry as well as molecular studies.

- Fixation should be not less than 6 hours and not more than 48 hours.
- Specimen should be embedded in a way to get the exact accurate section all along the long axis of the specimen.
- 3 – 5 μm thick serial sections should be prepared
- H & E stain for all sections and special stains whenever required help in rendering accurate histopathological diagnosis.[24]

Determination of extent and severity of the lesion can be documented by assessing the biopsy. It helps in monitoring of disease with specific reference to recurrence and effect of therapy.

Diagnostic approach to interpret gastric biopsies

Acute bleeding or obstruction and the differing levels of endoscopic experience are all important factors to be considered during interpretation of endoscopic biopsies.[25]There are chances that endoscopic procedures may cause variable degrees of edema, vascular dilation, focal lamina propria hemorrhage, and surface cell flattening in a biopsy specimen. These should be distinguished from the mucosal lesions by presence or absence of epithelial degeneration and acute inflammation.

The number of endoscopic samples taken increases the diagnostic accuracy.

- Multiple biopsies from the edge of the lesion, can detect minute carcinomas less than 5 mm in diameter.

  The limitation of endoscopic biopsy lies in its inability to document invasion. Hence, other techniques can be incorporated to document invasion, such as,

- Special stains for intracytoplasmic mucin.
- Immunohistochemical stain for cytokeratin and Ki67.

Endoscopic ultrasonography (EUS) may be employed as an adjuvant to estimate the tumor stage prior to definitive therapy. This procedure yields 82% accuracy as to the depth of tumor invasion, but is less accurate in detecting lymph node metastases.[26]

Spectrum of neoplastic lesions of esophagus and stomach diagnosed on upper GI endoscopic biopsies [27,28]

I. Neoplastic lesions of esophagus

1. Epithelial tumors

a. Squamous cell papilloma

b. Intraepithelial neoplasia

i. Squamous

ii. Glandular (adenoma)

c. Carcinoma

i. Squamous cell carcinoma

ii. Verrucous (squamous) carcinoma

iii. Basaloid squamous cell carcinoma

iv. Spindle cell (squamous) carcinoma

v. Adenocarcinoma

vi. Adenosquamous carcinoma

vii. Mucoepidermoid carcinoma

viii. Adenoid cystic carcinoma

ix. Small cell carcinoma

x. Undifferentiated carcinoma

xi. Others

d. Carcinoid tumor

2. **Non-epithelial tumors**
    - **a.** Leiomyoma
    - **b.** Lipoma
    - **c.** Granular cell tumor
    - **d.** Gastrointestinal stromal tumor
        - **i.** Benign
        - **ii.** Uncertain malignant potential
        - **iii.** Malignant
    - **e.** Leiomyosarcoma
    - **f.** Rhabdomyosarcoma
    - **g.** Kaposi sarcoma
    - **h.** Malignant melanoma
    - **i.** Others

II. Neoplastic lesions of stomach

1. **Epithelial tumors**
    - Intraepithelial neoplasia – Adenoma
    - Carcinoma
        - **i.** Adenocarcinoma
            a. Intestinal type
            b. Diffuse type
        - **ii.** Papillary adenocarcinoma
        - **iii.** Tubular adenocarcinoma
        - **iv.** Mucinous adenocarcinoma
        - **v.** Signet-ring cell carcinoma
        - **vi.** Adenosquamous carcinoma
        - **vii.** Squamous cell carcinoma
        - **viii.** Small cell carcinoma

ix. Undifferentiated carcinoma

x. Others

- Carcinoid (well differentiated endocrine neoplasm)

2. Non-epithelial tumors

- Leiomyoma
- Schwannoma
- Granular cell tumor
- Glomus tumor
- Leiomyosarcoma
- GI stromal tumor

i. Benign

ii. Uncertain malignant potential

iii. Malignant

- Kaposi sarcoma
- Others
- Malignant lymphomas

i. Marginal zone B-cell lymphoma of MALT-type

ii. Mantle cell lymphoma

iii. Diffuse large B-cell lymphoma

iv. Others

3. Secondary tumors

Considering the pathogenesis, it has been found that there is a sequential evolution of all gastric carcinomas from an initial stage of *in situ* carcinoma which is confined to mucosal layers. This is called early gastric cancer (EGC).[29]

Early Gastric Cancer:

Japanese classification for gastric carcinoma was published in 1998. The Japanese classification and staging system is more detailed and places emphasis on the distinction between clinical, surgical, pathologic, and "final" staging.[10]

Early gastric cancer is defined as a cancer that is limited to the mucosa or submucosa, regardless of the presence of lymph node metastases. They also gave macroscopic classification of the different forms of early cancer which is now accepted worldwide. On the basis of gross tumor morphology, gastric carcinomas are conventionally classified as either superficial or advanced type gastric cancer.[30] The superficial type is typical of T1 tumors while T2-4 tumors eventually penetrate the muscularis propria or beyond, resulting in advanced type gastric carcinoma **(Fig. 2).**

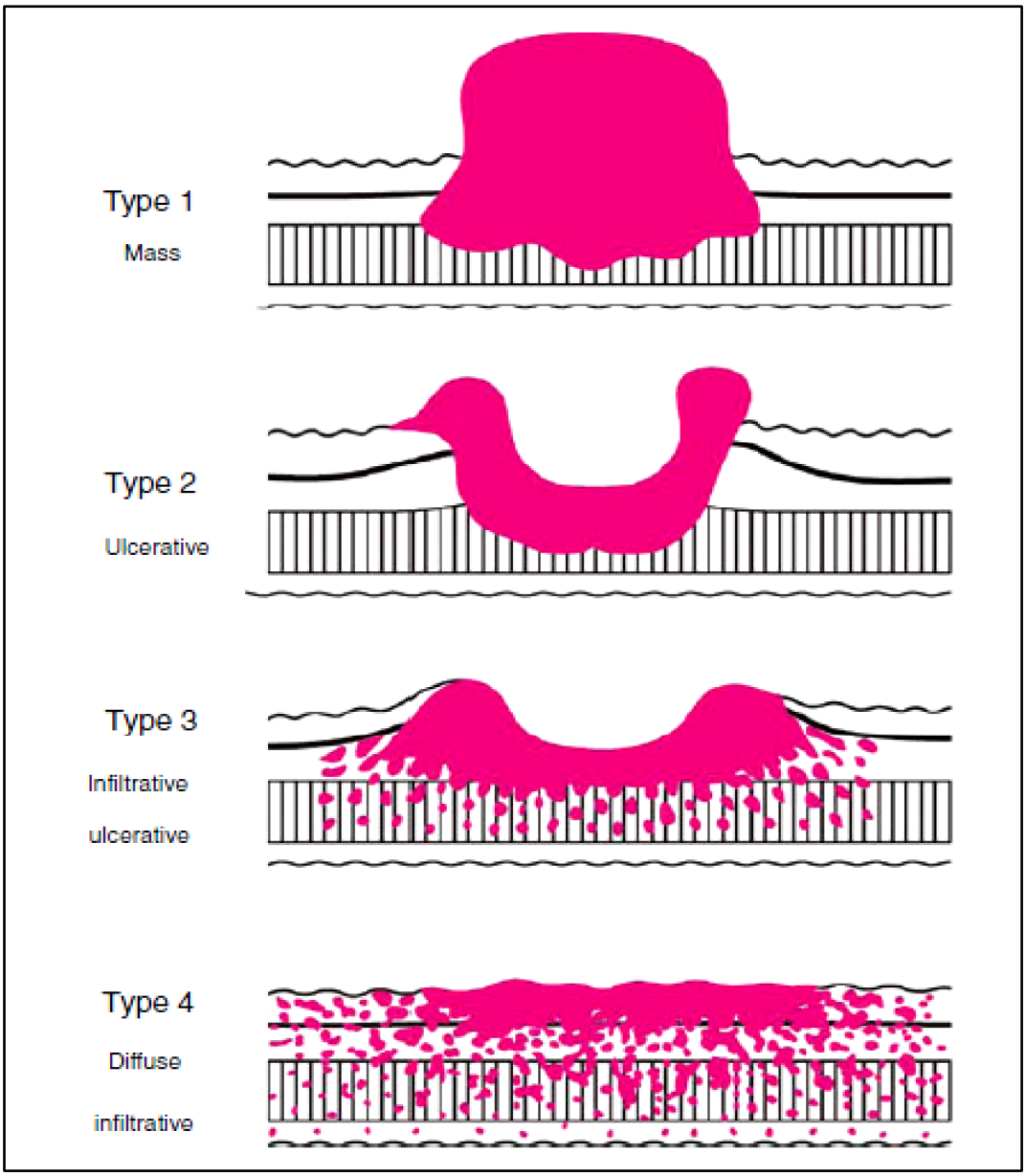

Fig. 2: Macroscopic types of advanced gastric cancer [30]

When visualised from the mucosal surface, the tumor is classified six types (**Table 1).** Depending on the Macroscopic classification of Early Gastric Cancer, the **s**uperficial type tumor (Type 0) is further sub-classified (**Table 2** and **Fig 3)**.

Table 1: Macroscopic classification of Early gastric cancer [30]

Type 0 (superficial).	Typical of T1 tumors
Type 1 (mass)	Polypoid tumors, sharply demarcated from the surrounding mucosa.
Type 2 (ulcerative)	Ulcerated tumors with raised margins surrounded by a thickened gastric wall with clear margins
Type 3 (infiltrative ulcerative)	Ulcerated tumors with raised margins, surrounded by a thickened gastric wall without clear margins.
Type 4 (diffuse infiltrative)	Tumors without marked ulceration or raised margins, the gastric wall is thickened and indurated and the margin is unclear.
Type 5 (unclassifiable)	Tumors that cannot be classified into any of the above types.

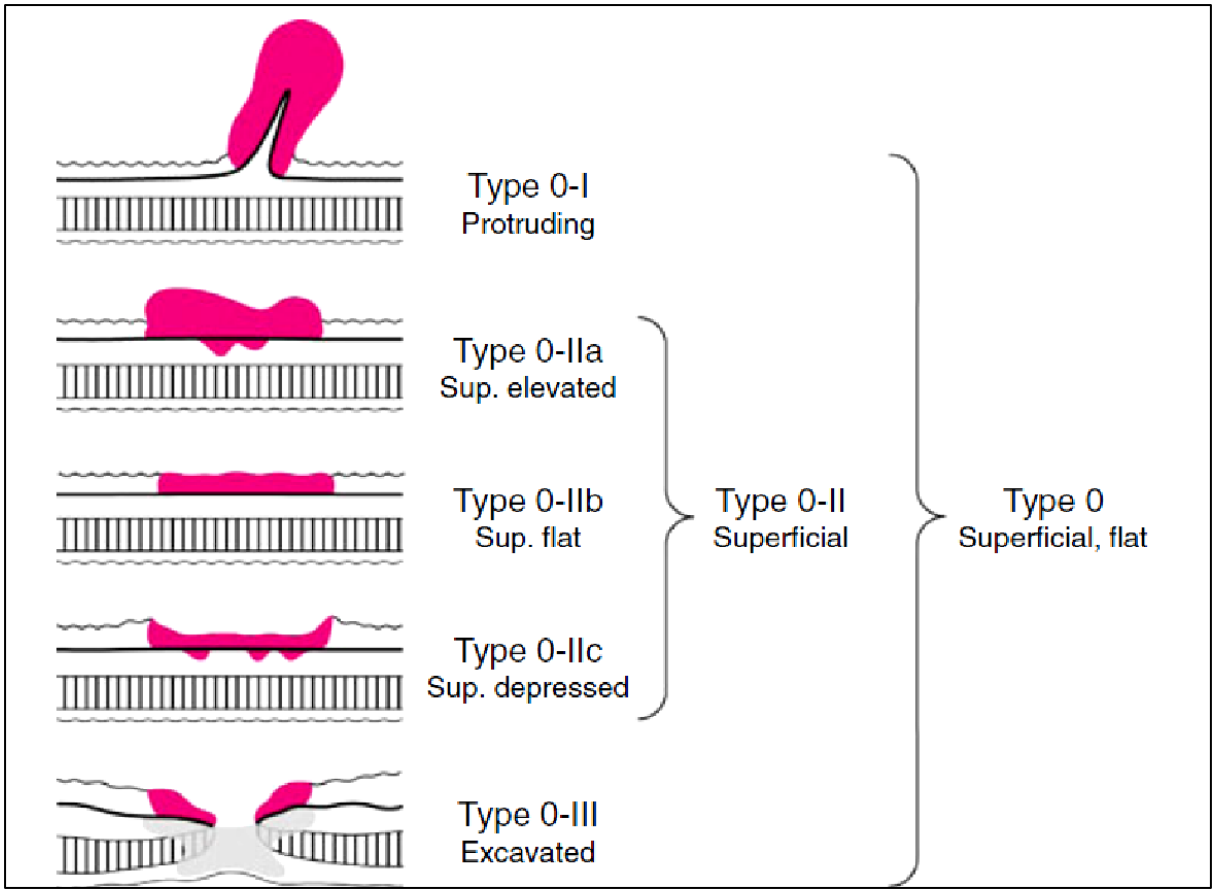

Fig. 3: Subclassification of Type 0 [30]

Diagnosing early gastric cancer helps the clinician to treat patients with endoscopic mucosal resection (EMR) and wedge resection which preserves the function of the organ.

Table 2 Subclassification of Type 0 (Superficial Type) [30]

Type 0-I (protruding)*	Polypoid tumors.
Type 0-II (superficial)	Tumors with or without minimal elevation or depression relative to the surrounding mucosa.
Type 0-IIa (superficial elevated)	Slightly elevated tumors.
Type 0-IIb (superficial flat)	Tumors without elevation or depression.
Type 0-IIc (superficial depressed)	Slightly depressed tumors.
Type 0-III (excavated)	Tumors with deep depression

*Tumors with less than 3mm elevation are usually classified as 0-IIa, with more elevated tumors being classified as 0-I

Advanced gastric cancer: [31]

There is a direct relationship between prognosis of gastric cancer and its morphological characteristics. Advanced gastric cancer is further divided into 4 types according to depending on macroscopic appearance. The gross appearance of advanced carcinomas forms the basis of Borrmann's classification: **(Fig. 4).**

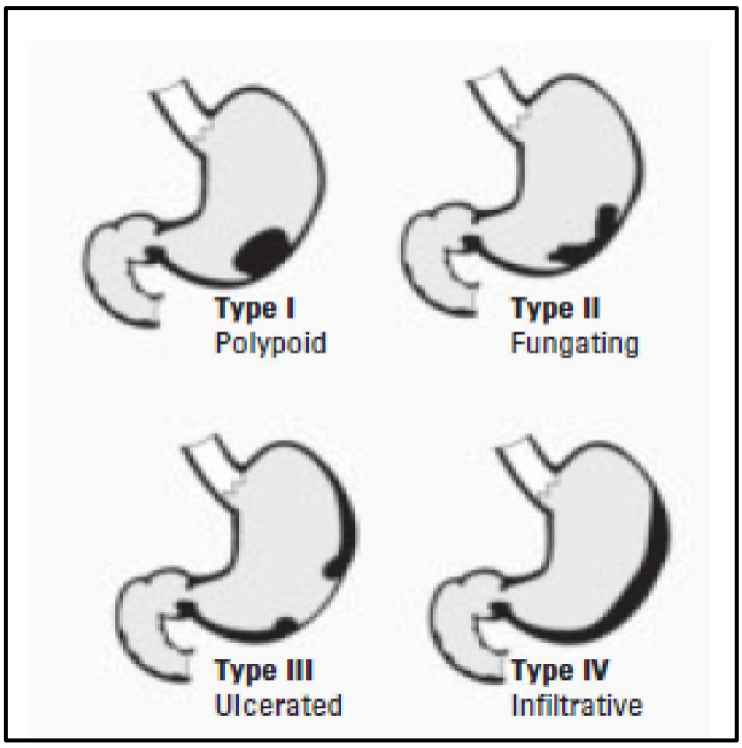

Fig. 3 Borrmann classification of advanced gastric carcinoma [28]

1. Borrmann Type I – Polypoid.
2. Borrmann Type II – Fungating, ulcerated with sharp raised margins.
3. Borrmann Type III- Ulcerated with poorly defined infiltrative margins.
4. Borrmann Type IV- Infiltrative, predominantly intramural lesion, poorly demarcated.

Lauren's classification [32], classified gastric cancer is classified into:

- Intestinal type
- Diffuse type.
- Mixed type

The characteristic features and the differences between the intestinal type and diffuse type of cancers are as given in **Table 3**. The mixed type exhibits features containing a mixture of intestinal and diffuse type of gastric cancers.

Table 3: Difference between Intestinal type and Diffuse type of gastric cancers[33]

	Intestinal Type	**Diffuse Type**
Epidemiological characteristics	Epidemic type seen in high-risk populations	Endemic - Incidence similar in most countries
Site	Gastric antrum	Body of stomach
Etiology and pathogenesis	Environmental factors, Helicobacter pylori (HP) infection	Genetic etiology, CDH1 mutations
Age and sex	Elderly male patients	Females, Younger adults
Precursor lesions	Multifocal atrophic gastritis	Active gastritis
Gross appearance	Polypoid, fungating	Linitis plastic
Pathological characteristics	Tubular pattern Glandular pattern Cohesive tumor cells Intestinal metaplasia often noted	Non‐ cohesive, scattered tumor cells Infiltration into stroma
Distant disease	Discrete hepatic metastases	Diffuse, trans peritoneal spread
Prognosis	Better prognosis.	Worse prognosis

Adenocarcinoma may be graded into: [28]

- Well-differentiated - greater than 95% of tumor composed of glands
- Moderately differentiated - 50% to 95% composed of glands
- Poorly differentiated - 49% or less composed of glands.

Based on the location, gastric cancers can be sub divided into those arising from

- Cardia
- Distal end of the stomach
- Diffusely involving whole stomach
- GEJ

Neoplasms at the gastroesophageal junction may have arisen either in the gastric cardia or in Barrett esophagus. The classification of tumors depends on the percentage of tumor tissue involving a particular site (either esophagus or stomach). The tumors are categorized as esophageal carcinomas if >50% of the tumor is located in the, whereas if more than 50% involves the gastric cardia, it is classified as gastric carcinoma. If the tumor is located equally above and below the anatomic gastroesophageal junction, it is designated as junctional.[24]

Of all the classifications, WHO classification is most reproducible. Lauren's classification has been used in numerous epidemiologic and clinical studies.

Predisposing factors for upper GI malignancies:

The etiopathogenesis of gastric malignancies relies on both environmental and genetic factors Dietary habits, smoking, Helicobacter pylori infection, radiation exposure and previous gastric surgery are amongst the important predisposing environmental factors. Important predisposing genetic changes are [9, 24 27, 34, 35]

I. Activation of oncogenes like

a. Cyclin D1

II. Activation of growth factors and growth factor receptors like

- **a.** EGFR
- **b.** TGF
- **c.** HER-2/neu
- **d.** C-met
- **e.** K-sam

III. Inactivation of tumor suppressor genes like

- **a.** p53
- **b.** p16
- **c.** APC
- **d.** Rb

IV. Inactivation of DNA repair genes like

- **a.** hMLH1
- **b.** hMLH2
- **c.** hMLH3
- **d.** hMLH6

V. Inactivation of cell adhesion molecules like

- **a.** E-cadherin

VI. Alterations in cell cycle regulatory genes

Detection of any of these genetic changes by use of novel molecular or immunohistochemical techniques might determine new prognostic and theranostic factors for gastric malignancies.

Almost all gastric carcinomas arise from the generative (stem/ basal) cells of the foveolae.[9]

SCC of the esophagus has been associated with various geographic, ethnic and lifestyle risk factors. It has a higher incidence as compared to adenocarcinoma in Asian countries. HPV infection is associated with almost 40% of SCC.[36]

Predisposing gastric lesions include, chronic gastritis, atrophic gastritis, intestinal metaplasia, autoimmune gastritis, gastric ulcer, heterotopic pancreatic tissue and various gastric polypoid lesions.[9, 24]

In earlier days, gastric cancer was endemic in developing countries with unsanitary conditions and crowded families; predisposing them to Helicobacter pylori infection which is one of the etiological factors in developing gastric cancer. Currently the incidence of adenocarcinomas is increasing in western countries. The increasing incidence of GE reflux diseases is considered one of the causes of this observation. Secondly, Barrett esophagus leading to adenocarcinoma is also one of the major reasons leading to this rise in incidence.[37]

Immunohistochemical markers

Carcinomas of the GIT present with distant metastasis and have a poor survival rate due to their advanced stage at initial presentation. The advanced stage and marked degree of undifferentiation, makes is difficult to identify these lesions without the help of ancillary techniques such as immunohistochemistry. Pancreatic and biliary tumors being an exception, most other GI tumors can be differentiated by their unique immunohistochemical profile. Immunohistochemistry plays a major role in determining the origin of GI carcinomas as and when the size of the biopsies reduce. Use of IHC stains is very popular in various malignancies. They can also be applied: [36]

- To assess the phenotypic heterogeneity of gastric cancers
- To identify tumor characteristics that might influence prognosis
- To determine whether these characteristics make a way for novel chemotherapy or targeted therapy.

Phenotypic markers help us to identify progenitor cell of specific cancer. Cytokeratin 20 (CK20) marks antral epithelium, Cytokeratin 7 (CK7) marks the columnar cells of the cardia. Hence, adenocarcinomas of the antrum and the gastroesophageal junction are more likely to express respective markers.[32]

The mucin core peptide cores (MUC), MUC1 and MUC5AC, are more frequent in carcinomas distal to the cardia than in cardia cancers.[9]

A prognostic and therapeutic marker like EGFR overexpression is associated with more frequent metastases and less favorable prognosis.

TC1, a novel regulator of the Wnt signaling pathway, is up-regulated in gastric cancers. It plays a role in poor differentiation and aggressive biologic behavior in gastric cancer.[37]

Cyclin D1, is not expressed in normal gastric mucosa, but is expressed in 40% of gastric cancers and is associated with a less favorable 5-year survival.

Immunohistochemical expression of MMP7, VEGF-C and VEGF-D are associated with poor prognosis.[36]

Androgen Receptor (AR) expressing gastric cancers are more likely to have nodal metastases and less favourable prognosis than those that do not.[38] Recently EGFR, a HER-2/neu is correlated with poor outcome and aggressive course of gastric cancer.[39] Yet there are very few studies regarding HER-2/neu as a prognostic marker in gastric cancer and many authors had encouraged further studies. [37, 40, 41]

HER-2/neu

The DNA of rats with neuroblastomas when chemically induced, first demonstrated the *neu* gene. It was homologous with EGFR and coded for a 185kd transmembrane oncoprotein. An independent human equivalent of *neu* was later cloned from a complementary DNA library, and was called "HER-2".[42]

The *HER2* (c-erbB2) gene for HER - protein is a proto-oncogene located on chromosome 17q11.2–12. It belongs to a family including EGFR, HER2, HER3 and HER4. **(Fig. 5)** These are a group of transmembrane glycoprotein all together known as receptor tyrosine kinases (RTKS).[43] The receptor has:

- A ligand-binding domain
- A transmembrane domain
- A cytoplasmic tyrosine kinase part.

The cytoplasmic domain has an enzyme activity – tyrosine specific phosphorylation.[44] At the ligand binding domain, the inactive monomeric receptors activate to homo/heterodimers. This causes phosphorylation of the receptor.[41]

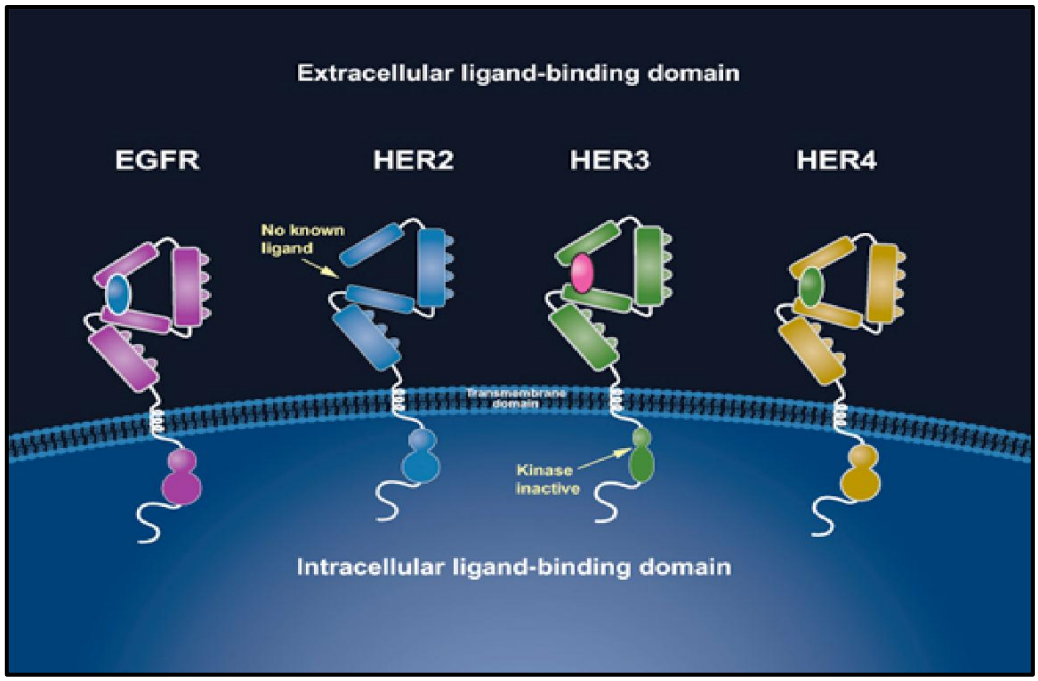

Fig. 5. The HER-2/neu receptor dimer transmembrane structure

The HER-2/neu receptor phosphorylation and signalling occurs via:

1. Different biochemical pathways like:[43]
    a. Mitogen-activated protein kinase (MAPK)
    b. Phosphatidylinositol 3- kinase (PI3K)
    c. Phospholipase C-γ
2. Transcription factors like:
    a. Signal transducers of transcription (STATs)
    b. SMAD proteins

These modules of cellular activation and the respective growth factors (GFs) are co-opted in several phases of tumor progression.

TK receptors control vital pathways such as cell survival, invasion, metastasis and angiogenesis. Hence, overexpression of HER- 2/ neu is responsible for both development and progression of cancer.[5]

Overexpression of HER- 2/*neu* has been associated with advanced disease, metastasis, and poor clinical outcome in

1. Oral and oropharyngeal SCC
2. Salivary gland and salivary duct carcinoma
3. Esophageal carcinomas
4. Gastric carcinomas
5. Non-small cell lung carcinoma
6. Primary and metastatic breast cancer
7. Pancreatic carcinoma
8. Endometrial carcinoma
9. Ovarian carcinoma
10. Bladder carcinoma

11. Colonic carcinoma

12. Prostatic carcinoma

The association of HER-2/neu and SCC of head and neck region is not well defined. Squamous cell carcinoma being one of the most common epithelial neoplasm of the head and neck region, a majority of them are derived from the oral cavity and oropharynx. A study done by Khan *et al*[41] reported 31% of SCC of the oral cavity and oropharynx demonstrated HER-2/neu overexpression.

Studies that reported the over-expression of HER-2/neu in carcinomas of the salivary gland stated that HER-2/neu amplification and immunostaining correlated with shorter disease free interval and a shorter overall survival.[45] Similar findings were also noted in case of salivary duct carcinomas.[46]

20-30% of human breast carcinomas, overexpress HER-2/neu oncoprotein. This overexpression is shown to be associated with resistance to hormone based therapy as well as overall poor survival.[47]

Research has been carried out to evaluate HER-2/neu expression with IHC in lung malignancies and have stated a higher association (27-57%) of its expression with non small cell lung carcinomas (NSCLC) with adenocarcinoma. NSCLC showing HER-2/neu overexpression were also proved to be resistant to chemotherapy.[48]

There is a wide variation (7-82%) in the reported cases of HER-2/neu overexpression in pancreatic adenocarcinomas.[49] A study done by Chou *et al* concluded that 2.1% of pancreatic adenocarcinomas that exhibited HER-2/neu overexpression were associated with atypical distant metastases.[50]

In 2004, research done by Brian *et al* concluded, 18% of uterine papillary serous adenocarcinomas displayed HER-2/neu overexpression on IHC however a

definite conclusion regarding its association with poor survival and reduced disease free survival rate could not be made. It also concluded, HER-2/neu overexpression was rarely associated with gene amplification.[51]

Studies done on human bladder transitional cell carcinoma (TCC) showed a statistically significant increase in the overexpression of HER-2/neu in grade 3 cancers. Gene amplification however was not found to be the mechanism of protein overexpression.[52]

HER-2/neu scoring system in gastric carcinomas

HER-2/neu analysis in breast cancer differs from that of gastric cancer because they both have inherent differences in biological characteristics. Gastric cancer exhibits unique immunostaining features that are not observed in breast cancer. The two important features of gastric carcinoma that calls for modification of the scoring system are: [41, 53]

1. Incomplete, basolateral ('U' shaped) or lateral staining in addition to complete staining: This staining pattern is due to apical (luminal) shedding of the membrane with the secretion in the glands. Hence, it was agreed that complete and incomplete immunoreactivity will be assigned same score depending upon the intensity of reactivity.
2. Heterogeneity (defined as <30% of tumor cells staining positive or only focal staining of tumor cells) was higher in gastric carcinomas (4.8%) as compared to breast carcinomas (1.4%). Hence, the 10% cut off for number of reactive cells for breast cancer, may be retained only for surgically resected specimens of gastric cancer and should not be applied to the biopsy specimens. Chromosomal instability is probably one of the major causes of heterogeneity.

Although both the surgical specimens and the biopsy samples can be subjected for HER-2/neu testing; biopsy samples are preferred for optimal results due to appropriate fixation of small specimen and the surgically resected specimens are preferred for the heterogeneity.

Ruschoff *et al* [54] recommend 6-8 biopsy samples to overcome the heterogeneity factor. They also discouraged the use of tissue microarray for the same reason. Considering the above mentioned biological differences, applying the HER-2/neu scoring system of breast cancer may result in underscoring of gastric carcinomas.

Hence, in 2008 Hofmann *et al*[53] came with a new scoring system for the gastric carcinomas. European Medical Agency[55] and the ToGA[11] trial also recommended the similar scoring system. Grabsch *et al*[56] scoring was based on protein expression levels only. They considered membrane staining regardless of pattern and intensity in >5% of tumor cells as positive.

Zhou *et al* [57] modified the Hofmann score criteria. They considered IHC 2+ as positive HER-2/neu overexpression since all the cases had HER-2/neu amplification with CISH test. However, a recent study by Mayo Clinic reported that considering the criteria for HER-2/neu expression in breast carcinomas, about 15% of IHC displaying a score of 2+ cases showed HER-2/neu amplification on subsequent FISH analysis.[58]

Hofmann[53] classified the intensity of reactivity as absent, faint, moderate or strong. But with this classification, inter observer variability was high. Hence, Ruschoff [54] came up with the 'magnification rule' which states that intensity should be considered as strong if the stain is visible at low magnification (x20), moderate if seen at x100 magnification and faint if appreciated only at x400 magnification.

HER-2/neu scoring system for gastric cancer as given by Hofmann *et al*[53] is mentioned in the **table 4**

Table 4: Difference between scoring of HER-2/neu in gastric and breast carcinomas

Gastric cancer	Breast cancer	Score
No reactivity or membranous reactivity in <10% of tumor cells (resected specimen) / <5 cells (biopsy specimen)	No reactivity or membranous reactivity in <10% of tumor cells	0
Faint/barely perceptible membranous reactivity in ≥ 10% of tumor cells (resected specimen)/ ≥ 5 cells (biopsy specimen)	Faint membranous reactivity in >10% of tumor cells	1+
Weak to moderate complete or basolateral membranous activity in ≥10% of tumor cells (resected specimen) / ≥ 5 cells (biopsy specimen)	Weak to moderate complete membrane staining in >10% of tumor cells	2+
Moderate to strong complete or basolateral membranous activity in ≥10% of tumor cells/ ≥ 5 cells (biopsy specimen)	Strong complete membrane staining in >10% of tumor cells	3+

Since, the cost of Trastuzumab (targeted therapy for HER-2 receptor) is high and side effects are significant, accurate selection of eligible patients for this therapy is crucial.

Hofmann *et al*[53] recommended that, IHC evaluation for HER-2/neu on the tumor sample should be the first step. 3+ score is considered as positive and an eligible candidate for Trastuzumab therapy. Samples scored as 2+ should be retested with FISH/CISH and treatment is decided on FISH/CISH result.

Schoppmann *et al*[58], Reichelt *et al* [59] and Marx *et al*[38] in their studies comparing HER-2/neu overexpression in primary tumor and metastatic deposits discovered that the HER-2/neu expression is similar in both the sites. Further Schoppmann *et al*[58] concluded that routine HER-2/neu testing is not required in metastatic deposit site unless the HER-2/neu status of primary site is negative or if primary site is inaccessible.

HER-2/neu amplification and overexpression [39, 37, 42, 60, 61]

In breast cancer, HER-2/neu overexpression is mainly due to gene amplification (i.e. increased number of copies of normal HER-2/neu gene); this results in increased transcription of gene, increased HER-2/neu receptors on the cell membrane (i.e. overexpression) and increased cell proliferation. The concordance between IHC and FISH assessment for HER-2/neu status in breast carcinoma is 73%-98%. But in gastric carcinomas it is controversial.

Many authors have detected HER-2/neu overexpression without amplification. This indicates that, as in breast cancer, gene amplification leading to protein overexpression may not be the case in gastric carcinomas. Hence, it can be concluded that HER-2/neu overexpression in gastric malignancies is not cell cycle dependent. So HER-2/neu over-expression in such cases can be attributed to either transcriptional activation by other genes or post-transcriptional events.

Studies done by Yan *et al*[62], Hofmann *et al*[53] and the ToGA[11] trial have reported a high (~87%) rate of concordance in IHC and FISH/CISH assessment. This was because of the scoring criteria given by Hofmann *et al*[53] which considers unique features (basolateral membrane staining and tumor heterogeneity) of gastric carcinomas.

Targeted therapy for HER-2 receptor

Initially, murine origin monoclonal antibodies (mAb) were developed but they were immunogenic and their half-life was short when injected to humans. In 1988, Winter *et al* generated a mouse-human chimeric antibody.[59] Later, panitumumab, first fully human antibody to EGFR was produced by genetically inactivating the immunoglobulin locus of transgenic mice.[11] Then, Trastuzumab, a monoclonal antibody to HER2 which carry all human immunoglobulin genes was approved for treating lymphoma and breast cancer patients. Till date only two drugs (Trastuzumab and lapatinib) targeting HER-2/neu are approved for clinical application.[40]

Trastuzumab

Trastuzumab is a recombinant humanized anti-HER2 mAb directed against the HER2 ECD. Its molecular weight is 145531.5 g/mol. It is engineered from a cloned human IgG, with structure and antigen-binding residues of a potent murine mAb4D5. The antibody was humanized to minimize the immunogenicity associated with murine mAb and to enhance endogenous immune anti-tumor effects. Postulated mechanisms of action are:[7]

- Blocking HER-2 receptor cleavage and inhibiting dimerization, thus reducing HER-2 signaling
- Increasing receptor destruction by endocytosis

- Inhibiting PI3K signaling pathway
- Anti-angiogenesis effect
- G1 phase arrest by inducing cyclin dependent kinase (CDK) inhibitor p27Kip1
- Cytostatic and cytotoxic activity due to immune system recruitment by antibody-dependent cell-mediated cytotoxicity (ADCC)

As a result of these, downstream signaling process shuts down cell proliferation, angiogenesis, invasive growth, resistance to apoptosis and DNA repair, thus sensitizing tumor cells to therapeutic mechanisms like chemotherapy, endocrine treatment and radiotherapy.

The US FDA approved Trastuzumab in 1998 for advanced breast cancer and later for early breast cancer as well. When given in combination with adjuvant chemotherapy in breast cancer, an improved overall survival is demonstrated. Hence, many studies were performed to demonstrate utility of targeted therapy in HER-2/neu positive cancers of gastrointestinal tract, ovary, salivary gland and lung.

Trastuzumab in gastric cancers

ToGA,[11] an open label, international, phase 3, randomized controlled trial was performed in 122 centers in 24 countries for gastric cancer. In this trial, Trastuzumab was combined with standard chemotherapy for HER-2/neu positive gastric cancer which demonstrated a significant improvement of gastric cancer survival. Patients with high IHC positivity for HER-2/neu had a trend for better and longer (16 months) survival as compared to chemotherapy alone (11.8 months).[56] There was 26% reduction in risk of death in Trastuzumab group. In this trial, it was reported that patients with amplified tumors without overexpression (IHC 0 or 1+) did not show a

substantial overall survival benefit suggesting that measuring HER-2/neu at protein level should be primary screening method. Also, there was no increase in toxicity noted. Hence, Trastuzumab was proved to be a safe and effective option to be considered for all HER-2/neu positive advanced gastric cancers.[60]

Currently, Trastuzumab is approved for treatment of gastric cancer in Europe, United States, Japan and other countries.[57]

Although all the above mentioned reports are encouraging, to use Trastuzumab against HER-2/neu positive gastroesophageal malignancies, the important point is - as in breast cancer, there can be resistance to Trastuzumab even among HER-2/neu positive gastric carcinomas. Hence, an understanding of other pathways like Wnt and TGFβ pathways is essential. This points to the need of combination targeted agents which target different "crosstalk" pathways.[37]

METHODOLOGY

This is a prospective study. All UGI endoscopic biopsies received at the department of Pathology, B.L.D.E. University's Shri. B. M. Patil Medical College, Hospital and Research Centre, Vijayapura were subjected to the present study. The study included 107 gastric and esophageal UGI endoscopic biopsies that were diagnosed as carcinoma on histopathology, from the period 1st December, 2015 to 30th June, 2017. Endoscopic biopsies were sent to pathology department in 10% neutral buffered formalin (NBF).

Inclusion criteria

Endoscopic biopsy specimens of patients with esophageal or gastric or duodenal carcinomas.

Exclusion criteria:

1. Biopsies with esophageal/gastric carcinomas that show association with histological changes of Barrett's esophagus.
2. Poorly fixed tissue.

The number and size of the biopsy specimen were noted; the entire tissue was wrapped in a filter paper and submitted for processing in a labelled (unique histopathology number of the laboratory) capsule. The tissue was embedded in paraffin wax.

- For sectioning, the paraffin blocks were initially cooled with an ice tray to give them a consistent temperature.

- The blocks were first trimmed with a coarse setting of 15-30 μm sections by advancing the coarse feed mechanism.
- Care was taken to avoid aggressive trimming.
- Routine sections with a thickness of 3-4 μm were cut to attain a continuous ribbon of serial sections.
- When a ribbon of several sections was ready, the first section was held using a forceps, or teasing needle and the last section was eased from the knife edge with a small brush.
- The ribbon was floated out in the hot water bath (preheated at 58^0C) by allowing the tailing end of the ribbon to make contact with the water first.
- If any folds in the section, they were removed with the help of forceps or teasing needle.
- The sections were allowed to float in the hot water bath for 10-15 seconds and later picked up on:
    - **i.** Slides that were pre-coated with egg albumin as an adhesive for H&E staining – 2 slides containing serial sections each.
    - **ii.** Slides that were pre-coated with poly L lysine in order to charge the slides for immunohistochemical analysis- 1 section.
- Care was taken so as to pick up the ribbon of the sections on to the slides within one attempt so as to prevent the washing away of the adhesive in the water bath.
- The slides were allowed to dry and were labelled with their specific Histopathology identification number.

Staining procedure (H&E) [63]

- Slide containing serial sections of the biopsy was initially dewaxed by placing it on the hot plate.
- These slides were later dipped in xylene 2 changes of xylene, 5 minutes each.
- In order to rehydrate the sections, the slides were further dipped in descending concentrations of graded alcohol solutions (90%, 70% and 50% alcohol) for 2 changes of 30 seconds each change so as to rehydrate the sections.
- Sections were placed in distilled water for 5 minutes.
- Slides were placed in Harris Haematoxyline for 2-3minutes.
- Slides were rinsed with tap water.
- For differentiation slides were dipped in 1% Acid Alcohol (1% HCL in 70 % alcohol) - 2 seconds
- For the purpose of blueing, the slides were placed under running tap water for 10 minutes
- Counter staining was done by placing the slides in alcohol preparation of eosin Y for 5 seconds.
- Slides were mounted with D.P.X as a mounting agent.

The microscopic features were assessed and diagnosis was rendered accordingly. The tumors were classified according to WHO and Laurens classification.

They were also graded according to the WHO criteria as well moderately and poorly differentiated carcinomas depending on the percentage of differentiation.

All the gastric /esophageal carcinomas were further subjected to IHC stain HER-2/neu primary antibody.

Staining procedure[64, 65, 66]

IHC was done using Peroxidase- Antiperoxidase method.

- NBF fixed paraffin embedded tissue sections of 3 to 4 μm were taken on pre-coated poly L lysine coated slides.
- Slides were incubated at 60^0 C for 30 minutes.
- De paraffinization was done with 3 changes in xylene and decreasing concentration of alcohol solutions.
- Hydration was done in running tap water then changed to distilled water for 5 minutes.
- Antigen retrieval was done with citrate buffer (pH 6.0 to 6.8) using microwave oven at 95^0C for 2 cycles of 10 minutes each.
- Slides were brought to room temperature and rinsed in distilled water.
- Slides were then treated with endogenous peroxidase block for 10 minutes.
- Further, slides were washed in wash buffer (phosphate), 3 times for 3 minutes each.
- Slides were coated with power block for 10 minutes, the solution was then allowed to drain.
- Primary antibody was applied for 45 minutes.
- Washed with wash buffer, 3 times for 3 minutes each.
- Super enhancer was added, for 20 minutes.
- Secondary antibody was applied for 30 minutes.
- Slides were washed with wash buffer, 3 times for 3 minutes each.

- Di-amine-Benzidine (DAB) chromogen was applied for 5 minutes.
- Slides were later washed with distilled water to stop chromogen reaction.
- Counter staining was done with Harri's Haematoxylin for 30 seconds and then washed with tap water.
- Slides were mounted with D.P.X as a mounting agent.

Note: Care should be taken that the slides should not dry in between any step to prevent artefacts.

Staining pattern

HER-2/neu is a cytoplasmic membrane stain. Staining pattern was compared with control slides (known HER-2/neu positive breast cancer).

Scoring

HER-2/neu protein expression on the cell membrane was scored as described by Hofmann[53] and colleagues for biopsy specimens, according to the following criteria:

Table 5: HER-2/neu score in upper GI biopsy

Interpretation	Score	Interpretation
No reactivity or membranous reactivity in $\leq$5 cells.	0	Negative
Faint/ barely perceptible membranous reactivity in $\geq$ 5 cells	1+	Negative
Weak to moderate complete or basolateral membranous activity $\geq$5 cells.	2+	Equivocal
Moderate to strong complete or basolateral membranous activity in $\geq$5 cells.	3+	Positive

RESULTS

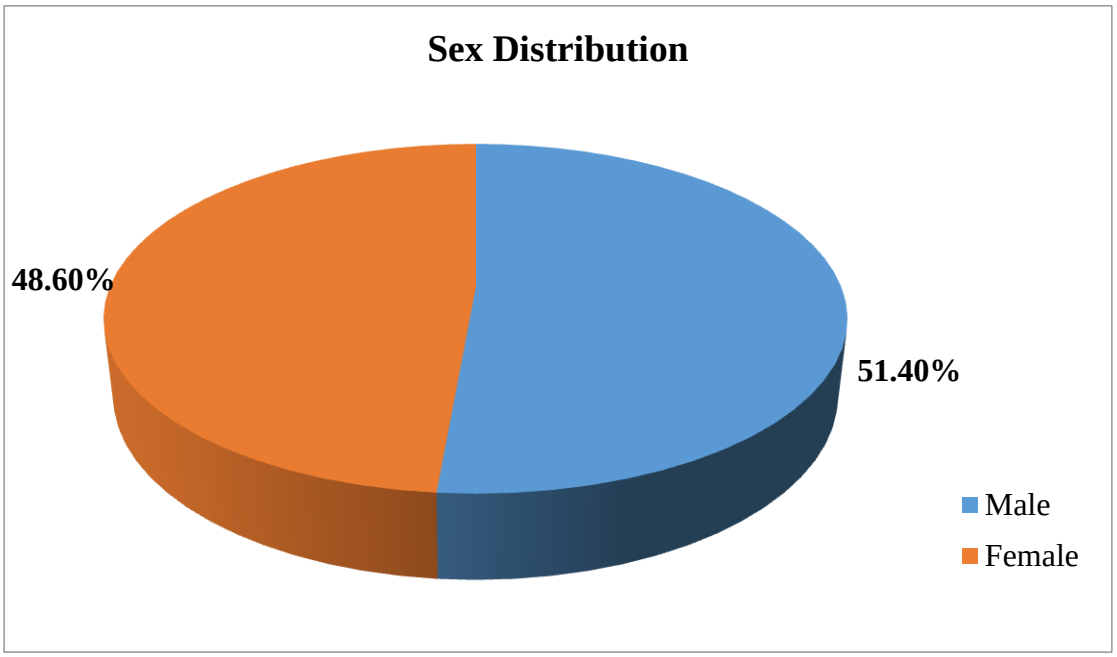

Fig. 6: Distribution of cases according to Sex

This study included 107 biopsies that were diagnosed as a malignant lesion on histopathology. Amongst them, 55 were from male patients and 52 belonged to female patients, M:F being 1.06:1. **(Fig. 6)**

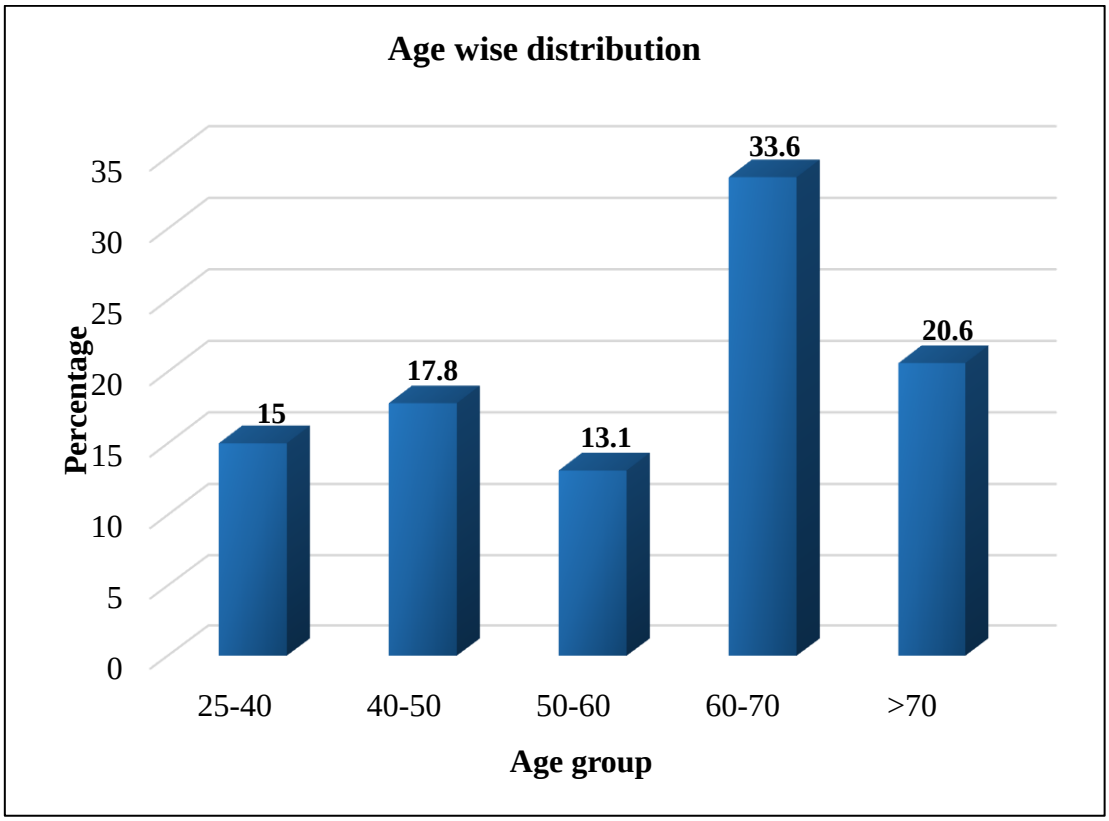

Fig. 7: Distribution of cases according to Age groups

Majority of the cases (54.2%) belonged to an age group above 60 years. **(Fig. 7)**

Out of all the malignancies that were diagnosed most of them were esophageal carcinomas followed by gastric carcinomas. GEJ accounted for only a minority of the cases. **(Fig. 8)**

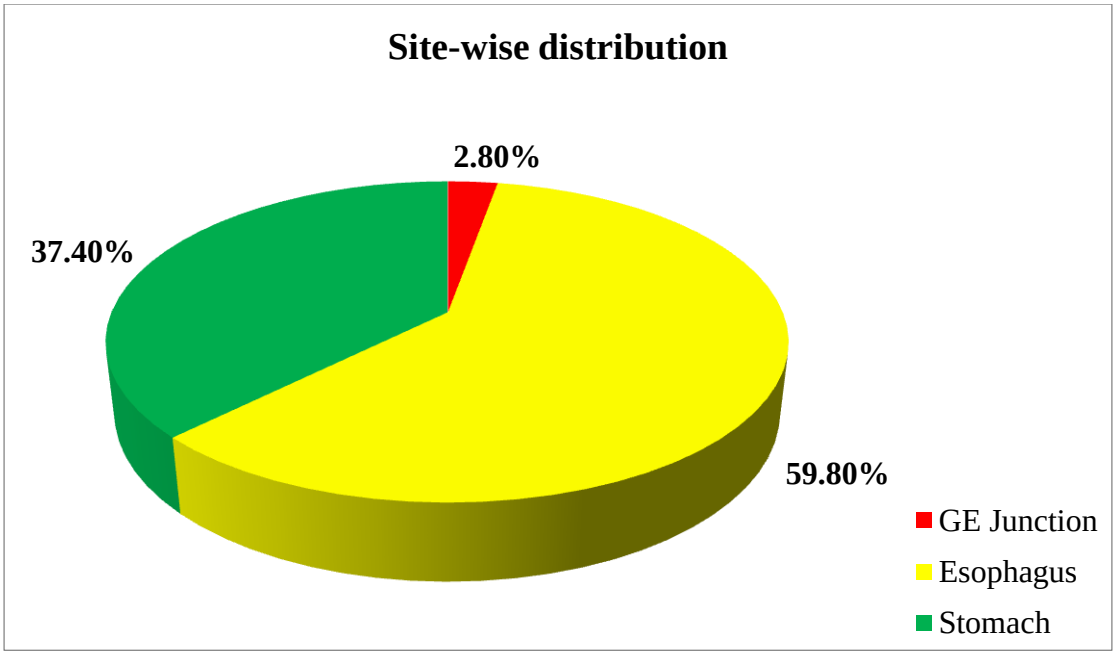

Fig. 8: Distribution of cases according to Site

Squamous cell carcinoma was the predominant lesion in the esophagus, followed by adenocarcinoma. In GEJ, all cases were of adenocarcinoma. Stomach had maximum of adenocarcinomas followed by signet ring cell carcinoma. (**Table 6 and Fig. 9**)

Table 6. Site-wise distribution of malignancies

Site	Adeno-carcinoma	Signet ring cell carcinoma	SCC	Un differentiated carcinoma	Adeno-squamous carcinoma	Small round blue cell
GE junction	3	0	0	0	0	0
Esophagus	5	1	50	5	2	1
Stomach	31	4	0	4	0	0

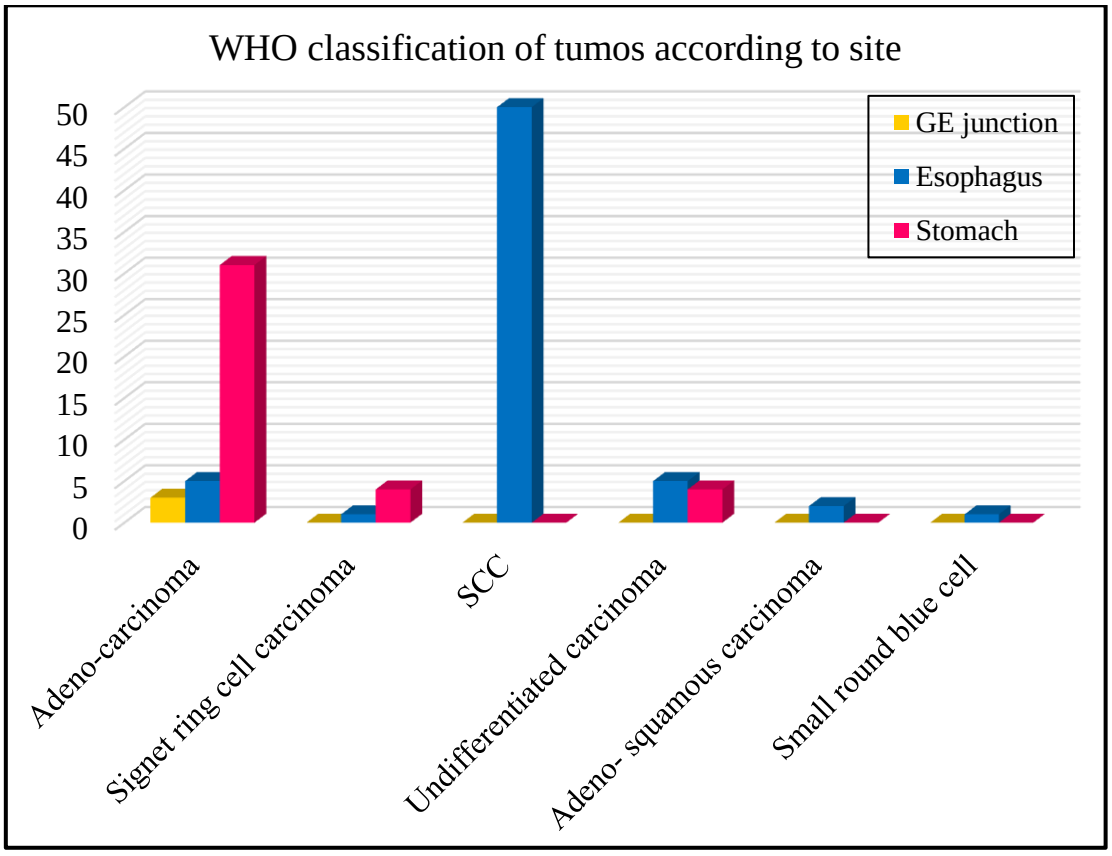

Fig. 9. WHO classification of the carcinomas according to site

Lauren's classification was applied to gastric adenocarcinomas that revealed majority of cases to be diffuse type. (**Fig. 10**)

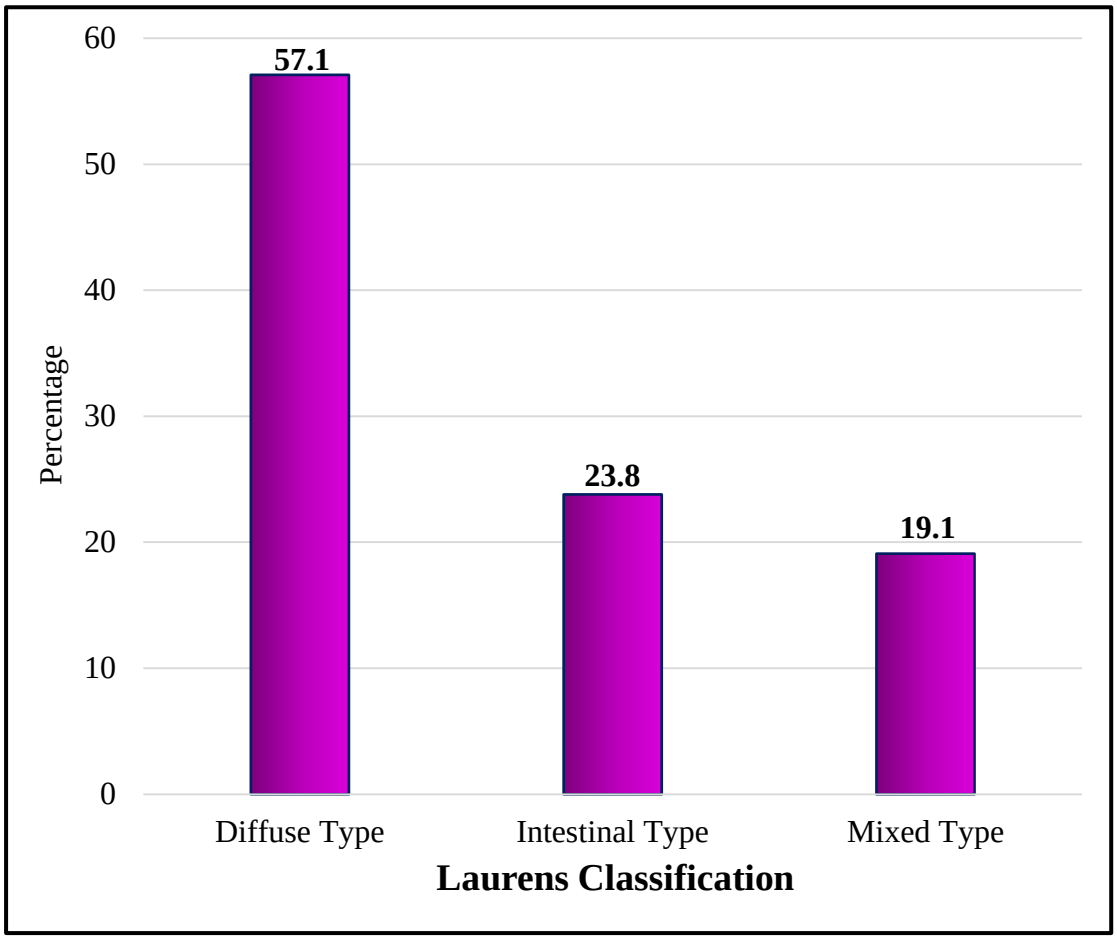

Fig. 10: Distribution of cases according to Laurens Classification

Site-wise distribution of malignancies classified according to Lauren's classification is shown in **Fig. 11.**

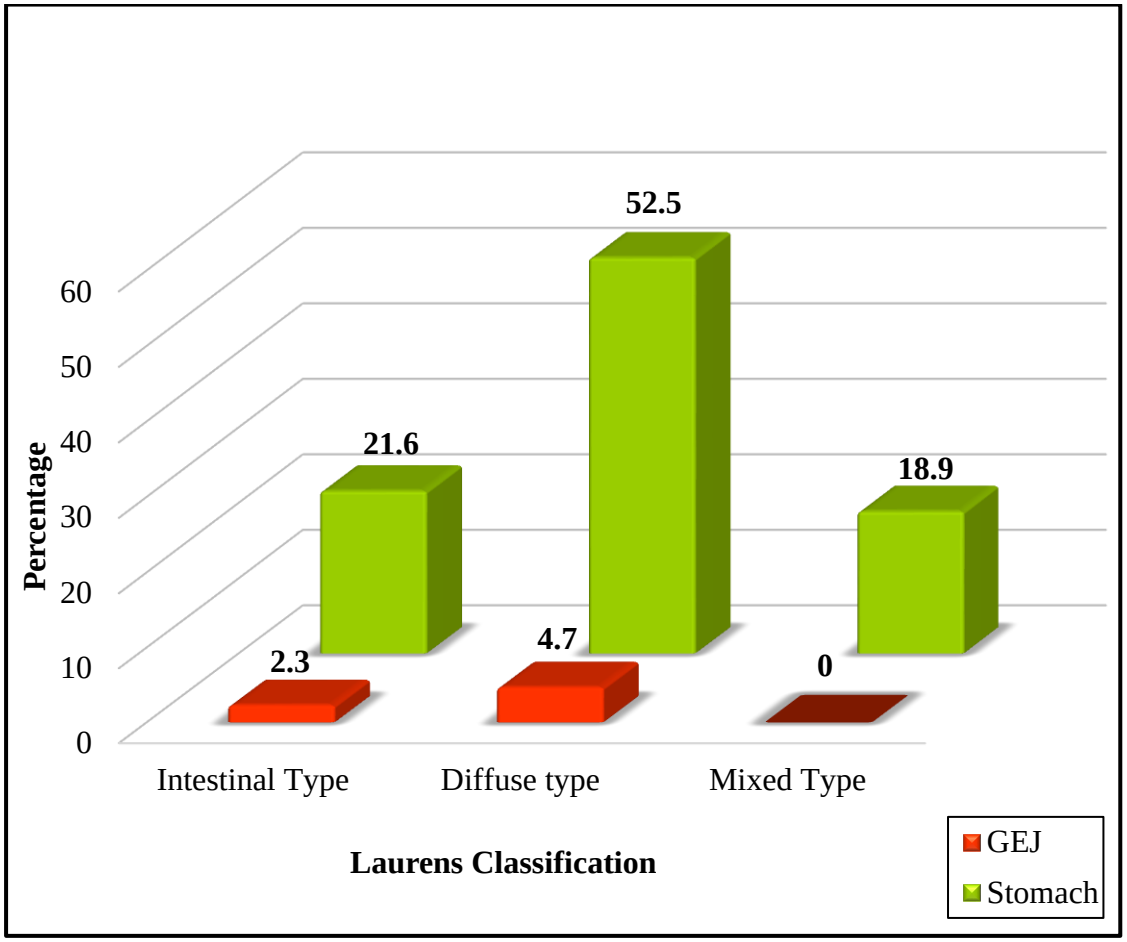

Fig. 11: Site-wise distribution of gastric carcinomas according to Lauren's classification

IHC staining of HER-2/neu was performed. Score of 3+ was granted if membrane staining was noted even in a single cluster of cells (5 cells) and was considered as positive. Of the 42 cases that were diagnosed as adenocarcinomas, 06 (14.2%) cases overexpressed HER- 2/neu, 4 of which were intestinal type (**fig. 19**) and one each of diffuse (**fig. 17**) and mixed type (**fig 18**). (**Table 7, Fig. 12**)

Table 7: HER-2/neu score and Laurens classification

IHC / **Laurens Classification**	**0**	**1+**	**2+**	**3+**	**p value**
Diffuse type	15	5	0	1	<0.001*
Intestinal type	0	1	3	4	
Mixed Type	2	0	3	1	

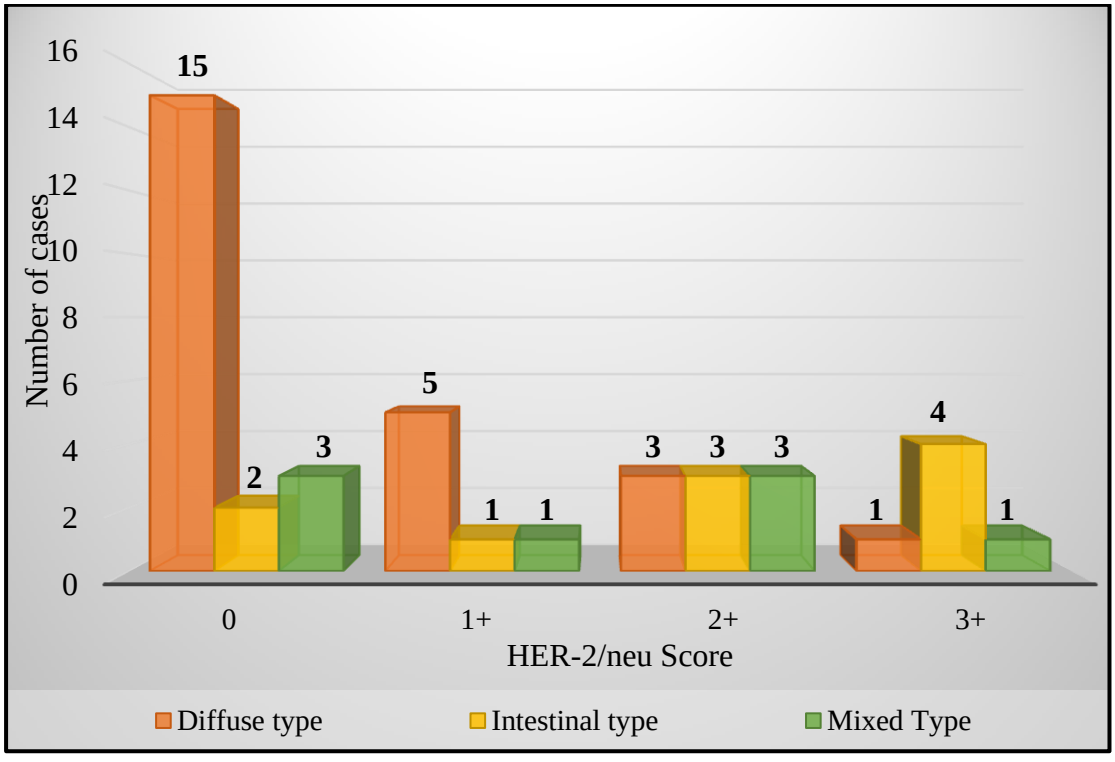

Fig. 12: HER-2/neu reactivity in gastric carcinomas

HER-2/neu analysis was also done on esophageal carcinomas. The frequency of HER-2/neu expression in these lesions was less than that seen in gastric carcinomas. Majority of squamous cell carcinomas were scored 0 (**fig. 16**). Only 02 cases displayed 3+ score (**fig 21**). (**Table 8**). Considering esophageal adenocarcinomas, 01/05 (20%) case (**fig. 20**) showed HER-2/neu positivity.

Table 8: Frequency of HER-2/neu expression in esophageal SCC

IHC	N	%
0	48	96
3+	2	4

Grading of the carcinomas was done on the basis of differentiation of tumor, as well (grade I- 54%), moderately (grade II-19%) and poorly (grade III- 27%) differentiated. Three cases were unclassifiable and hence could not be graded according to WHO criterion. (**Fig. 13**)

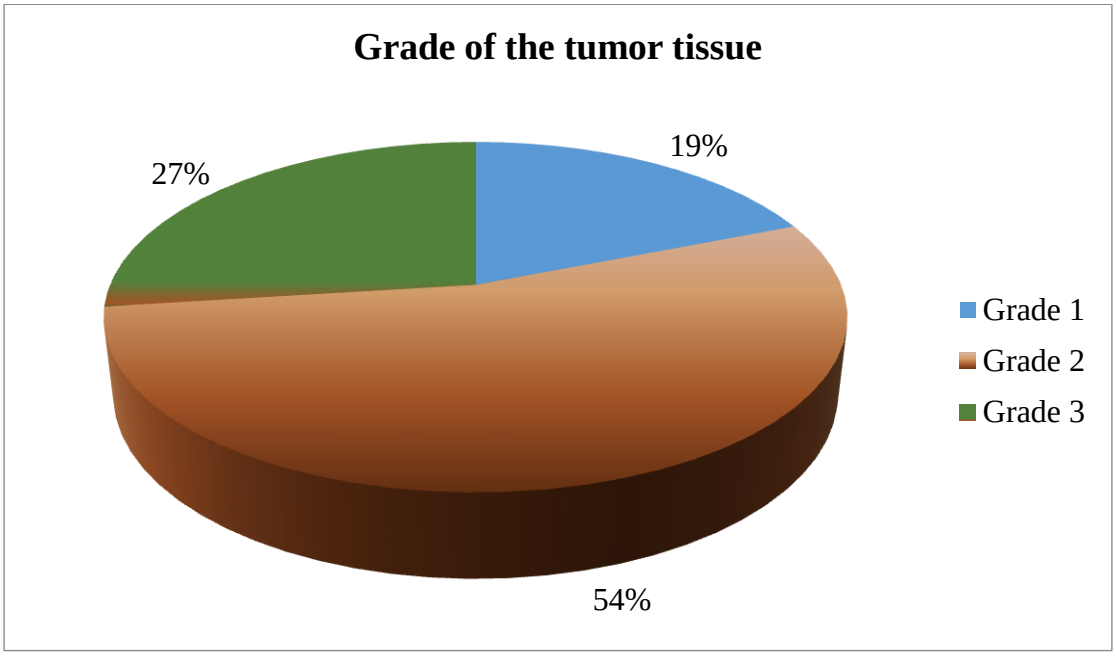

Fig. 13: Grading of upper GI carcinomas

Carcinomas in esophagus and stomach were predominantly moderately differentiated. (**Fig. 14**)

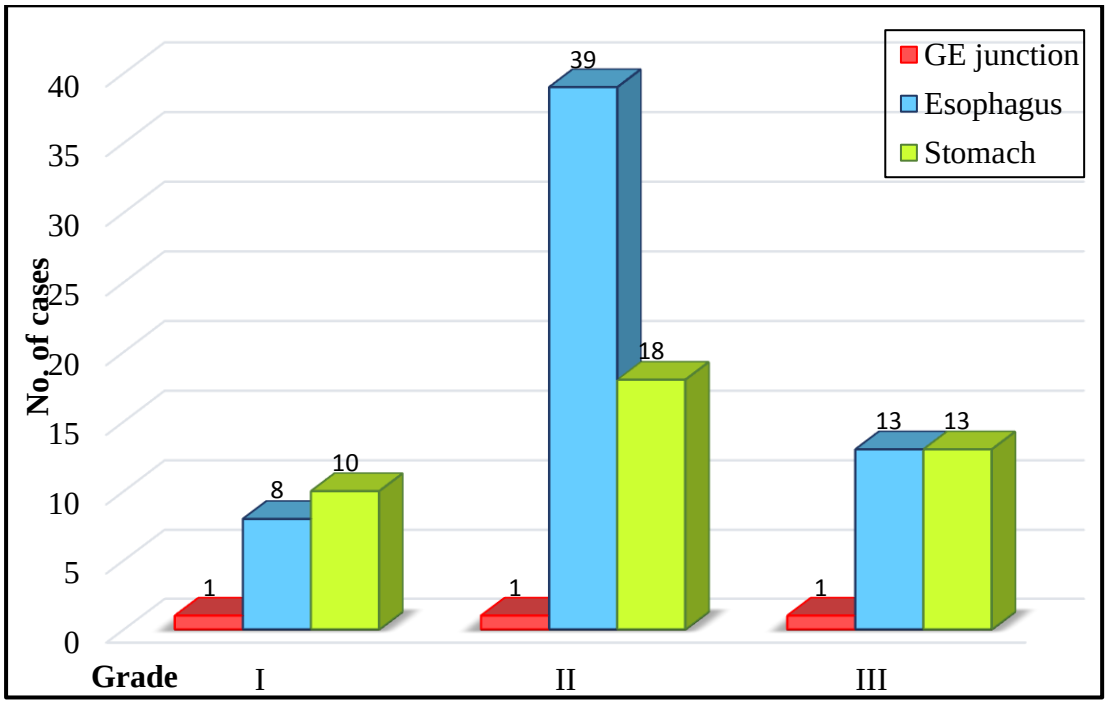

Fig. 14: Site-wise grading of upper GI Carcinomas

Association of age of the patients, sex of the patients and grade of cancer with the HER-2/neu status (positive or negative) was done using Chi square test of independence. P value was not statistically significant (>0.05)

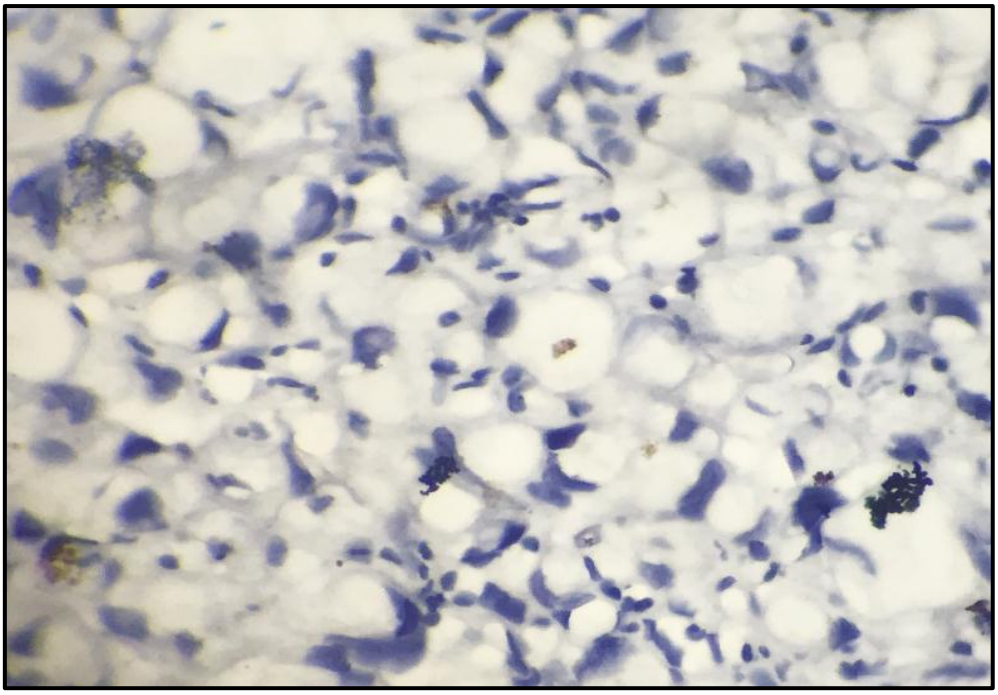

Fig. 15: IHC HER-2/ neu Score 0 in signet ring cell carcinoma- Stomach (IHC HER-2/ neu, 400x)

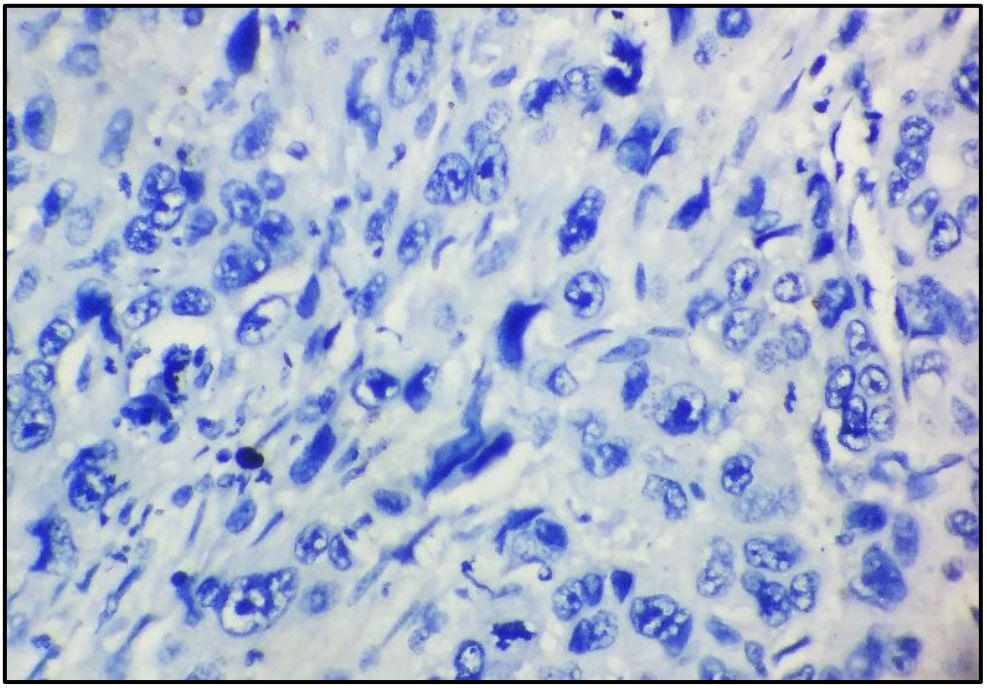

Fig. 16: IHC HER-2/ neu Score 0 in squamous cell carcinoma- Esophagus. (IHC HER-2/ neu, 400x)

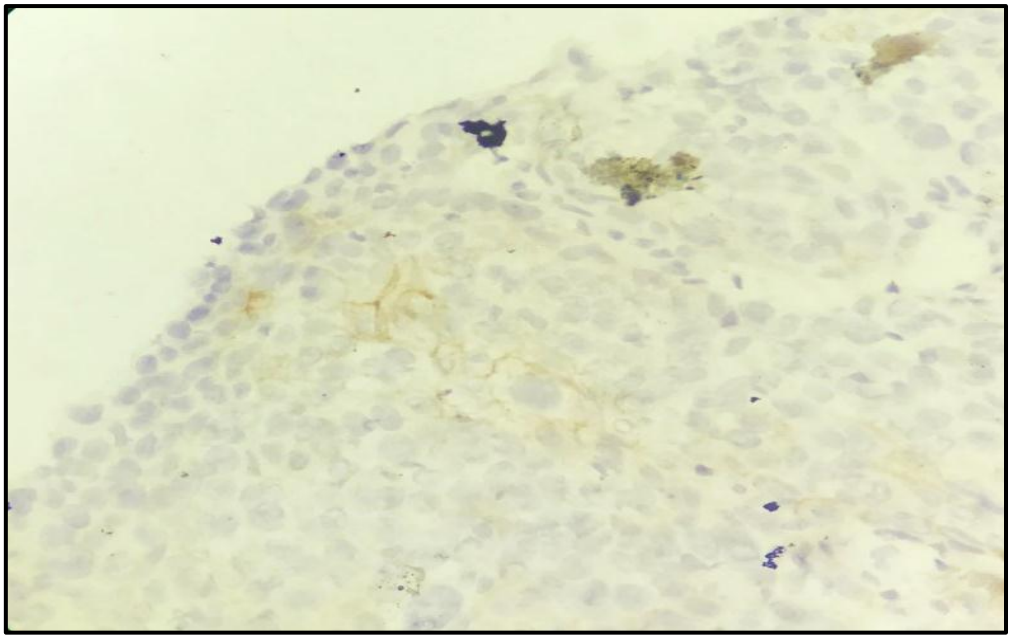

Fig. 17: IHC HER-2/ neu Score 1+ in diffuse type adenocarcinoma-Stomach. (IHC HER-2/ neu, 400x)

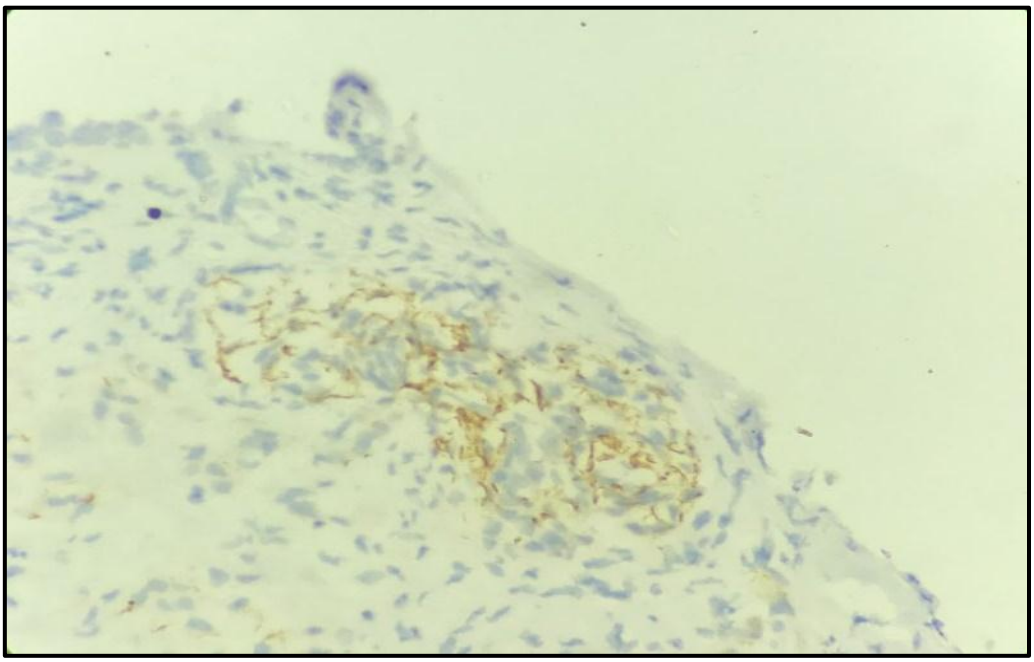

Fig. 18: IHC HER-2/ neu Score 2+ in mixed type adenocarcinoma-Stomach. (IHC HER-2/ neu, 400x)

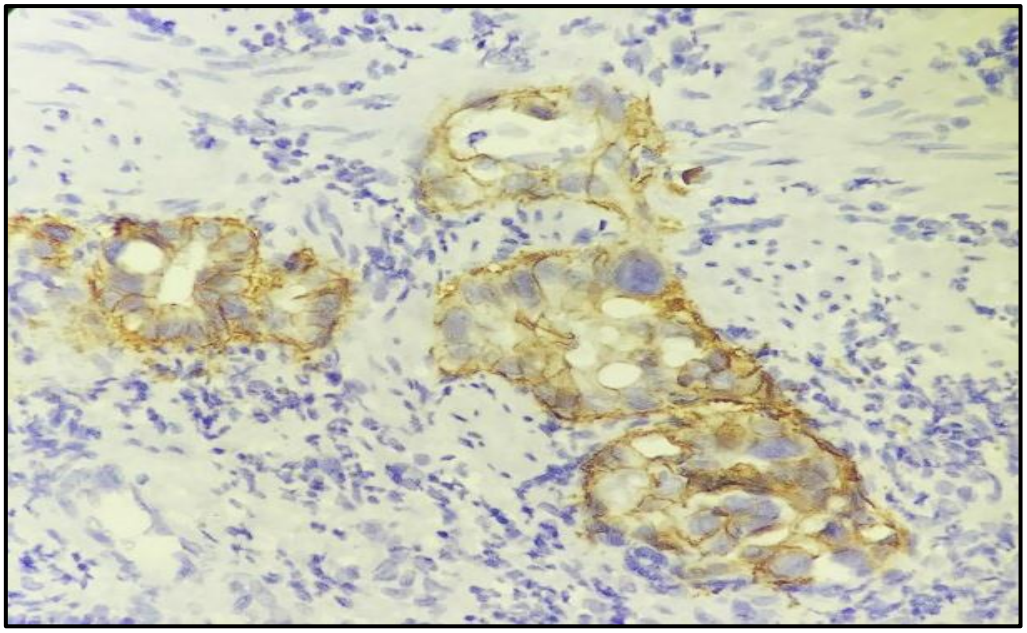

Fig. 19: IHC HER-2/ neu Score 3+ in intestinal type adenocarcinoma- Stomach. (IHC HER-2/ neu, 400x)

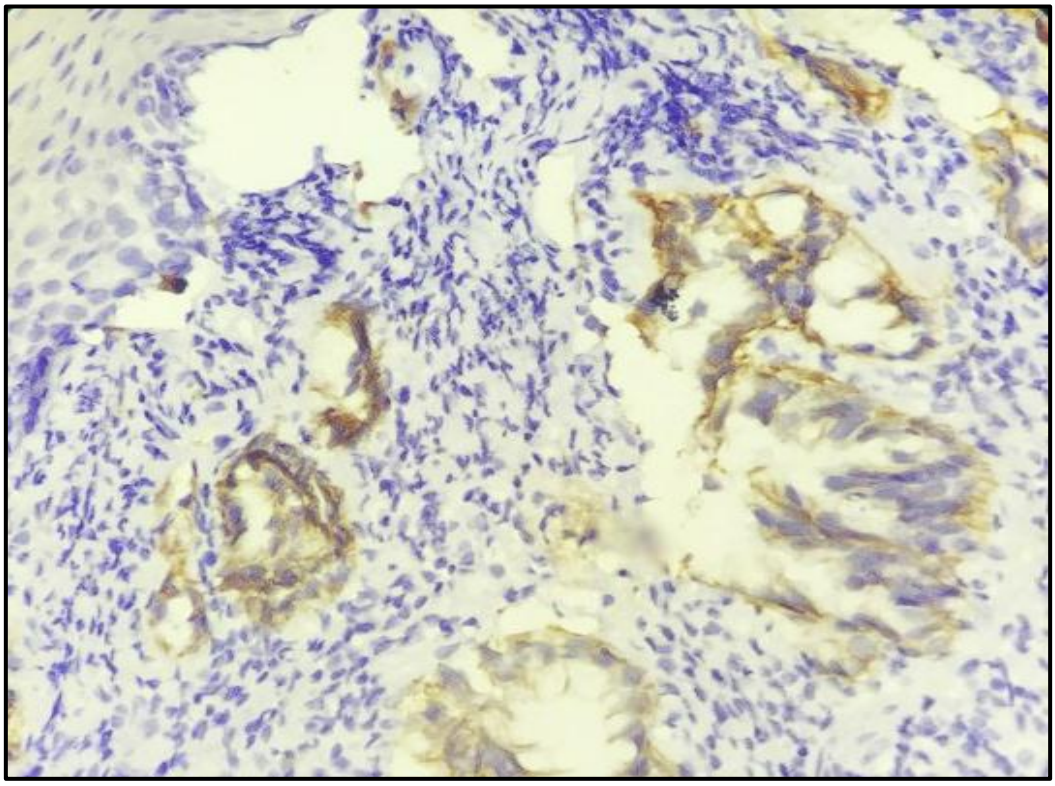

Fig. 20: IHC HER-2/ neu Score 3+ in adenocarcinoma- Esophagus. (IHC HER-2/ neu, 400x)

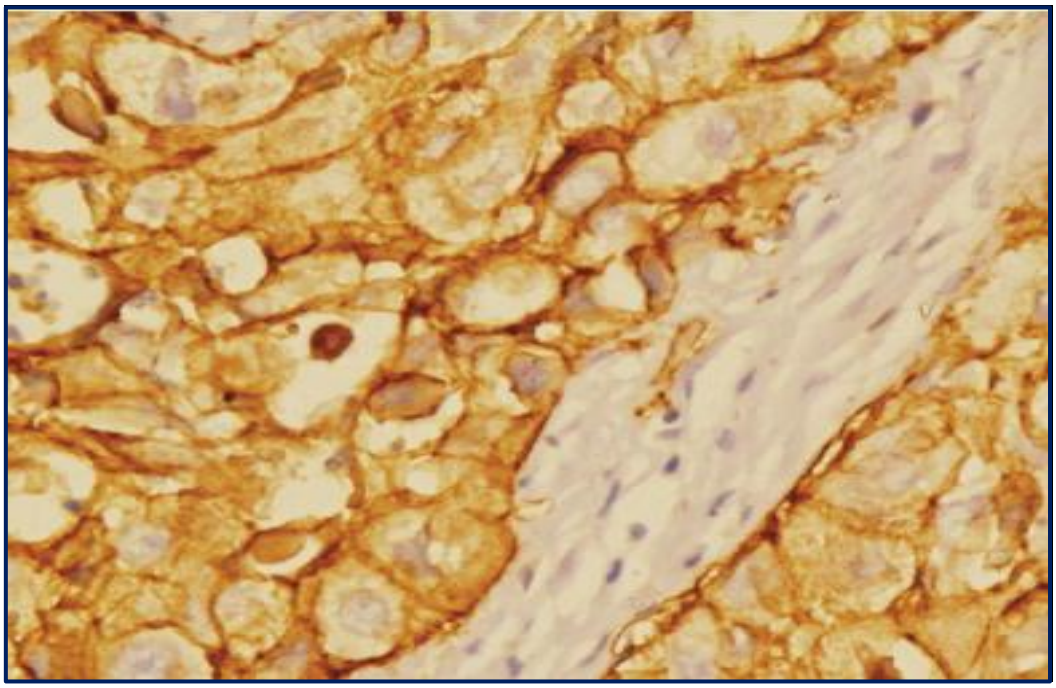

Fig. 21: IHC HER-2/ neu Score 3+ in well differentiated squamous cell carcinoma- Esophagus. (IHC HER-2/ neu, 400x)

DISCUSSION

Endoscopic biopsies are common procedures done at the out-patient department to diagnose a variety of conditions. Such biopsies remain the mainstay for diagnosis of both non-neoplastic lesions and malignancies. Important information gained from biopsies can guide the surgeon to plan future management.

In the present prospective study, a total of 107 endoscopic biopsies that were diagnosed as esophageas/gastric carcinoma on histopathology, were studied and analysed.

The age distribution was wide, ranging from 25 years to 85 years old. The mean age being 56.9 years. The majority of cases belonged to 60 to 70 years age group. Similar findings were seen in various studies shown in **Table 9**

Table 9: Age distribution in comparison to other studies

Authors	**Minimum Age (Years)**	**Maximum Age (Years)**	**Maximum cases in age range (Years)**	**Mean Age (Years)**
Qiu *et al*[67]	18	86	50-70	59
Indu *et al*[68]	31	85	61-70	46.7
Ahmadi *et al*[69]	39	80	50-60	56.46
Present Study	**25**	**85**	**60-70**	**56.9**

The number of males diagnosed as carcinoma on endoscopic biopsy was slightly more than females. The gender difference observed in upper gastrointestinal malignancies in the present study was consistent with that in the literature. (**Table 10**) High susceptibility in males can be due to personal habits of smoking and alcohol consumption; which are more common in males as stated by Krishnappa *et al.* [70]

Table 10: Gender distribution according to literature

Authors	**Male**	**Female**	**Male:Female**
Qui *et al*[67]	554	284	1.95:1
Krishnappa *et al*[70]	67	33	2.03:1
Dang *et al*[71]	66	18	3.66:1
Jawalkar *et al*[72]	136	60	2.6:1
Present Study	**55**	**52**	**1.06:1**

In view of increasing trend of upper gastrointestinal malignancies and associated poor survival of advanced carcinomas, it is important to evaluate these carcinomas for expression of prognostic or theranostic molecular markers which can direct the use of specific targeted therapy. Various markers available for evaluation are enlisted in the literature review. Amongst them HER-2/neu is a recent marker in the domain of gastric and esophageal carcinomas. Assessing HER-2/neu in these carcinomas not only predicts behaviour of carcinoma but also helps in deciding the chemotherapeutic drug to be used.

HER-2/neu in upper gastrointestinal carcinomas

There is a wide variation in the HER-2 overexpression in gastric cancers. Reported rates of HER-2/neu expression in gastroesophageal cancer vary from the range of 2% to 45%.[3,37,41,73] The largest data set of >3800 esophageal and gastric cancer samples found HER-2/neu protein positivity rate of 23%. Hence, for clinical trial and treatment, it is very important to develop a standard HER- 2/neu detection test to recruit eligible patients for Trastuzumab treatment.

Schoppmann *et al*[74], in their study of HER-2/neu expression in esophageal carcinomas compared HER-2/neu status using Hofmman[53] scoring system (15.3%) and Grabsch[56] scoring system (33.9%). It showed a drastic difference between the results by the two methods. This observation reaffirms the importance of standardization of scoring as a prerequisite for selecting patients for targeted therapy. Hofmann[53] scoring is similar to the guidelines given by EMA[55] and ToGA[11] and hence was used in the present study.

In the present study, HER-2/neu overexpression was seen in only 06 (14.2%) cases of gastric carcinomas, of which 04 (9.5%) were of intestinal type followed by one case each of diffuse and mixed type. Among the esophageal malignancies, 2/50 (4%) of squamous cell carcinomas and 1/5 (20%) of adenocarcinomas displayed HER-2/neu positivity.

Various authors who studied the HER-2/neu overexpression are listed in **table 13**. The wide variation in the overexpression of HER-2/neu can be explained by various factors such as, interpretation of data (considering only adenocarcinomas in the study) and the specimen (endoscopic biopsy/surgically resected) used for analysis. Even different geographic areas may exhibit variation in HER-2/neu expression (**Table 11**).

Table 11: World-wide variation in HER-2/neu overexpression

Authors	n	Geographic area	% of HER-2/Neu Overexpression	
			Esophagus	Stomach*
Silva *et al*[3]	463	Portugal	--	9.3%
Lee *et al*[6]	178	Australia	--	20.2%
Marx *et al*[38]	166	Germany	--	16%
Gravalos *et al*[39]	166	Europe	--	13%
Ruschoff *et al*[41]	--	--	--	22.8%
Hu *et al*[43]	116	USA	12%	--
Hofmann *et al*[53]	168	Japan	--	22.1%
Reichelt *et al*[59]	255	Germany	5% (SCC) 15% (AC)	--
Yan *et al*[62]	128	Singapore	--	9.4%
Schoppmann *et al*[74]	341	Austria	3.9% (SCC) 15.3% (AC)	--

Tanner *et al*[75]	131+100	Europe	--	12.2% + 24%
Dreilich et al[76]	97	Sweden	13% (SCC) 30% (AC)	--
Kuwabara *et al*[77]	185	Brazil	36.8%	--
Present study	**107**	**India**	**4% (SCC)** **20% (AC)**	**14.2%**

*****gastric + GE junction. AC- adenocarcinoma, SCC- squamous cell carcinoma

Overexpression of HER- 2/neu also depends on the type of carcinoma, whether it is squamous cell carcinoma or adenocarcinoma. It is more commonly associated with adenocarcinoma as studied by Schoppmann *et al*[58] and Reichelt *et al*[59]. These differences in expression of HER-2/neu in different histologies of adenocarcinoma and squamous cell carcinoma suggest that HER-2/neu might have different prognostic implications in the two types. Allgayer *et al*[78] reported HER-2/neu overexpression to be an independent functional prognostic parameter for overall survival in gastric cancer.

In gastric adenocarcinomas, variation is seen in the subtypes as classified by Laurens classification. HER-2/neu overexpression is more commonly seen in intestinal type followed by diffuse and mixed type of adenocarcinomas. This observation was consistently seen in various studies by Moelans *et al*[37]**,** Marx *et al*[38]**,** Gravalos *et al*[39] and Tanner *et al*[75]. (**Table 12**) The findings of the present study are in concordance with the other studies.

The above mentioned variation in expression is because, intestinal and the diffuse types of adenocarcinomas have different molecular pathways of development and distinct genetic alterations. They also have different histological features. Lack of E-cadherin is inversely related to HER-2/neu expression which is more commonly seen in the intestinal type of adenocarcinoma.[37]

The signet ring cells characteristically do not show HER-2/neu expression.[41] In the present study as well, signet ring cell carcinoma was HER-2/neu negative (**fig. 15**). Bakkelund *et al*[79] proposed that signet ring cell carcinomas develop by gradual de-differentiation from ECL (enterochromaffin like) cells which indicate neuroendocrine origin of signet ring cells and hence they express markers like synaptophysin and chromogranin.

Silva *et al*[3] demonstrated HER-2/neu expression in both the components (isolated cells and glandular component) of mixed type of carcinoma. This suggests –

- Common clonal origin of both the histological patterns and
- HER-2/neu amplification is an early genetic alteration acquired before other epigenetic alterations associated with phenotypic divergence.

As per the study by Yan *et al*[62], among the mixed type of carcinomas, all the 3 cases displayed amplification in intestinal component, while one displayed amplification in diffuse component. In the present study, 1 case of mixed type of carcinomas had an intestinal component that exhibited HER-2/neu overexpression, whereas the diffuse component was negative.

Table 12: Variation in HER-2/neu overexpression according to subtypes of Carcinomas

Authors	Intestinal	Diffuse	Mixed
ToGA *trial*[11]	32%	6%	20%
Moelans *et al*[37]	16.34%	2-7%	5-20%
Marx *et al*[38]	21%	4%	-
Gravalos *et al*[39]	16%	7%	14%
Yan *et al*[62]	15.8%	13%	0%
Tanner *et al*[75]	21.5%	2%	5%
Jeung *et al*[80]	29%	15%	0%
Present study	**9.5%**	**2.3%**	**2.3%**

In the present study HER-2/neu was overexpressed in 4% oesophageal squamous cell carcinoma and 20% of esophageal adenocarcinomas. Dreilch *et al*[76] in their study concluded HER-2/neu overexpression was a poor prognostic factor for squamous cell carcinoma of the esophagus but had no effect on survival of patients with esophageal adenocarcinoma. Similarly, Akamatsu *et al*[81] stated HER-2/neu oncoprotein expression is associated with resistance to chemotherapy and radiotherapy in esophageal squamous cell carcinoma

Marked heterogeneity of HER-2/neu overexpression by gastric tumor tissue is documented in literature by various authors: Lee *et al*[6]**,** Hofmann *et al*[53], Ruschoff *et al*[41], Zhou *et al*[57] and Tanner *et al*[75]**.** On the contrary, only Marx *et al*[38] in their study of 166 gastric cancers and 69 lymph node metastases concluded that HER-2/neu overexpression was highly homogenous in the primary tumor as well as in their metastases. In the present study, HER- 2/neu overexpression was heterogenous, and focal overexpression of HER- 2/neu, was seen. **(Fig. 22)**

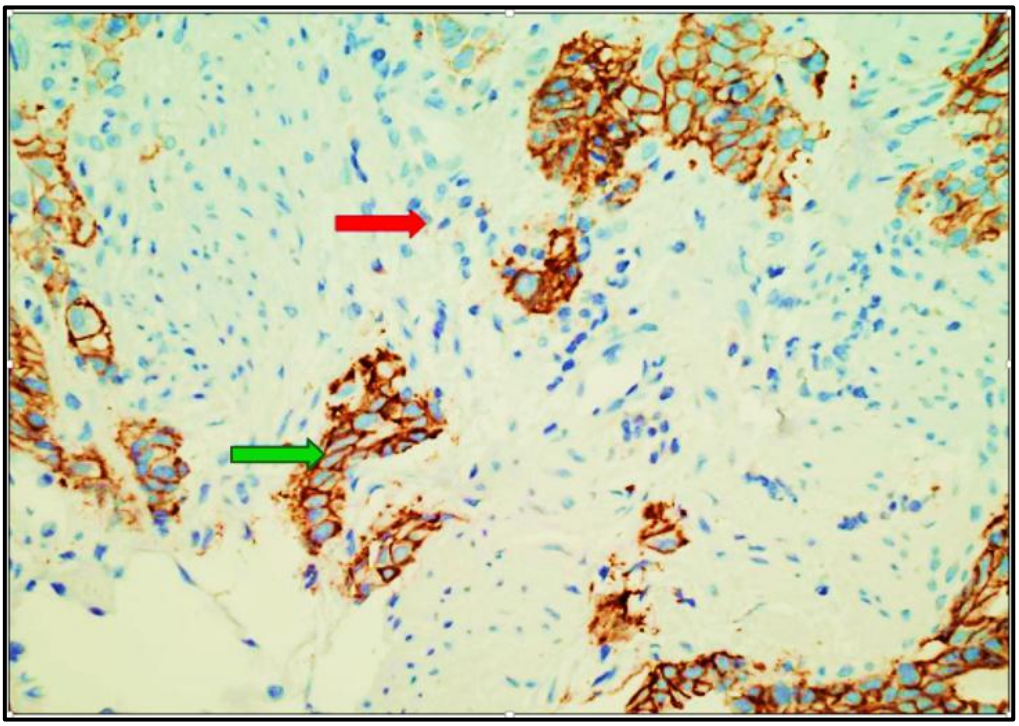

Fig. 22: Heterogenecity in Her-2 expression in mixed type gastric adenocarcinoma (Focal HER-2/neu over-expression in green and HER-2/neu negative in red arrow)

HER-2/neu and conventional treatment

Majority of the upper GIT malignancies are treated with radiotherapy or chemotherapy. Cells exposed to radiation induce different signalling pathways. Some may also induce HER-2/neu signalling. PI-3k signalling pathway (which is activated by HER-2/neu) and down streaming activated factors are suggested to be part of cellular response to radiation therapy.[82] Dreilich *et al*[76] stated that HER- 2/neu overexpression causes resistance to radiotherapy. Further, in a study by Akamatsu *et al*[81] esophageal carcinomas, HER-2/neu overexpression was associated with resistance to chemotherapy and radiotherapy. From studies in head and neck squamous cell carcinomas, addition of the HER-2/neu antibody - Trastuzumab in HER-2/neu positive cases seemed to enhance the effect of irradiation. Sato *et al*[83] reported that Trastuzumab acts synergistically with irradiation in esophageal carcinomas (both adenocarcinoma and squamous cell carcinoma).

Schoppmann *et al*[74] in their study of esophageal carcinomas observed that HER-2/neu status of patients with adenocarcinomas who had received neoadjuvent chemotherapy differed between biopsy and corresponding surgical specimen in about 25% of the cases. This can be explained due to

- Neoadjuvant chemotherapy might alter HER-2/neu status
- Sampling error

HER-2/neu overexpression correlating with clinicopathological features and overall survival

The prognosis of HER-2/neu overexpression in gastric cancer and esophageal carcinomas is controversial. Also, association of HER-2/neu positive gastric cancer with clinicopathological features is not consistent.

In a study done by Wang *et al*[84], it was observed that there was a higher frequency of HER-2/neu positivity in grade 2 carcinomas than in grade 3 because; many of the diffuse carcinomas are included in grade 3. Similar features were noted by Hu *et al*[43], Zhou *et al*[57], and Yan *et al.*[62] In the present study, 107 gastric and esophageal carcinomas were studied and HER-2/neu overexpression was independent of grade of the carcinoma.

As stated by various authors,[3, 6, 37- 38, 41, 76, 84] in their respective studies, in the present study as well, there was no correlation between the gender and the age of the patients of gastric and esophageal carcinoma with HER-2/neu overexpression.

Dreilich *et al*[76] showed that HER-2/neu 3+ score on IHC in esophageal squamous cell carcinomas is associated with poor survival whereas it had no effect on survival in patients with adenocarcinoma. Similarly in a study by Reichelt *et al*[59], HER-2/neu gene amplification was unrelated to survival, grading and pTNM staging in esophageal adenocarcinomas. Also, Schoppmann *et al*[74] studied HER-2/neu expression in esophageal carcinomas and concluded that there is no association between the overall survival and squamous cell carcinoma or adenocarcinoma. However, Moleans *et al*[37] described association of HER-2/neu overexpression with tumor invasion and tumor metastases in gastro-esophageal adenocarcinoma.

Yan *et al*[62] described a significant inverse correlation between overall survival and HER-2/neu overexpression in intestinal type of gastric adenocarcinoma. Gravalos *et al*[39] demonstrated poor 10 years survival in HER-2/neu positive gastric carcinomas. Tanner *et al*[75] and Zhou *et al*[57] also concluded HER-2/neu as a poor prognostic indicator. In the present study, patient follow-up and survival was not studied.

Jorgensen and Hersom[85] in 2012 reviewed previous studies with more than 100 patients. Forty-two publications with a total of 12,749 patients were studied. The

majority of the publications (71%) showed that HER-2/neu positive status measured either by IHC or ISH were associated with poor survival or clinicopathological features.

Based on current analysis a clear trend towards a potential role for HER-2/neu as a negative prognostic factor in gastric and esophageal carcinomas was shown, suggesting that HER-2/neu overexpression or amplification is a molecular abnormality that might be linked to the development of gastric cancer.

CONCLUSION

Endoscopy has emerged as an important diagnostic procedure in the investigation of gastrointestinal disorders. Amongst the various markers, HER-2/neu is a recent marker in the domain of upper gastrointestinal carcinomas.

Different scoring system for scoring HER-2/neu in UGI carcinomas is required because it has distinct biological behaviour as compared to breast carcinoma.

HER-2/neu overexpression is seen predominantly in adenocarcinomas especially intestinal subtype and is associated with poor patient survival. A minor percentage of esophageal squamous cell carcinomas also express HER-2/neu oncoprotein. However, there is no association between the grades of the tumor, age and sex of the patient with HER-2/neu status.

The level of HER-2/neu protein predicts well for the response of the carcinoma to monoclonal antibody, Trastuzumamb and is associated with poor prognosis.

Evaluation of HER-2/neu as a routine diagnostic work up in UGI carcinomas may the useful. So IHC is recommended as the initial testing methodology.

SUMMARY

The present study entitled "Clinicopathological Significance Of Human Epidermal Growth Factor Receptor- 2 (Her-2/neu) Over-Expression In Gastric And Esophageal Carcinomas Of Upper Gastrointestinal Endoscopic Biopsies" was a prospective study carried out in the Department of Pathology, BLDEU's Shri B. M. Patil Medical College, hospital and Research Centre, Vijayapura, between 1st December 2015 to 30th June 2017.

A total of 107 endoscopic biopsies from upper GIT that were diagnosed as gastric/ esophageal carcinoma on histopathology were included in this study, of which around 59.8% cases were from the esophagus, 37.4% cases were from the stomach while GE junction constituted only 2.8% of the cases.

These carcinomas were further subjected for immunohistochemical detection of HER-2/neu oncoprotein. Hofmann (2007) scoring system was used for assessing HER-2/neu reactivity. HER-2/neu overexpression was seen in 14.2% of gastric adenocarcinomas, 20% of esophageal adenocarcinomas and 4% of esophageal squamous cell carcinomas.

9.5% intestinal type of gastric adenocarcinoma showed HER-2neu overexpression followed by 2.3% of diffuse and 2.3% of mixed type.

There was no co-relation between age, sex and grade of the tumor with HER-2/neu overexpression.

LIMITATIONS OF THE PRESENT STUDY

- HER-2/neu overexpression of the tumor immunohistochemically was not further confirmed by ISH.
- As only endoscopic biopsy specimens were included in study, the rate of false negative cases can be high due to known heterogeneity of the HER-2/neu expression by the tumor.
- Follow up study was not possible to correlate HER-2/neu positivity with overall survival and other clinicopathologic features.

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ANNEXURES

ETHICAL CLEARANCE

B.L.D.E.UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR – 586103
INSTITUTIONAL ETHICAL COMMITTEE

No/58/2015
20/11/15

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 17-11-2015 *at* 03 pm *scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has accorded Ethical Clearance.*

Title "clinicopathological significance of human epidermal growth factor receptor-2 (HER-2/NEU) over expression in Gastric & Oesophageal carcinomas of upper Gastrointestinal biopsies"

Name of P.G. Student : Dr Lynda Dennis Rodrigues
Dept of pathology

Name of Guide/Co-investigator : Dr. S.B. Hipparagi, Professor

DR.TEJASWINI VALLABHA
CHAIRMAN

CHAIRMAN
Institutional Ethical Committee
BLDEU's Shri B.M. Patil
Medical College,BIJAPUR-586103.

Following documents were placed before E.C. for Scrutinization
1)Copy of Synopsis/Research Project
2)Copy of informed consent form.
3)Any other relevant documents.

ANNEXURES

INFORMED CONSENT FOR PARTICIPATION IN DISSERTATION/RESEARCH

I, the undersigned,_____ ,S/O or D/O _____,aged____years, ordinarily resident of _____do hereby state/declare that Dr. of _____Hospital has examined me thoroughly on _____ at _____ (place) and it has been explained to me in my own language that I am suffering from _____ disease (condition) and this disease/condition mimic following diseases . Further Doctor informed me that he/she is conducting dissertation/research titled _____under the guidance of Dr _____requesting my participation in the study. Apart from routine treatment procedure, the pre-operative, operative, post-operative and follow-up observations will be utilized for the study as reference data.

Doctor has also informed me that during conduct of this procedure like adverse results may be encountered. Among the above complications most of them are treatable but are not anticipated hence there is chance of aggravation of my condition and in rare circumstances it may prove fatal in spite of anticipated diagnosis and best treatment made available. Further Doctor has informed me that my participation in this study help in evaluation of the results of the study which is useful reference to treatment of other similar cases in near future, and also I may be benefited in getting relieved of suffering or cure of the disease I am suffering.

The Doctor has also informed me that information given by me, observations made/ photographs/ video graphs taken upon me by the investigator will be kept secret and

not assessed by the person other than me or my legal hirer except for academic purposes.

The Doctor did inform me that though my participation is purely voluntary, based on information given by me, I can ask any clarification during the course of treatment / study related to diagnosis, procedure of treatment, result of treatment or prognosis. At the same time I have been informed that I can withdraw from my participation in this study at any time if I want or the investigator can terminate me from the study at any time from the study but not the procedure of treatment and follow-up unless I request to be discharged.

After understanding the nature of dissertation or research, diagnosis made, mode of treatment, I the undersigned Shri/Smt _____ under my full conscious state of mind agree to participate in the said research/dissertation.

Signature of patient:

Signature of doctor:

Witness: 1.

2.

Date:

Place

PROFORMA FOR STUDY

- **Demographic Details:**

Name: Age: Religion:

Occupation **:** Residence **:** OPD / IPD No. :

Laboratory number:

- **Chief complaints:**

- **History of present illness:**

- **Clinical diagnosis:**

- **Histopathological diagnosis:**

Classification of tumors based on type of carcinoma:

Site	Adeno-carcinoma	Signet ring carcinoma	Squamous cell carcinoma	Un-differentiated carcinoma	Carcinoid	Round cell tumor
Oesophagus						
Gastro-esophageal junction						
Stomach						

Site wise distribution of malignancies classified according to Lauren's classification:

Site	Intestinal	Diffuse	Mixed
Stomach			
Gastro-esophageal jucnction			

Grading of carcinomas according to differentiation of tumors:

Site	Well differentiated (I)	Moderately differentiated (II)	Poorly differentiated (III)
Esophagus			
Gastroesophageal junction			
Stomach			

- **Immunihistochemistry Analysis:**

IHC Score:

Grade of cancer and HER-2/neu status:

Grade	HER-2/neu Positive	HER-2/neu Positive
I		
II		
III		

IHC scoring according to type of carcinoma:

Type of carcinoma	0	1+	2+	3+	Total
Intestinal					
Diffuse					
Mixed					

KEY TO MASTER CHART

Y	Years
M	Male
F	Female
IHC	Immunohistochemistry
Diff	Differentiated
Adeno	Adenocarcinoma
Mod	Moderately
SCC	Squamous cell carcinoma
GE	Gastro-esophageal

MASTER CHART

Sr. No	IP No	Lab No.	Sex	Age (Y)	Name	Site	Hpr diagnosis	Laurens Classification	Grade	IHC Score
1	262989/15	4802/15	M	85	Hanumath M	Esophagus	Well diff adeno	intestinal type	1	0
2	23204/15	4805/15	M	85	Sidappa M	Stomach	Mod diff adeno	Mixed Type	2	3
3	23081/15	4806/15	F	75	Bangeramma	Esophagus	Poorly diff carcinoma	-	3	0
4	27633/15	5744/15	M	60	Bhimappa	Stomach	Mod Diff adeno	diffuse type	2	0
5	28454/15	5778/15	F	72	Ningawwa N	Stomach	Signet ring cell carcinoma	diffuse type	3	0
6	28484/15	5780/15	F	75	Kalawwa L	Esophagus	Mod diff SCC	-	2	0
7	28901/15	5901/15	F	70	Balawwa	Esophagus	Poorly diff SCC	-	3	0
8	28995/15	6008/15	F	28	Sujatha Yallappa	Esophagus	Mod diff SCC	-	2	0
9	30944/15	6170/15	M	40	Basamma	Esophagus	Poorly diff SCC	-	3	0

10	32584/15	6480/15	F	60	Bhimaraya Shahapur	Stomach	Mod Diff adeno	diffuse type	2	1
11	405638/15	6893/15	F	80	Chand Rabza	Esophagus	Mod diff SCC	-	2	0
12	35741/15	6927/15	M	60	Basalingappa Kumbar	Esophagus	Poorly diff SCC	-	3	1
13	420450/15	7094/15	F	60	Laxmibai	Esophagus	Well diff SCC	-	1	0
14	38282/15	7212/15	M	72	Sidappa Biradar	GE junction	Mod diff adeno	diffuse type	2	0
15	38810/15	7298/15	M	57	Malappa K	Stomach	Mod diff adeno	diffuse type	2	1
16	38282/15	7339/15	F	63	Siddappa Biradar	GE junction	Mod diff adeno	intestinal type	1	3
17	40794/15	7643/15	M	82	Balappa Hatti	Stomach	Mod diff adeno	diffuse type	2	1
18	41745/15	7755/15	M	50	Veerapakshayya	Esophagus	Mod diff SCC	-	2	1
19	734/16	185/16	M	40	Datta Linkar	Esophagus	Mod diff SCC	-	2	0
20	1890/16	465/16	F	35	Yallawwa	Esophagus	Mod diff SCC	-	2	3

21	8857/16	1738/16	F	60	Shantabai Hadapad	Esophagus	Poorly diff adeno	intestinal type	3	2
22	8927/16	1745/16	F	45	Kashabai	Esophagus	Mod diff SCC	-	2	0
23	122453/16	2116/16	M	75	B K Kulkarni	Esophagus	Mod diff SCC	-	2	0
24	131351/16	2307/16	F	30	Kavita Gunapur	Stomach	Mod diff adeno	Mixed Type	2	2
25	143123/16	2473/16	M	40	Prakash Kasagi	Esophagus	Mod diff SCC	-	2	0
26	144572/16	2504/16	F	64	Sidawwa Ganagiri	Esophagus	Mod diff SCC	-	2	0
27	149224/16	2584/16	F	58	Dwarakabai	Esophagus	Mod diff SCC	-	2	0
28	158322/16	2745/16	M	58	Irayya Swami	Esophagus	Mod diff SCC	-	2	0
29	160841/16	2794/16	M	70	Shantgouda	Esophagus	Well diff SCC	-	1	0
30	162602/16	2830/16	M	63	Chandbasappa Pujari	Esophagus	Mod diff SCC	-	2	0
31	19628/16	3637/16	F	60	Janabai	Stomach	Mod diff adeno	Mixed Type	2	2

32	183059/16	3128/16	F	45	Kashabai	Esophagus	Mod diff SCC	-	2	0
33	183351/16	3158/16	M	50	Sangappa	Esophagus	Mod diff SCC	-	2	3
34	184733/16	3191/16	M	65	Raghunath More	Esophagus	Mod diff SCC	-	2	0
35	190650/16	3283/16	M	83	Niingangouda	Esophagus	Mod diff SCC	-	2	0
36	192886/16	3309/16	M	65	Chanagondappa	Esophagus	Poorly diff SCC	-	3	0
37	194236/16	3329/16	F	40	Shantawwa Ullagaddi	Esophagus	Poorly diff SCC	-	3	0
38	199064/16	3430/16	F	60	Janabai Kale	Stomach	Papillary adeno	intestinal type	1	2
39	18712/16	3456/16	F	35	Sumitra	Stomach	Well diff adeno	intestinal type	1	2
40	53311/17	304/17	M	32	Basavaraj	Esophagus	Well diff SCC	-	1	-
41	220232/16	3798/16	M	68	B K Kulkarni	Esophagus	Mod diff SCC	-	2	0
42	20386/16	3803/16	F	40	Anil Rathod	Esophagus	Mod diff SCC	-	2	0
43	95880/16	3811/16	M	65	Ishwarappa	Esophagus	Mod diff SCC	-	2	0

44	227585/16	3902/16	M	60	Yamanappa Ullagaddi	Esophagus	Mod diff SCC	-	2	0
45	22888/16	3930/16	F	58	Renuka Pujari	Esophagus	Mod diff SCC	-	2	0
46	234869/16	4026/16	F	70	Mallawwa Depagal	Esophagus	Mod diff SCC	-	2	0
47	420703/16	4121/16	M	72	Chandrashekhar	Esophagus	Mod diff SCC	-	2	0
48	22324/16	4125/16	F	72	Neelamma	Esophagus	Mod diff adeno	diffuse type	2	3
49	223547/16	4139/16	M	25	Mahesh	Esophagus	Mod diff SCC	-	2	0
50	15052/16	2849/16	F	35	Sumitra	Stomach	Well diff adeno	intestinal type	1	0
51	14431/16	2850/16	F	30	Roopa	Stomach	Well diff adeno	intestinal type	1	2
52	254189/16	4340/16	F	62	Shobha	Esophagus	Mod diff SCC	-	2	0
53	23602/16	4341/16	M	58	Jaganam	Esophagus	Small round blue cell tumor	-	-	0
54	139816/17	2719/17	F	42	Rukayya	Stomach	Poorly diff adeno	diffuse type	3	0

55	17736/17	2756/17	M	30	Subhash	Esophagus	Adenosquamous	-	-	0
56	17652/17	3632/17	M	60	Madivalayya	Stomach	Signet ring cell carcinoma	diffuse type	3	0
57	139821/17	2721/17	F	30	Roopa	Stomach	Poorly diff adeno	diffuse type	3	1
58	262947/16	4460/16	M	68	Ramalingappa	Esophagus	Mod diff SCC	-	2	0
59	28860/16	5764/16	M	70	Gurappa	Stomach	Poorly diff carcinoma	-	3	0
60	335250/16	5768/16	F	38	Tangamma	Stomach	Mod diff adeno	Mixed Type	2	0
61	335247/16	5766/16	M	47	Anarayya	Esophagus	Mod diff adeno	diffuse type	2	0
62	333227/16	6054/16	M	65	Sidappa T	Esophagus	Mod diff SCC	-	2	0
63	30321/16	6057/16	F	55	Girija K	Esophagus	Mod diff SCC	-	2	0
64	8857/16	1738/16	F	70	Champabai	Esophagus	Mod diff SCC	-	2	0
65	8927/16	1745/16	F	34	Zubeda	Esophagus	Mod diff SCC	-	2	0
66	359057/16	6527/16A	F	39	Vijayalaxmi C	Stomach	Well diff adeno	intestinal type	1	3

67	359057/16	6527/16B	F	39	Vijayalaxmi C	Stomach	Mod diff adeno	diffuse type	2	0
68	359059/16	6525/16	F	32	Savitri	Esophagus	Signet ring cell carcinoma	diffuse type	1	0
69	18712/16	3456/16	M	42	Hanumanthray	Stomach	Mod diff adeno	diffuse type	1	0
70	15052/16	2849/16	F	53	satyawwa	Stomach	Mod diff adeno	diffuse type	1	0
71	14431/16	2850/16	M	82	siddarayya	Stomach	Mod diff adeno	diffuse type	1	0
72	34791/16	6718/16	M	75	Hanadu	Esophagus	Mod diff adeno	diffuse type	2	0
73	35027/16	6722/16	F	45	Mahadevi N	Esophagus	Well diff SCC	-	1	0
74	37026/17	7093/16	M	64	Chandrashekar K	Stomach	Mod diff adeno	diffuse type	2	0
75	432412/17	7820/16	M	75	VS Hiremath	Stomach	Mod diff adeno	diffuse type	2	0
76	424241/17	7715/16	F	75	Shakuntala	Esophagus	Mod diff SCC	-	2	0
77	42229/17	7881/16	M	70	Chandrashekhar	Esophagus	Poorly diff carcinoma	-	3	0
78	424238/17	7717/16	F	45	Indrabai M	Stomach	Mod diff adeno	diffuse type	2	1

79	439788/17	7999/16	M	65	Sangappa J	GE junction	Poorly diff adeno	diffuse type	3	0
80	445955/17	8079/16	F	39	Marityamma	Esophagus	Poorly diff carcinoma	-	3	2
81	445954/17	8051/16	M	76	Siddangouda	Stomach	Mod diff adeno	intestinal type	2	3
82	441505/17	8041/16	M	55	Adiveppa	Stomach	Poorly diff adeno	diffuse type	3	0
83	53311/17	980/17	F	48	Laila B	Esophagus	Poorly diff carcinoma	-	3	0
84	49567/17	918/17	M	30	Ningappa	Esophagus	Poorly diff carcinoma	-	3	0
85	53311/17	304/17	M	70	Lata Kattimani	Esophagus	Poorly diff carcinoma	-	3	0
86	432412/17	7820/16	M	75	VS Hiremath	Stomach	Mod Diff adeno	diffuse type	3	0
87	154356/17	2622/17	F	45	Shantawwa	Stomach	Mod diff adeno	Mixed Type	2	2
88	461007/16	8124/16	M	56	Moulali	Stomach	Poorly diff adeno	diffuse type	3	0
89	372740/16	6808/16	F	70	Shantabai	Esophagus	Mod diff SCC	-	2	0
90	185253/17	3637/16	F	50	Janabai	Stomach	Mod diff adeno	Mixed Type	2	0
91	171776/17	3359/17	F	70	Sonawwa	Esophagus	Poorly diff SCC	-	3	0

92	181237/17	3506/17	F	80	Damawwa	Esophagus	Poorly diff SCC	-	3	0
93	115309/17	2220/17	F	80	Badibee K	Esophagus	Well diff SCC	-	1	0
94	119121/17	2250/17	F	52	Rajiya	Stomach	Signet ring cell carcinoma	diffuse type	3	0
95	4529/17	995/17	M	58	Chidannad	Esophagus	Mod diff SCC	-	2	0
96	4169/17	965/17	M	85	Sidarayya	Stomach	Mod diff adeno	intestinal type	2	3
97	49563/17	952/17	M	61	Yashwanthrai	Esophagus	Well diff SCC	-	1	0
98	5730/17	114/17	M	60	Hanumanthray	Esophagus	Mod diff SCC	-	2	0
99	10298/17	2157/17	F	45	Savitri	Esophagus	Mod diff adeno	diffuse type	2	0
100	108444/17	2076/17	F	48	Shankarewwa M	Stomach	Mod diff adeno	diffuse type	2	0
101	165893/17	3230/17	F	70	Champabai	Esophagus	Basaloid SCC	-	1	0
102	266560/17	5306/17	F	34	SV Kase	Stomach	Well diff adeno	intestinal type	1	2
103	124712/17	2409/17	F	34	Zubeda	Stomach	Well diff adeno	Mixed Type	1	0

104	115609/17	2471/17	F	84	Manowwa	Esophagus	Poorly diff SCC	-	3	0
105	266881/17	5305/17	F	35	Laxmibai	Stomach	Mod diff adeno	Mixed Type	2	0
106	156522/17	2610/17	F	42	Rukayya	Stomach	Poorly diff adeno	intestinal type	3	1
107	265229/17	4242/17	M	60	Revappa H	Stomach	Signet ring cell carcinoma	diffuse type	3	0