A Dissertation on

"ANALYSIS OF HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR -2 EXPRESSION IN GASTRIC ADENOCARCINOMA"

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CERTIFICATE

This is to certify that this dissertation entitled **"ANALYSIS OF HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR -2 EXPRESSION IN GASTRIC ADENOCARCINOMA"** is the bonafide record of the research work done by **Dr. V. LOKESH KUMAR,** submitted , in partial fulfilment of the regulations laid down by the TamilnaduDr.M.G.R. Medical University, Chennai, for M.D. (Pathology) Degree Examination to be held in April 2017.

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DECLARATION

I, **Dr.V. LOKESH KUMAR,** declare that I carried out this work on**"ANALYSIS OF HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR -2 EXPRESSION IN GASTRIC ADENOCARCINOMA"** at Department ofPathology, Government Kilpauk Medical College Hospital . I also declare that this bonafide work or a part of this work was not submitted by me or any other for any award, degree, and diploma to any other university.

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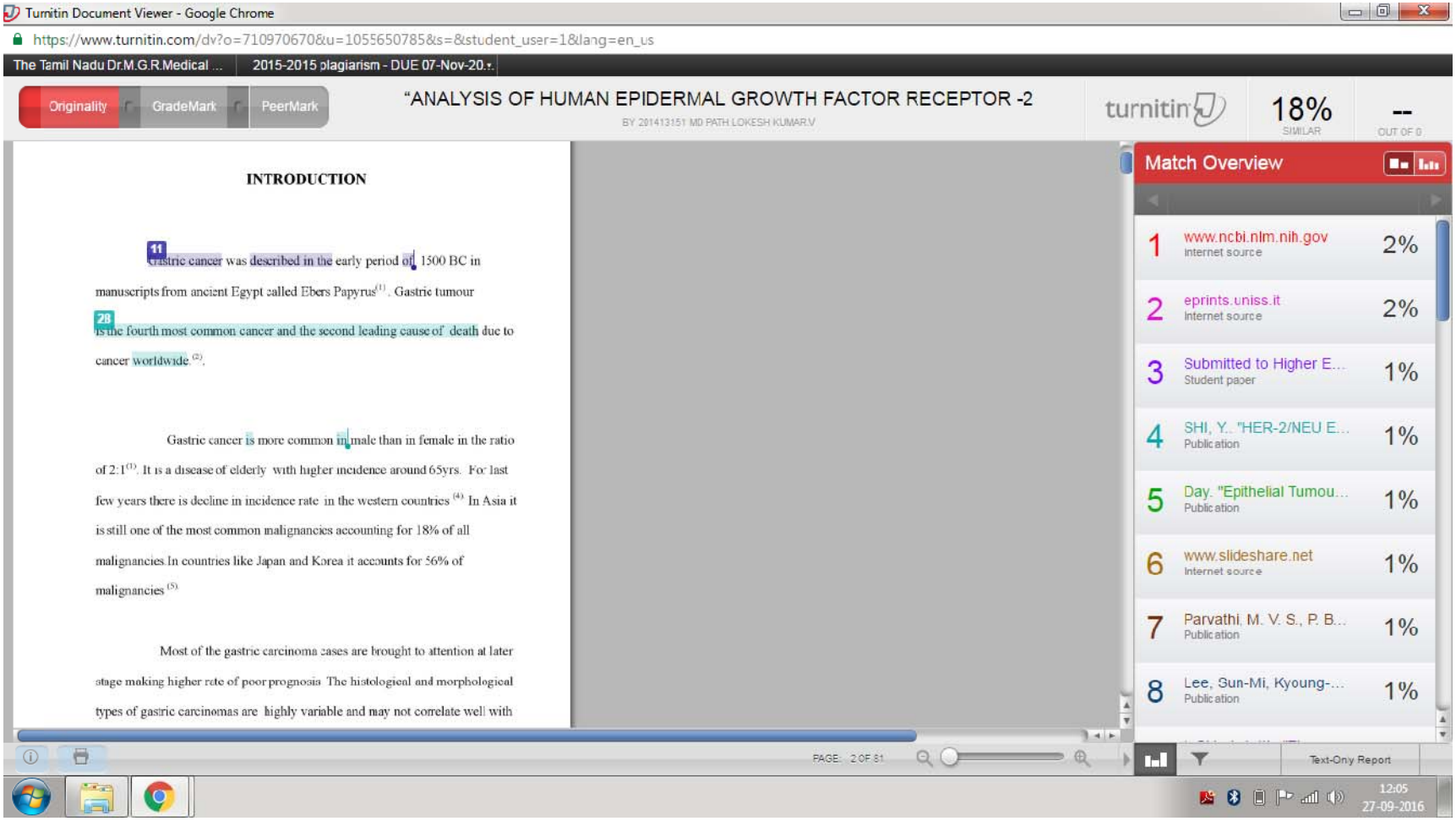
CONTENTS

ABBREVIATIONS

EGFR	:	Epidermal Growth Factor Receptor
HER-2	:	Human epidermal growth factor receptor -2
WHO	:	World Health Organization
MLH - 1	:	MutL Homolog – 1gene
hPMS:	human Protein Homolog gene	
hMSH	:	human MutS Homolog gene
AJCC	:	American Joint Committee on Cancer
cDNA	:	Complementary Deoxyribo Nucleic Acid
PCR	:	Polymerase Chain Reaction
FISH	:	Fluorescent In Situ Hybridisation
IHC	:	Immunohistochemisrty
HRP	:	Horse Radish Peroxidase
GIST	:	Gastro Intestinal Stromal Tumour
CI	:	Confidence Interval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Gastric cancer was described in the early period of 1500 BC in manuscripts from ancient Egypt called Ebers Papyrus(1). Gastric tumour
28
is the fourth most common cancer and the second leading cause of death due to cancer worldwide.(2).
Gastric cancer is more common in male than in female in the ratio of 2:1(3). It is a disease of elderly with higher incidence around 65yrs. For last few years there is decline in incidence rate in the western countries (4). In Asia it is still one of the most common malignancies accounting for 18% of all malignancies.In countries like Japan and Korea it accounts for 56% of malignancies (5).
Most of the gastric carcinoma cases are brought to attention at later stage making higher rate of poor prognosis. The histological and morphological types of gastric carcinomas are highly variable and may not correlate well with
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INTRODUCTION

Gastric cancer was described in the early period of 1500 BC in manuscripts from ancient Egypt calledEbers Papyrus[1] . Gastric tumouris the fourth most common cancer and the second leading cause of death due to cancer worldwide.[2].

Gastric cancer is more common in malethan in female in the ratio of 2:1[1]. It is a disease of elderlywith higher incidence around 65yrs. For last few years there is decline in incidence rate in the western countries [4]. In Asia it is still one ofthe most common malignancies accounting for 18% of all malignancies.In countries like Japan and Korea it accounts for 56% of malignancies [5].

Most of the gastric carcinoma cases are brought to attention at later stage making higher rate of poor prognosis. The histologicaland morphological types of gastric carcinomas are highly variable and may notcorrelate well with the prognosis of the patients.[15]

The poor prognosis of gastric adenocarcinoma is due to its late presentation, nonspecific symptoms like dyspepsia in early stage and limitations in treatment options. Molecular markers are vital in determining the disease progression and hence disease outcome, survival and prognosis.[15] An association between clinicopathologicalfeatures and molecular markers of

gastric adeno carcinomawould give a clue toward the relationship between them and hence provide usan extra tool to combat the high mortality due to these carcinomas[8].

HER-2/Neu receptor also known as c-Erb-2, encodes a transmembrane tyrosine kinase receptor;which is similar to epidermal growth factor receptor.Protein encoded by this gene is a growth factor receptor involved in growth and metastasis of malignant cells [10].

Though many studies have been conducted in gastric carcinoma all over the world for the expression of HER-2/Neu and their prognostic significance, the results are still contradictory. Some found a statistically significant association of these markers with prognosis and survival, while others found no such association.

Targeted therapy toward HER-2/neucan be justifiedonly when sufficient data regarding the role of these moleculesin gastric adenocarcinoma is available.

The aim of this study was to find the prevalence of HER-2/neu expression in Gastric adeno carcinoma and to correlate it with various clinico-pathologicalvariables

AIMS AND OBJECTIVES

1. To study the various clinicopathological factors of gastricadenocarcinoma including age of incidence, sex predilection, location of tumour, clinical features, Endoscopic appearance, gross appearance and histologic grade.

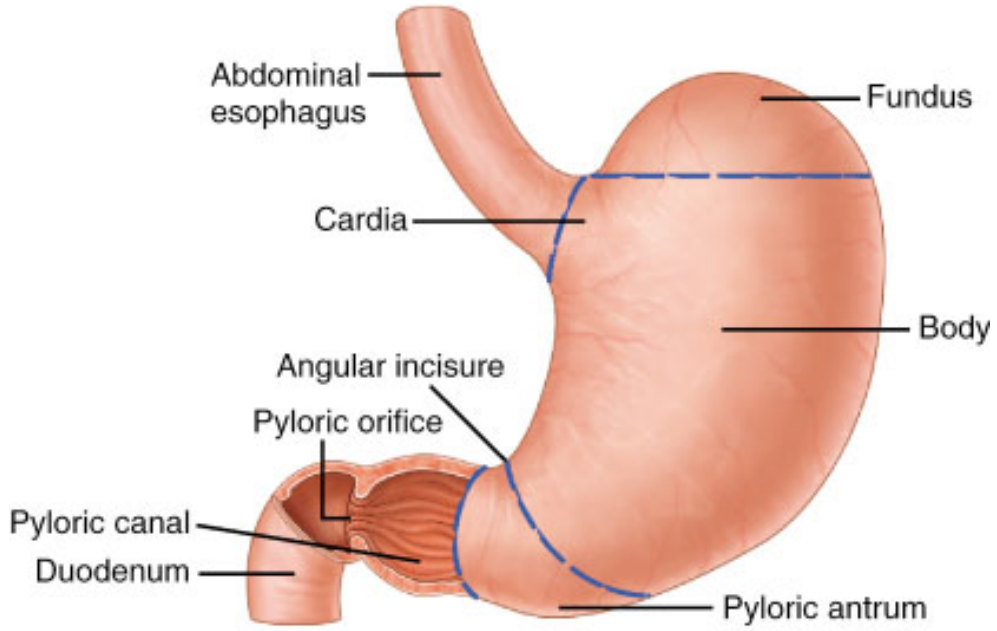
2. To determine the immunohistochemical expression of HER-2/Neu in Gastric adenocarcinoma.

3. To study the association of HER-2/Neu in Gastric adeno carcinoma with known prognostic factors like age, sex, histological grade and other variables like gross appearance and type of specimen received.

4. To study the prognostic significance of HER-2/Neu in Gastric carcinoma and its association with survival

REVIEW OF LITERATURE

Gastric adenocarcinoma is a malignant neoplasm arising from the glandular epithelial lining of stomach mucosa. The stomach is divided grossly into the following regions: *cardia*, *fundus*, *corpus* or *body*, (*pyloric*) *antrum*, and *pylorus*[(9)]. The superomedial margin is termed the *lesser curvature* and the inferolateral margin is termed the *greater curvature*. The junction between the corpus and the antrum on the serosal aspect ,in the lesser curvature is called as *incisura*. The mucosal folds are called as *rugae*[(9)]



These anatomic regions show some correspondence to the three traditionally recognized microscopic types of gastric mucosa : cardiac , fundic and pyloric (antral) . All of these types of gastric gland are comprised of two major components foveola and secretory portion. The foveolae represent the

most important area for the genesis of gastric carcinoma, in particular the layer of generative (stem) cells located at their base.

Epidemiology:

The incidence of gastric adenocarcinoma is increasingwith age and the peak incidence occurs at 60-80 years[(1)]. Incidence of age younger than 30 years are very rare. In India, the age range for stomach cancer is 35-55 years in the South and 45-55 years in the North. The disease shows a male preponderance in the ratio of 2:1 to 4:1.[(5)]

Gastric adenocarcinoma develops both in the proximal and the distal region. Incidence of distal gastric cancers is more in developing countries, blacks, and lower socio-economic groups[(7)]. Dietary factors and H. pylori infection are major risk factors for the development of distal tumors. Proximal tumors are more common in developed countries, whites, and in higher socio-economic classes[(9)]. The risk factors for proximal cancers are gastroesophageal reflux disorder and obesity. Recently prevalence of proximal tumors in the rest of the world is increased according to studies conducted.

The highest incidence of gastric adenocarcinoa was noted in Eastern parts of Asia and Europe, and South America, while North America and Africa show the lowest recorded rates[(8)]. Approximately 934,000 cases are detected each year . Japan and Korea have the highest gastric cancer rates in the world.

The Linxian province in China has highest incidence rate of gastric cardia cancer in the world.

In India, southern and north-eastern states has higher incidence of gastric carcinomawith Mizoram being highest.In view of study conducted by the National Cancer Registry in 2001, the number of new gastric adenocarcinoma cases were estimated to be approximately 35,675. The incidence rate of gastric adenocarcinomas was four times higher in Southern India compared with Northern India.

The age-standardized incidence rates in Chennai were 13.6 per 100,000 in male and 6.5 per 100,000 in female. The rates in rural population are lower than the urbanpopulation. Early gastric cancer has a higher five year survival rate (up to95%) than those of advanced gastric cancer (10% -20%).A recent assessment of 556 400 deaths due to cancer in India in 2010 based on a nationally representative survey found that stomach cancer with a mortality rate of 12.6% is the second most common fatal cancer.

Clinical features:

Gastric adenocarcinomas have non-specific symptoms likeepigastric distress or pain, vomiting or regurgitation, hematemesis, melena, anorexia,weight loss and fatigue[9]. Early gastric cancers are usually

asymptomatic. Mostly proximal gastric cancer causes dysphagiaand distal gastric cancer causes gastric outlet obstruction.

Etiopathogenesis:

Etiology of gastric cancer is multifactorial. The risk factors associated are diet, lifestyle, genetic, socioeconomic status and other factors contribute to gastric carcinogenesis.

Diet:

Most consistent association with dietary factor is observed inintestinal type of gastric carcinoma. Fresh fruits and vegetables lower therisk due to the antioxidant actions of ascorbic acid, carotenoids, folates,tocopherols[(9).] Salt intake, smoked foods, pickled vegetables, chillipepper are found to be associated with high risk.

Helicobacter pylori infection:

"H.Pylori is a Gram-negative microaerophilic, spiral bacterium seen in the mucosa of stomach in those with severe &chronic atrophic gastritis .Many studies showed evidence of strong association with H.Pyroliinfection.Gastricadenocarcinoma had anti H Pylori antibodies in their serum stored 10 years before the diagnosis of cancer[(9)"]

“*H. pylori* causes sequential progression of normal gastric epithelium into atrophic gastritis, intestinal metaplasia, and dysplasia to carcinoma. The bacterium produces several products like urease that cause gastric mucosal damage . *H. pylori* disrupts gastric barrier function *via* urease-mediated myosin II activation.The formation ofsevere gastritis with atrophy and intestinal metaplasia is correlated with infection by CagA-positive strains of the bacillus[(9).]”

Hypochlorhydria:

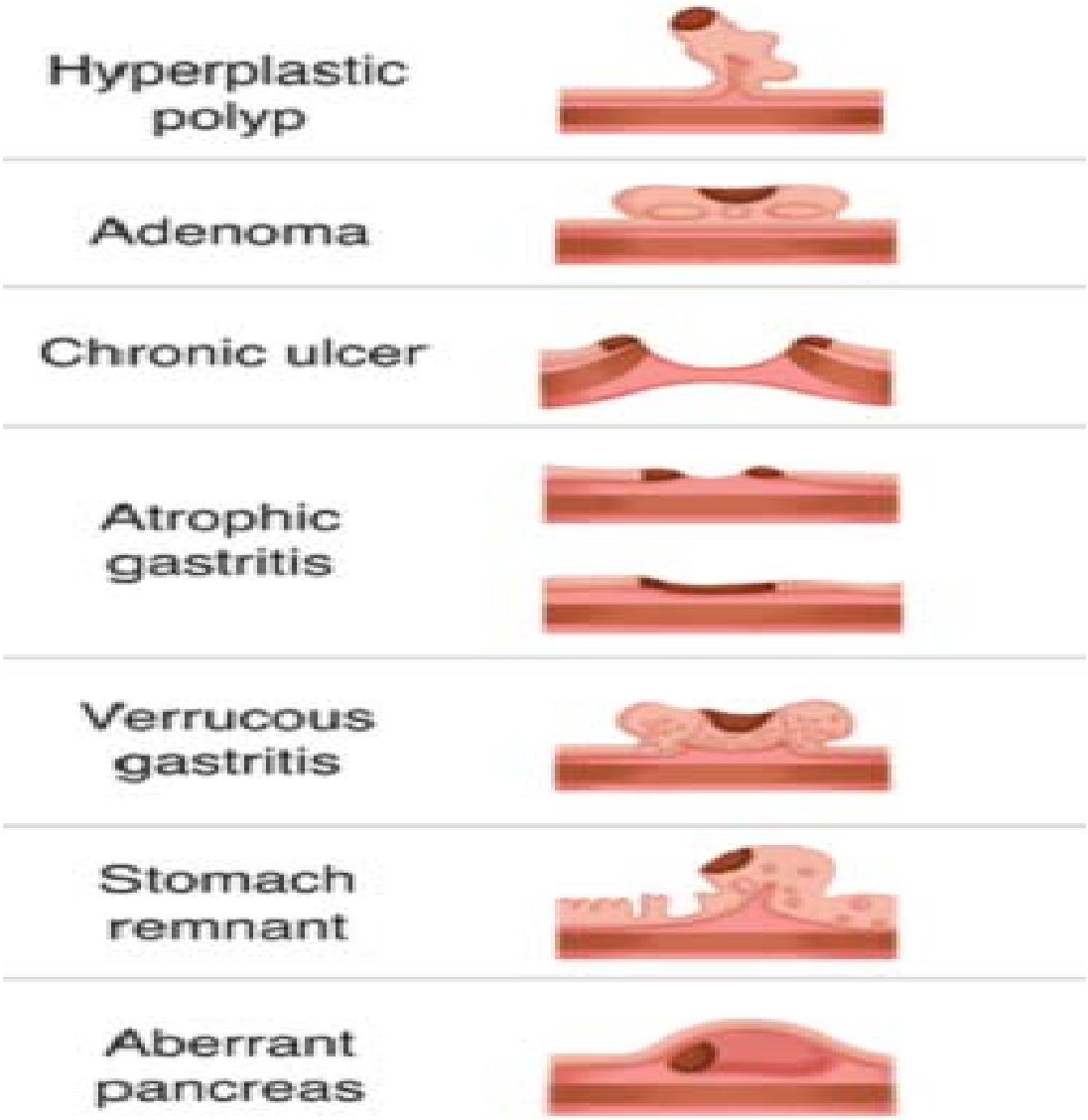
Gastric carcinoma is associated with hypochlorhydria in 85–90% of the cases. Hypochlorhydriapromotes the growth of bacteria which were thought to reduce dietary nitrate to nitrite and convert dietary amines into carcinogenic *N*-nitroso compounds[(9)].

Molecular genetics:

“Allelic loss has been identified at a variety of loci on various chromosomes. The earliest molecular events appear to be methylation and silencing of genes such as P16, MLH1, MGMT, and Runx 3 . These events are not specific to the histologic subtypes of carcinoma, although a loss on 7q is associated with peritoneal metastases. Microsatellite instability is encountered in 10% to 44% of cases, and tends to occur more frequently in antral intestinal carcinomas that are characterized by low clinical stage, less frequent lymph

node metastases, and better clinical prognosis[10] . Gastric carcinoma is now regarded as a component of the Lynch syndrome (hereditary nonpolyposis colorectal cancer) . Germline E-cadherin mutations had been detected infamilies with hereditary diffuse gastric adenocarcinoma .”

PRECURSOR LESIONS OF GASTRIC CANCER:



EARLY GASTRIC CANCER[17,18,19]

This term was first coined in the Japanese literature to describe infiltrating adenocarcinomas in which the growth is confined to the mucosa or submucosa of the stomach with or without lymph node metastasis. Early gastric adenocarcinoma is not the same as carcinoma in situ or gastric dysplasia conditions in which tumor cells have not penetrated the basement membrane and have no metastatic potential. Some cases of early gastric cancer may have isolated local lymph node metastases or even hepatic metastases, but most cases are still potentially curable by surgery.

A subclassification of the gross appearances of early gastric cancer was devised by the Japanese Gastroenterological Endoscopic Society.[18]

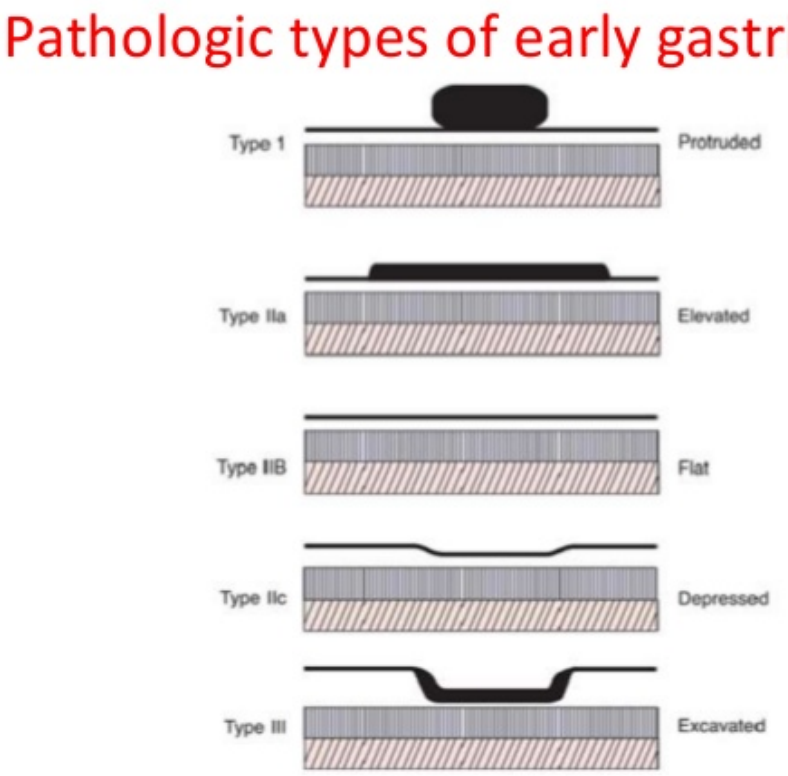
- Type I Exophytic lesion extending into the gastric lumen
- Type II Superficial variant

IIA Elevated lesions with a height no more than the thickness of the adjacent mucosa

IIB Flat lesions

IIC Depressed lesions with an eroded but not deeply ulcerated appearance

- Type III Excavated lesions that may extend into the muscularispropria without invasion .



These terminologies correlates weakly with microscopic appearances and prognosis. Early gastric cancer is mainly identified in the distal stomach, particularly along the lesser curve . The incidence of multicentricity has been estimated at 10%[(19)]. Most tumors are 2 cm or less in diameter, although cases as large as 8 cm have been described. The histology of early gastric cancer is similar to that of advanced cancer, with intestinal, diffuse, and mixed forms described.

For intramucosal tumour, the cure rate is 93% when no regional lymph node metastases are present, and 91% when they are present. For early cancers with submucosal involvement, the overall cure rate is 89%, which is 80% in cases with lymph node metastases .

Advanced Gastric adenocarcinoma:

When the tumour invades beyond submucosa of stomach wall, it is called as advanced gastric carcinoma. It implies that resection and cure of the tumour is difficult and does not indicate that the tumour is of higher stage.

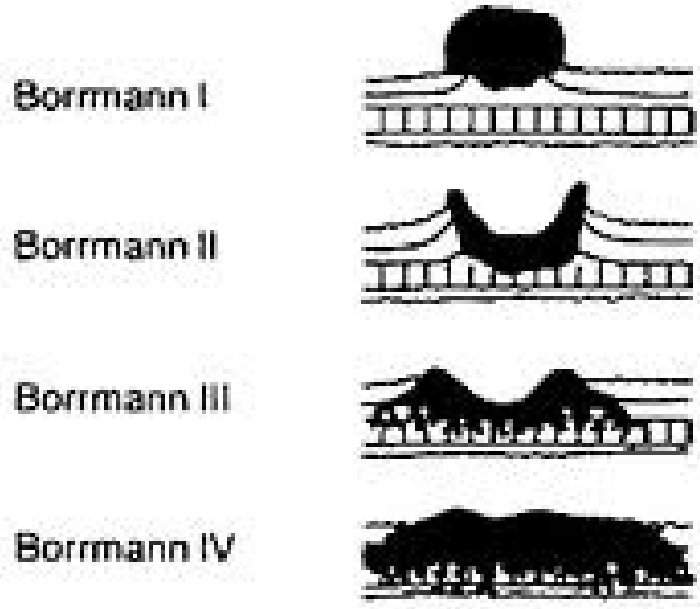
Dr.R.Borrman classification (Based on gross appearance)[9]

Type I – Polypoid / Nodular

Type II – Fungating

Type III – Ulcerative

Type IV – Diffusely infiltrative (linitisplastica)



Ulcers are common in the antrum, on the lesser curve. The ulcers are large with irregular margins, raised rolled out edges, necrotic shaggy base. Fungating and nodular tumours are common in the body of the stomach or fundus. Infiltrative tumours spread superficially, producingplaque-like lesions that causes thickening of the entirestomach wall producing the so-called linitisplastica (leather bottle)stomach.Gelatinous appearance occurs in tumours producing mucin.

Several classifications based on the histological picture exist forgastric carcinoma. A few of the commonly used ones are the following

Lauren's classification: (1965)[23]

Lauren divides gastric adenocarcinoma into two main types –

1) Intestinal

2) Diffuse.

Those with approximately equal portion of intestinal and diffuse components and those too undifferentiated are called indeterminate/unclassified carcinomas. Of the 1344 tumours initially described by Lauren, 53% were intestinal type, 33% were diffuse type, and others were indeterminate/unclassified type.[23]

Intestinal carcinoma:

This type is common in males and older age group.They have a glandular pattern with tubules, papillary formation or solid components. The glands are of welldifferentiated to moderately differentiated grade. The epithelium consists of pleomorphiccells with large hyperchromatic nuclei . The adjacent mucosa often shows chronic gastritis, intestinal metaplasia or dysplasia.

Diffuse carcinoma:

This type is common in younger age group and composed of dyscohesive and diffusely infiltrating tumour cells with indistinct cytoplasm and hyperchromatic nuclei. Desmoplasia is more pronounced and there is no accompanying dysplasia or metaplasia.

Mulligan and Rember classification(1975)

Extends the Lauren classification with third type , pyloric gland carcinoma. They are commoner in men than women. Histologically shows glands with tubular or papillary pattern containing cells showing vacuolation that stain well with PAS stain.

Ming's Classification (1977)[25]:

Ming classifies gastric tumour into 2 types

Expanding type

Infiltrating type

Expanding type tumurs are with pushing margins and tumour nodules.

Infiltrative type tumous are ill defined with widely infiltrative tumour cells and collagenous stroma. It is more common under the age of 50.

WHO Classification (2010[28]):

WHO classification of Gastric tumours is givenas follows,

1) EPITHELIAL TUMOURS

PRE MALIGNANT LESIONS

Intraepithelial neoplasia (dysplasia) , low grade

Intraepithelial neoplasia (dysplasia) , high grade

Adenoma

CARCINOMA

Adenocarcinoma

Papillary adenocarcinoma

Tubular adenocarcinoma

Mucinous adenocarcinoma

Poorly cohesive carcinoma (including Signet-ring cell carcinoma)

Adenosquamous carcinoma

Carcinoma with lymphoid stroma (medullary carcinoma)

Hepatoid carcinoma

Squamous cell carcinoma

Small cell carcinoma

Undifferentiated carcinoma

Neuro endocrine tumours (NET)

- NET G1 (carcinoid)
- NET G2

Neuroendocrine Carcinoma (NEC)

- Large cell NEC
- Small cell NEC

Mixed adenoneuroendocrine carcinoma

EC cell serotonin producing NEC

Gastrin producing NET (Gastrinoma)

2) **MESENCHYMAL TUMOURS**

Leiomyoma

PlexiformFibromyxoma

Granular cell tumour

Glomus tumour

Leiomyosarcoma

GI stromal tumour

Kaposi sarcoma

Synovial sarcoma

3) **LYMPHOMAS**

4) **SECONDARY TUMOURS**

The Goseki Classification (1992) :

Based on the degree of tubular differentiation and the amount of intracellular mucin present,

Group I - well differentiated tubules & low intracellular mucin

Group II - well differentiated tubules & plenty intracellular mucin

Group III - poorly differentiated tubules & low intracellular mucin

Group IV - poorly differentiated tubules & plenty intracellular mucin

Carneiro Classification (1997) :

1) Glandular,
2) Isolated cell carcinomas,
3) Solid variety
4) mixed type

Grading Of Gastric Carcinoma[9]:

1)Well differentiated:

shows well-formed glands, often resembling metaplastic intestinal epithelium.

2)Moderately differentiated:

intermediate between well differentiated andpoorly differentiated.

3)Poorly differentiated:

shows highly irregular glands that are recognized

with difficulty, or shows single cells that remain isolated or arranged in clusters with mucin secretions.

Spread and metastases [9]

Distal carcinomas of the stomach invade the duodenum and tumours of proximal stomach involve the esophagus. Serosal spread is common in infiltrative tumours than expanding types.Local extension occurs in omentum, colon, pancreas, and spleen. The rich mucosal and submucosal (Borrman) lymphatic plexus of the stomach is often invaded and cause the tumor to spread to perigastric, periaortic, and celiac axis nodes.Tumors of the distal third often involve hepatoduodenal nodes. Mucosal lymphangiectasia is found to be statistically associated with the presence of regional lymph node metastases. Invasion of the blood vessel walls by the tumor (‘*vasculitiscarcinomatosa*’) can also occur.

The most frequent sites of distant metastases are , peritoneum, liver, adrenal gland, lung and ovary. Bilateral ovarian metastases from gastric carcinoma is known as *Krukenbergtumor*. Metastases can also develop in the uterine body and cervix. Cutaneous metastases of gastric carcinoma can be produced by gastric tumours.

The diffuse type of gastric carcinoma shows more frequent involvement of peritoneum, lungs, and ovary.Liver metastases are more common with intestinal-type tumors.

STAGING OF GASTRIC TUMOURS[10]

TNM Staging of Gastric Carcinoma

Tis	Carcinoma in situ
T1a	Tumor invades lamina propria
T1b	Tumor invades submucosa
T2a	Tumor invades muscularispropria
T2b	Tumor invades subserosa
T3	Tumor penetrates visceral peritoneum
T4	Tumor invades adjacent structures
N0	No regional nodes involved
N1	Tumor involves 1-6 regional nodes
N2	Tumor involves 7-15 regional nodes
N3	Tumor involves more than 15 regional nodes
M0	No distant metastases
M1	Distant metastases present

PROGNOSIS:

"The prognosis of gastric carcinoma varies from country to country.The overall survival rate in the Western countries is 4 to 13% which is poor compared to Japan which shows the best results with an overall 5-year survival rate of 89% for early carcinoma and 46% for advanced carcinoma. This is atleast partly by the greater frequency of superficial carcinomas, and aggressive Japanese surgical approach to treatment with extensive and meticulous lymph node dissection."

PROGNOSTIC FACTORS[9]:

Prognostic factors includeclinical factors, morphological factors and/or genetic / molecular factors.

The clinical factors which indicate poor prognosis are young age and proximal location of gastric cancers[24]. Some of the important Pathologic factors are as follows,

1. *Tumour size:* Small size is associated with a better prognosis but this isclosely linked to depth of penetration.

2. *Tumour stage:* This is the most significant prognostic factor. Depth of invasion is considered in staging which is directly proportional to the chance of distant metastasis.

3. *Microscopic type and grading:* Intestinal type tumours has relatively better prognosis than diffuse types.

4. *Lymphocytic response:* Presence of inflammatory infiltrate at tumour and normal tissue interface is associated with good patient survival.

5. *Lymphovascular invasion:* Indicates infiltration of tumour cells into vascular spaces increasing the risk of recurrence and distant metastasis.Hence associated with poor prognosis.

6. *Perineural invasion:* It is associated with poor prognosis.

7. *Regional lymph node involvement*: When nodal involvement is present, 5 Year survival rate drops to below 10% and it is 50% in the node negative cases. The number of nodes involved is also significant. Overallsurvival rate decreases as the number of positive node increases.

Other factors found to have poor prognosis are tumour necrosis, infiltrative margins of tumour and involvement of surgical margins

Molecular biomarkers play an important prognostic role in gastric carcinoma management. These markers are HER -2 , E- Cadherin , P-53. Aneuploidy has been reported in about 40–50% of gastric carcinomas and show lower survival rates compared to diploidcancers. Over expression of HER-2/Neu which is a transmembraneepidermal growth factor receptor protein

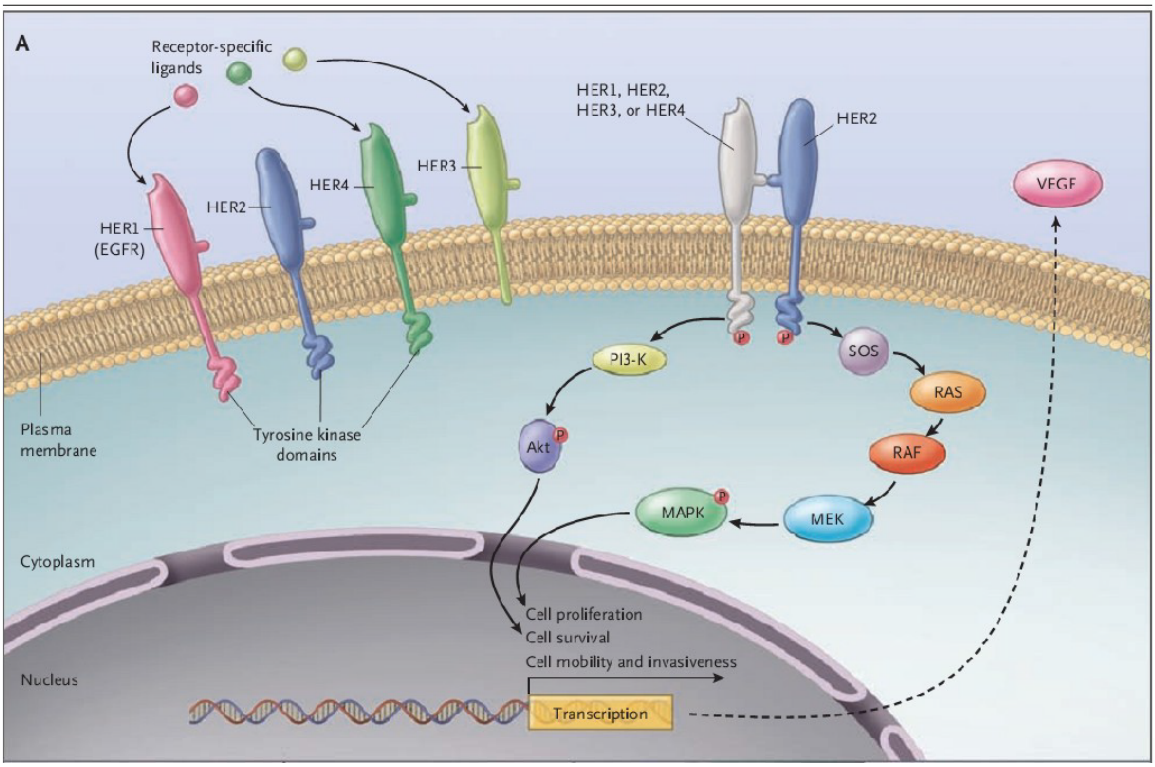
is reported to have poorer prognosis, but some other studies showed no such association. p53 protein over expression is associated with decreased survival. E-cadherin, a transmembrane protein plays a significant role in maintenance of intercellular connections. Mutations in Ecadherin gene is associated with aggressive behaviour.. Increased proliferation indices like Ki-67 are shown to be associated with reduced survival.

HER2 protein and gene

"The human epidermal growth factor receptor family of receptors plays a central role in the pathogenesis and treatment of several human cancers[(11)]. They regulate cell growth, survival, and differentiation by way of multiple signal transduction pathways and play a role in cellular proliferation and differentiation. HER1 (EGFR), HER2, HER3 (ErbB-3), and HER4 (ErbB-4) are four members of this gene family.All four HER receptors comprise a cysteine-rich extracellular ligand binding site and intracellular domain with tyrosine kinase activity."

The Her -2 / neu oncogene was found by scientists at Massachusetts Institute of Technology, Rockefeller, and Harvard University .The binding of various ligands to the extracellular domain causes a signal transduction cascade that can control cell proliferation, apoptosis, differentiation.adhesion, and migration.

“The HER2 receptor is a 1255 amino acid, 185 kDtransmembrane glycoprotein located at the long arm of human chromosome 17 (17q12) . HER2 is expressed in many tissues including the breast,Kidney, gastrointestinal tract, heart and its major use is to facilitate excessive/uncontrolled cell growth and tumorigenesis .”



Signal Transduction by the HER Family

There are receptor-specific ligands for HER1, HER3, and HER4. An intracellular tyrosine kinase domain exists for HER1, HER2, and HER4.Phosphorylation of the tyrosine kinase domain by means of dimerization causes cell proliferation and survival signaling. HER2 is the preferred dimerization partner for the other HER family members.

“The phosphorylated (activated) tyrosine residues on the intracellular domain of HER2 activate the lipid kinase phosphoinositide 3-kinase (PI3-K), which phosphorylates a phosphatidylinositol that in turn binds and phosphorylates the enzyme Ak transforming factor (Akt), driving cell survival. One of many other downstream effects is the production of vascular endothelial growth factor (VEGF) supporting angiogenesis[11]“

In carcinomas, HER2 acts as an oncogene, mainly because high-level amplification of the gene induces protein overexpression in the cellular membrane and subsequent acquisition of advantageous properties for a malignant cell .

HER2 protein overexpression in gastric cancer (11)

Overexpression of HER2 in gastric cancer is very much correlated with bad prognosis. It is also correlated with increased risk of local growth and distant metastasis(11). Prevalence Studies on HER2 positivity with gastric cancer revealed the frequency of HER2-positive gastric cancer ranging from 6.0 to 36.6 % . Studies, which determines HER2 overexpression by IHC using monoclonal antibody and/or gene amplification by FISH found similar results.

HER2 overexpression was found in 23% cases by IHC study and 27% cases by gene amplification (FISH) in a study of 200 specimen conducted by Yano et al(51). Gravalos and Jimeno found that HER2 overexpression is most

commonly found in gastroesophageal junction (GEJ) tumors and tumors with intestinal type histology . Various studies also showed a higher rate of HER2 over expression in GEJ tumors and intestinal subtype . HER2 as a prognostic factor in gastric cancer is controversial because some initial studies failed to find an association with prognosis.

Some studies showed that HER2 over expression was correlated with worse prognosis, while others found no association between the two. In a study involving 260 gastric cancers, HER2 positivity was an independent negative prognostic factor and HER2 staining intensity was correlated with tumor size, serosal invasion, and lymph node metastases . Another retrospective study involving 108 cases, HER2 overexpression was associated with a poorer 10-year survival[(11)].

HER2 positivity is considered as the second poorest prognostic variable in early gastric carcinoma according to Nakajima et al[(13)]

“Intestinal-type gastric cancers showed higher rates of HER2 overexpression than the diffuse-type cancers ($P<0.05$). Tumors with HER2 amplification are correlated with poor mean survival rates (922 vs 3243 days) and 5-year survival rates (21% vs 63%;$P< 0.05$). Age, TNM stage, and amplification of HER2 were found to be independently related to survival by multivariate analysis.”

Immunohistochemically, a positive reaction is considered in the presence of brown transmembrane staining and the scoringsystem to identify HER-2/Neu over expression is as follows,

	Surgical specimen staining pattern	Biopsy specimen staining pattern	HER2 overexpression assessment
0	No reactivity or membranous reactivity in <10% of tumour cells	No reactivity or no membranous reactivity in any tumour cell	Negative
1+	Faint or barely perceptible membranous reactivity in ≥10% of tumour cells; cells are reactive only in part of their membrane	Tumour cell cluster with a faint or barely perceptible membranous reactivity irrespective of percentage of tumour cells stained	Negative
2+	Weak to moderate complete, basolateral or lateral membranous reactivity in ≥10% of tumour cells	Tumour cell cluster with a weak to moderate complete, basolateral or lateral membranous reactivity irrespective of percentage of tumour cells stained	Equivocal
3+	Strong complete, basolateral or lateral membranous reactivity in ≥10% of tumour cells	Tumour cell cluster with a strong complete, basolateral or lateral membranous reactivity irrespective of percentage of tumour cells stained	Positive

HER2=human epidermal growth factor receptor 2 (also known as ERBB2).

Immunohistochemistry[11]

Albert Coons et al in 1941 first labelled antibodies directly with fluorescent isocyanate. Nakane and Pierce et al in 1966, introducedindirect labelling technique in which unlabelled antibody is followed bysecond antibody or substrate. Various stages of development ofImmunohistochemistry include peroxidase – antiperoxidase method(1970), alkaline phosphatase labelling (1971), avidin biotin method(1977) and two layer dextrin polymer technique (1993).

Antigen Retrieval:

Antigen retrieval can be done by the following different techniques to unmask the antigenic determinants of fixed tissue sections.

1. Proteolytic enzyme digestion
2. Microwave antigen retrieval
3. Pressure cooker antigen retrieval
4. Microwave and trypsin antigen retrieval

Proteolytic Enzyme Digestion:

Huank et al in 1976 introduced this technique to breakdown formalin cross linkages and to unmask the antigen determinants. The most commonly used enzymes include trypsin and proteinase. The disadvantages include over digestion, under digestion and antigen destruction.

Microwave Antigen Retrieval:

This is a new technique most commonly used in current practice.Microwave oven heating involves boiling formalin fixed paraffin sectionsin various buffers for rapid and uniform heating .

Pressure Cooker Antigen Retrieval[51]

Miller et al in 1995 compared and proved that pressure cookingmethod has fewer inconsistencies, less time consuming and can be used toretrieve large number of slides than in microwave method.

Pitfalls of Heat Pre-treatment:

Drying of sections at any stage after heat pre-treatment destroysantigenicity. Nuclear details are damaged in poorly fixed tissues. Fibersand fatty tissues tend to detach from slides while heating. Not all antigensare retrieved by heat pre-treatment and also some antigens like PGP 9.5show altered staining pattern.

Detection Systems*:*

After addition of specific antibodies to the antigens, next step is tovisualize the antigen antibody reaction complex. The methods employedare direct and indirect methods. In the direct method, primary antibody isdirectly conjugated with the label. Most commonly used labels areflourochrome, horse radish peroxidase and alkaline phosphatase.

Indirect method is a two-step method in which labelled secondary antibody reactswith primary antibody bound to specific antigen. The use of peroxidise enzyme complex or avidin biotin complex further increases the

sensitivityof immunohistochemical stains. In 1993, Pluzek et al introducedenhanced polymer one step staining, in which large numbers of primaryantibody and peroxidase enzymes are attached to dextran polymer backbone. This is the rapid and sensitive method. Dextran polymerconjugate two step visualization system is based on dextran technology inEpos system. This method has greater sensitivity and is less timeconsuming.

MATERIALS AND METHODS

This study is a retrospective study of gastric carcinoma conducted in the Department of Pathology, Government Kilpauk Medical College, Chennai during the period of July 2014 to June 2016. Endoscopic biopsy from stomach as well as resected specimens (subtotal, total, radical and palliative gastrectomy) from the Departmentof Surgery and Surgical Gastroenterology , Govt. Kilpauk Medical college Hospital, which were received in Department of Pathology, Govt. Kilpauk Medical College and reported as adenocarcinoma were included for the study.

Study population: Patients diagnosed as having gastric adenocarcinoma by Histopathological examination.

Inclusion Criteria:

Histopathologically proven cases of Gastric adenocarcinomas.

Exclusion Criteria:

Patients diagnosed with gastric neoplasms other than gastric adenocarcinoma like,

- Gastric lymphomas
- Neuroendocrine tumours
- Mesenchymal neoplasms
- Poorly differentiated tumours
- Metastatic tumours

Data collection and Methodology:

Data of Gastric adenocarcinoma patients will be collected from the registers and case records .

Retrospectively patient's tissue blocks will be analysed by immunohistochemical study for the expression of Human Epidermal Growh Factor Receptor -2 and graded appropriately.

Variables studied

The following clinical and pathological parameters were evaluated. Age, gender, location (cardia, body, pyroloantrum, fundus), gross appearance (ulcerative, nodular, ulceroproliferative, diffuse),endoscopic appearance ,histological grade (well differentiated, moderately differentiated,poorly differentiated) and Her -2 receptor expression.

Immunohistochemical evaluation

Immunohistochemical analysis was done in paraffin embedded tissue samples using supersensitive polymer HRP system based on nonbiotin polymeric technology. 4 micron thick sections from formalin fixed paraffin embedded tissue samples were transferred onto gelatin coated slides. Heat induced antigen retrieval was done. The antigen was bound with mouse monoclonal antibody HER-2/Neu proteins and then detected by the addition of

secondary antibody conjugated with horse radish peroxidase polymer and diaminobenzidine substrate.

The immunohistochemically stained slides were analyzed for the presence of reaction, cellular localization, percentage of cells stained and intensity of staining. Cytoplasmic membrane staining was assessed for HER-2/Neu positivity.

Data entry

All the data collected and the results obtained were entered into Excel 2007.

Statistical analysis

The collected data were analysed with IBM.SPSS statistics software 23.0 Version.To describe about the data descriptive statistics frequency analysis, percentage analysis were used for categorical variables and the mean & S.D were used for continuous variables. To find the significant difference between the bivariate samples in Independent groups the Unpaired sample t-test was used. To find the significance in categorical data Chi-Square test was used. In both the above statistical tools the probability value(P value) .05 is considered as significant level.

OBSERVATION AND RESULTS

SEX DISTRIBUTION

Among the 100 specimen of study, 68 cases (68%) were males and 32 cases (32%) were females showing male preponderance in incidence. (Table 1 and Chart 1)

Sex	No. of cases	Percentage
Males	68	68%
Females	32	32%
TOTAL	100	100%

Table.1 – Sex Distribution of study population

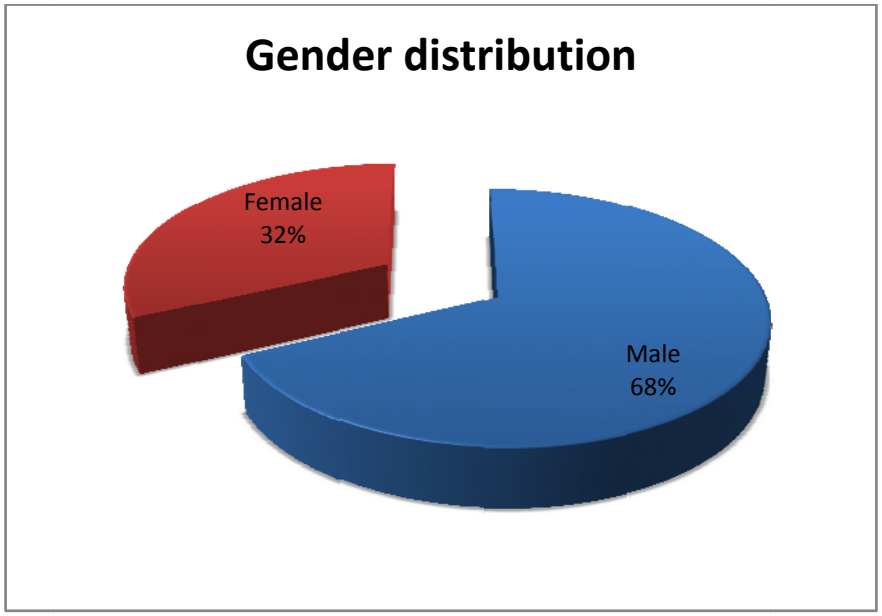


Chart.1 – Gender Distribution of study population

AGE DISTRIBUTION

Regarding age distribution, the mean age of incidence is 60.3yrs, the youngest age being 27 yrs and oldest age being 80 yrs. The percentage of people under 50 yrs of age is 23%(23 cases) and above or equal to 50 is 77% (77Cases). (Table .2 and Chart 2)

Age	No.of cases	Percentile
< 50 yrs	23	23%
>50 yrs	77	77%

Table.2 – Age distribution in study population

SITE DISTRIBUTION

In the study population , most of the gastric carcinomas were located at pyloro- antral region , the percentage being 60%, least cases occurred in cardia with 6%.(Table .3 and Chart 3)

Table.3- Site wise distribution Gastric cancer

SITE	Percentage of incidence
Cardia	6%
Body	27%
Fundus	7%
Pyloro - antrum	60%
TOTAL	100%

Chart.2 – Age distribution in study population

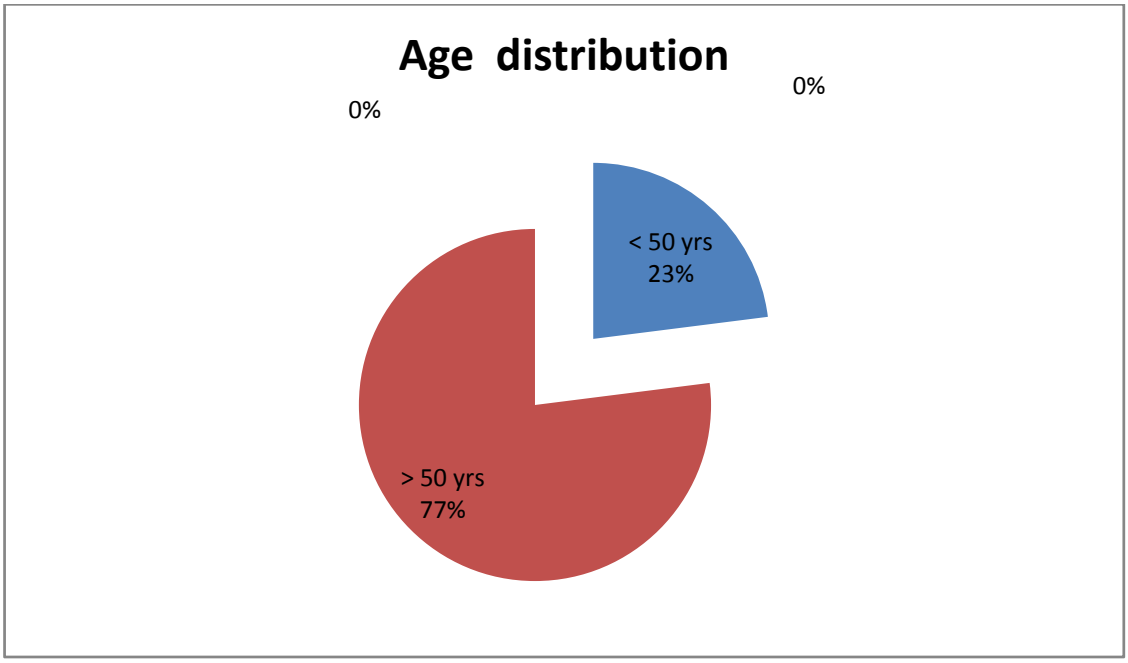
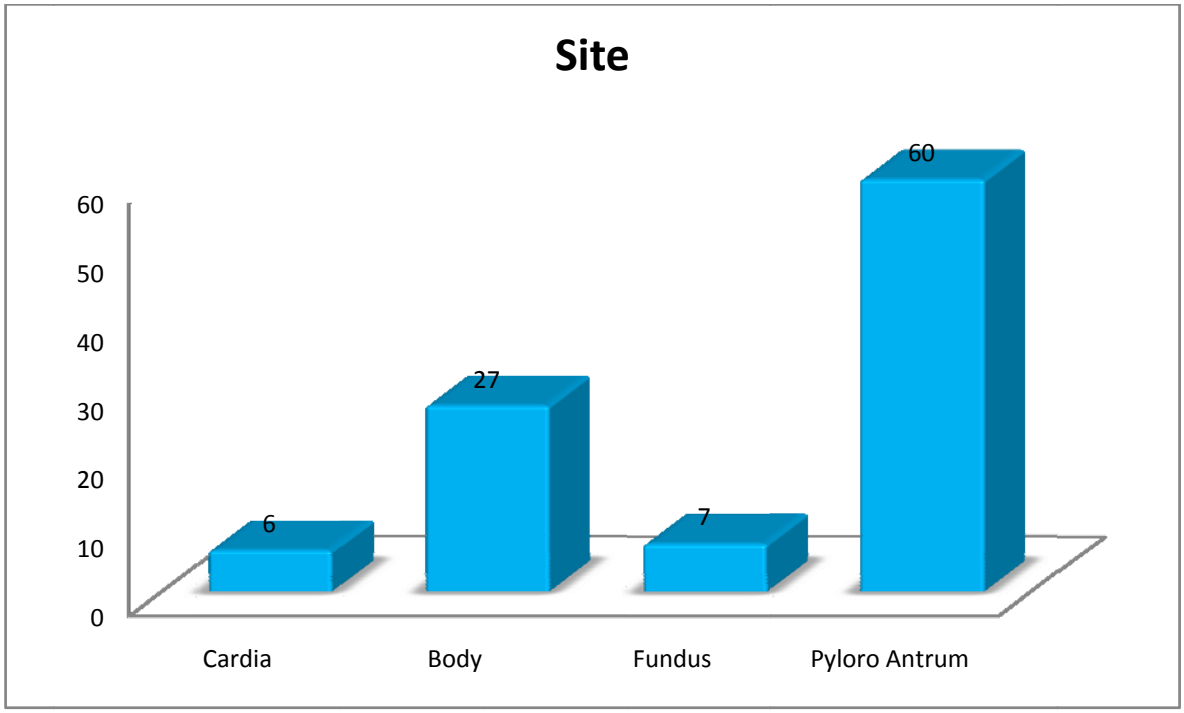


Chart.3- Site wise distribution Gastric cancer



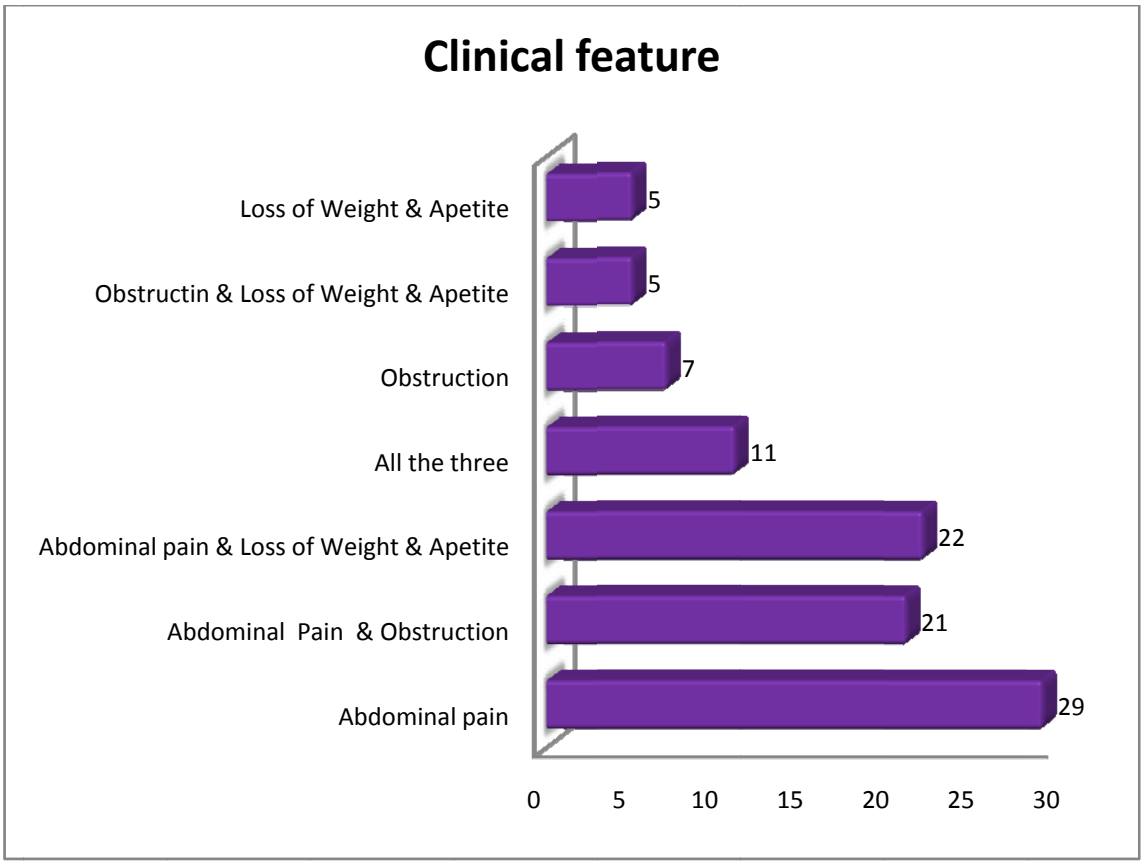
CLINICAL SYMPTOMS

Among the presenting complaints of the patients, abdominal pain(only) tops the list with 29% and the least being only loss of weight and apetite – 5%(Table. 4 and Chart .4)

Table .4 – Clinical features of study population

Clinical Features	**Percentage %**
Abdominal pain	29
Obstruction	7
Loss of Weight &Apetite	5
Abdominal pain & Loss of Weight and Apetite	22
Abominal pain & obstruction	21
Obstruction & Loss of Weight&Apetite	5
All the three	11
TOTAL	100

Chart .4 – Clinical features of study population



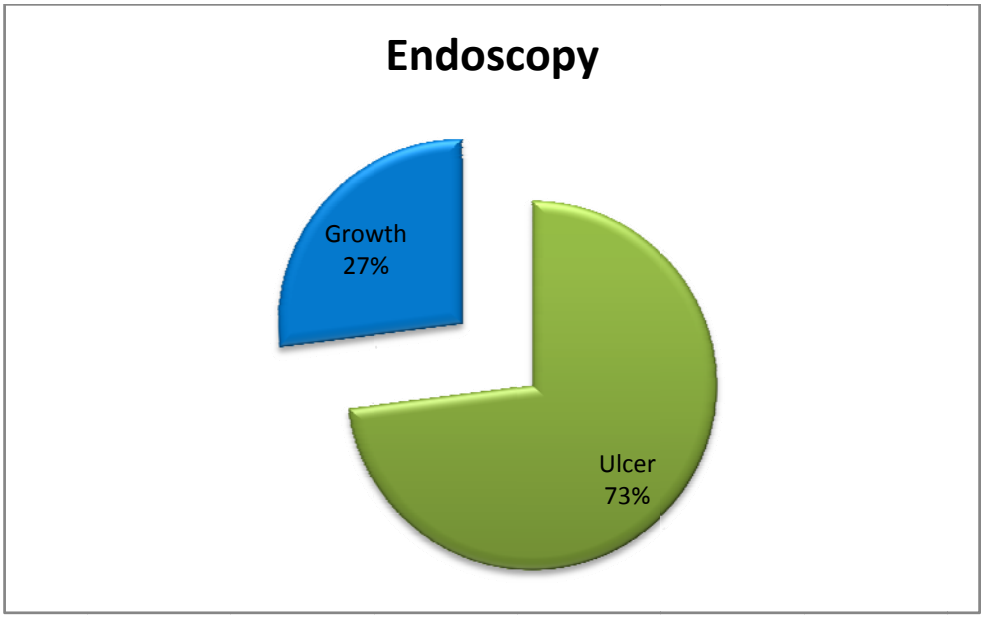
Endoscopic appearance

Most of cases are reported as Ulcers (73%). Remaining 23% cases were reported as growth .(Table5 &Chart5)

Table -5 showing endoscopic appearance of study population

Endoscopic appearance	Frequency	Percent
Ulcer	73	73.0
Growth	27	27.0
Total	100	100.0

Chart -5 showing endoscopic appearance of study population



GROSS APPEARANCE:

In my study population, the common gross pattern is proliferative (50%), the next being ulcerative (45%) and the least one is Polypoidal (2%). (Table.6 and Chart,6)

Table 6 Showing gross appearance distribution

Gross appearance	Frequency	Percent
Ulcerative	45	45.0
Proliferative	50	50.0
Polypoidal	2	2.0
Infiltrative	3	3.0
Total	100	100.0

HISTOLOGIC GRADE:

In the study population, most of the cases are moderately differentiated(42%). Poorly differentiated tumours accounts for 41% and 17 % cases were well differentiated.(Table .7 and Chart .7)

Histologic Grade	Frequency	Percent
Well differentiated	17	17.0
Moderately differentiated	42	42.0
Poorly differentiated	41	41.0
Total	100	100.0

Table .7 Distribution of different histological grade

Chart 6 Showing gross appearance distribution

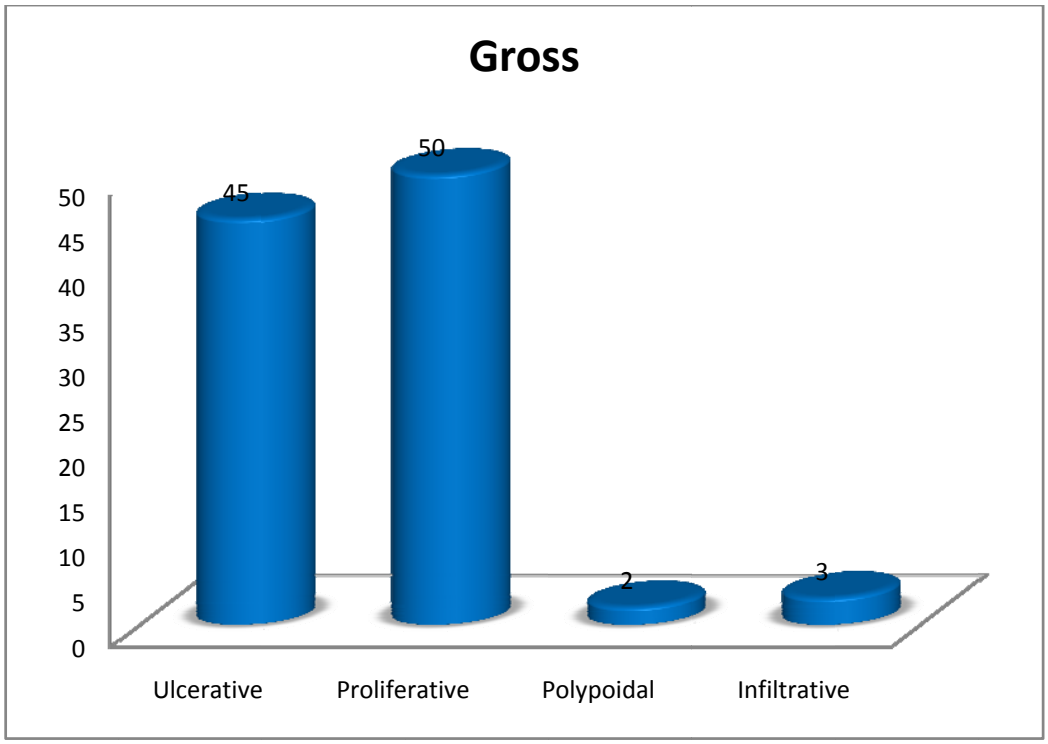
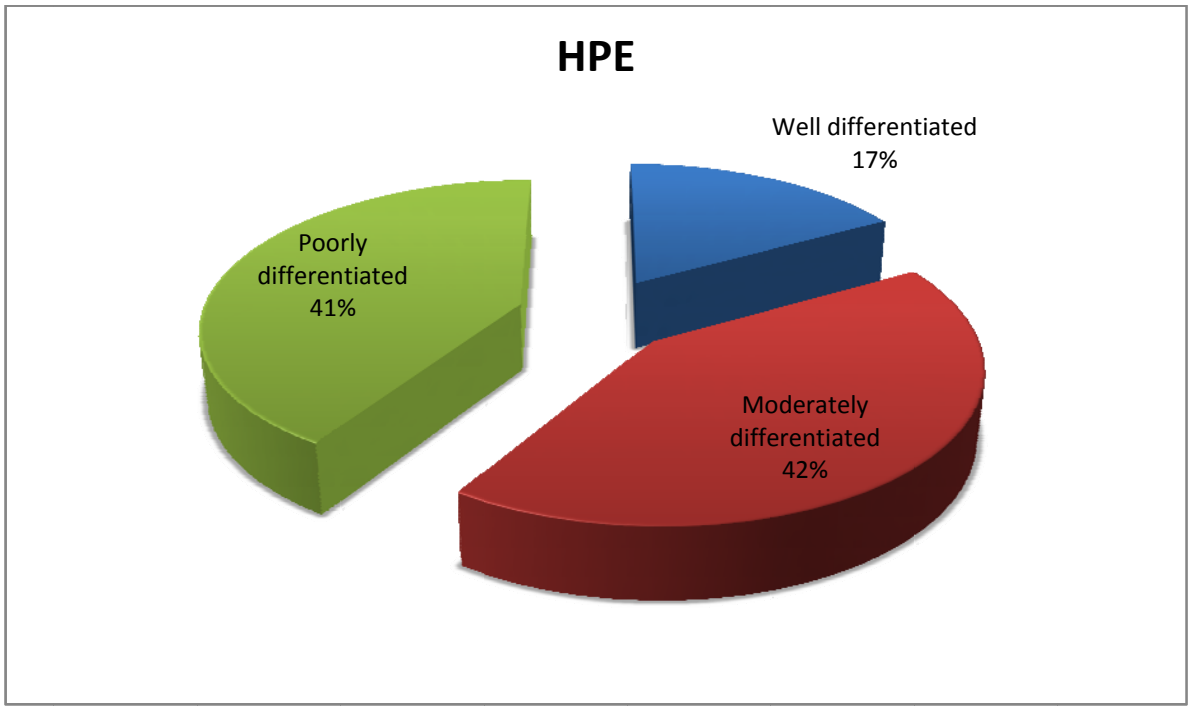


Chart .7 Distribution of different histological grade



HER -2 / NEU RECEPTOR EXPRESSION:

25% of cases were positive for Her-2 receptor. Moderately differentiated(52%) and well differentiated(28%) tumours shows more positivity than poorly differentiated tumours(20%). (Table.8 & Chart.8)

Table -8 Expression of Her -2 receptor in study population

Her – 2 Status	Frequency	Percent
Positive	25	25.0
Negative	75	75.0
Total	100	100.0

In the study population , 73 cases were endoscopic biopsies and 27 are gastrectomy specimen.(Table.9 and chart 9).In endoscopic biopsies, 54 cases are male and 20 were female.amonggastrectomy specimen, 15 cases are male and 11 were female.

Table -9 Type of specimen obtained in study population

Small	73	73.0
Gastrectomy	27	27.0
Total	100	100.0

Chart -8 Expression of Her -2 receptor in study population

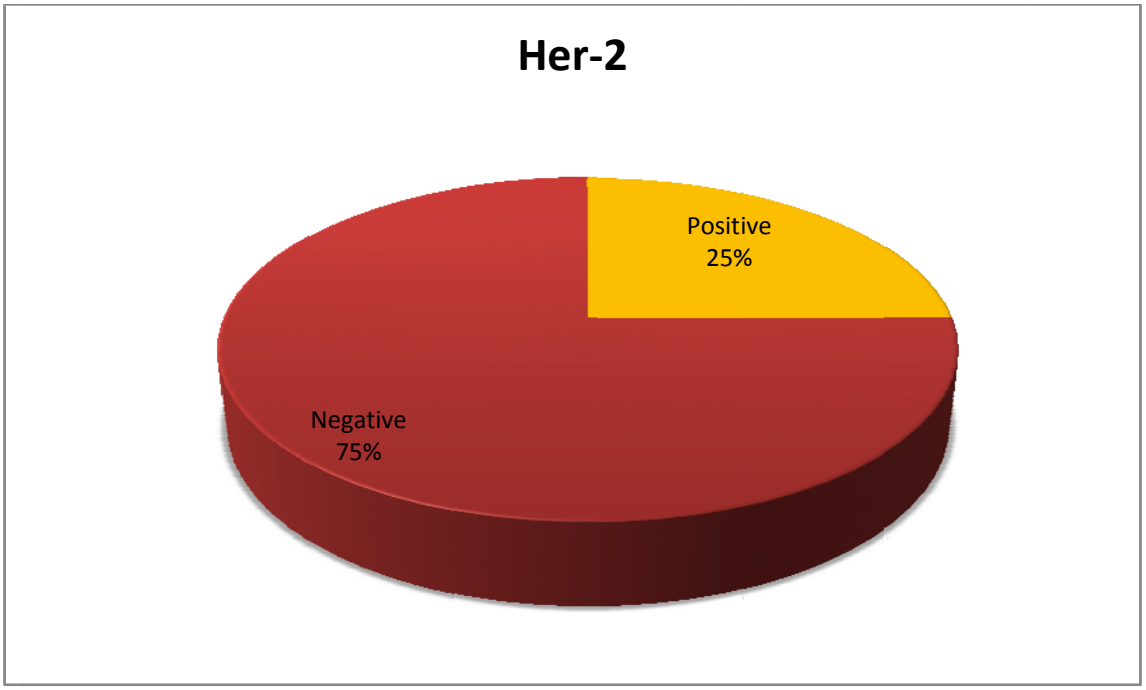
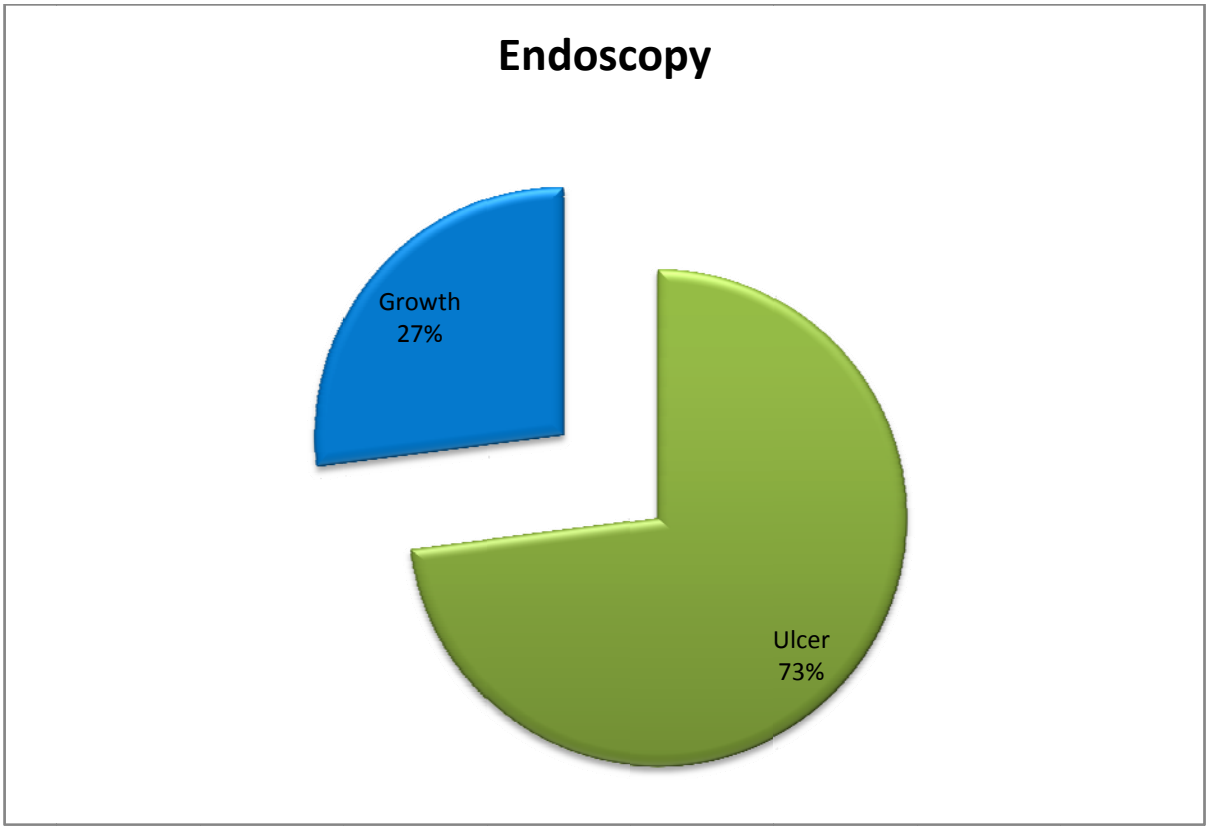


Chart -9 Type of specimen obtained in study population



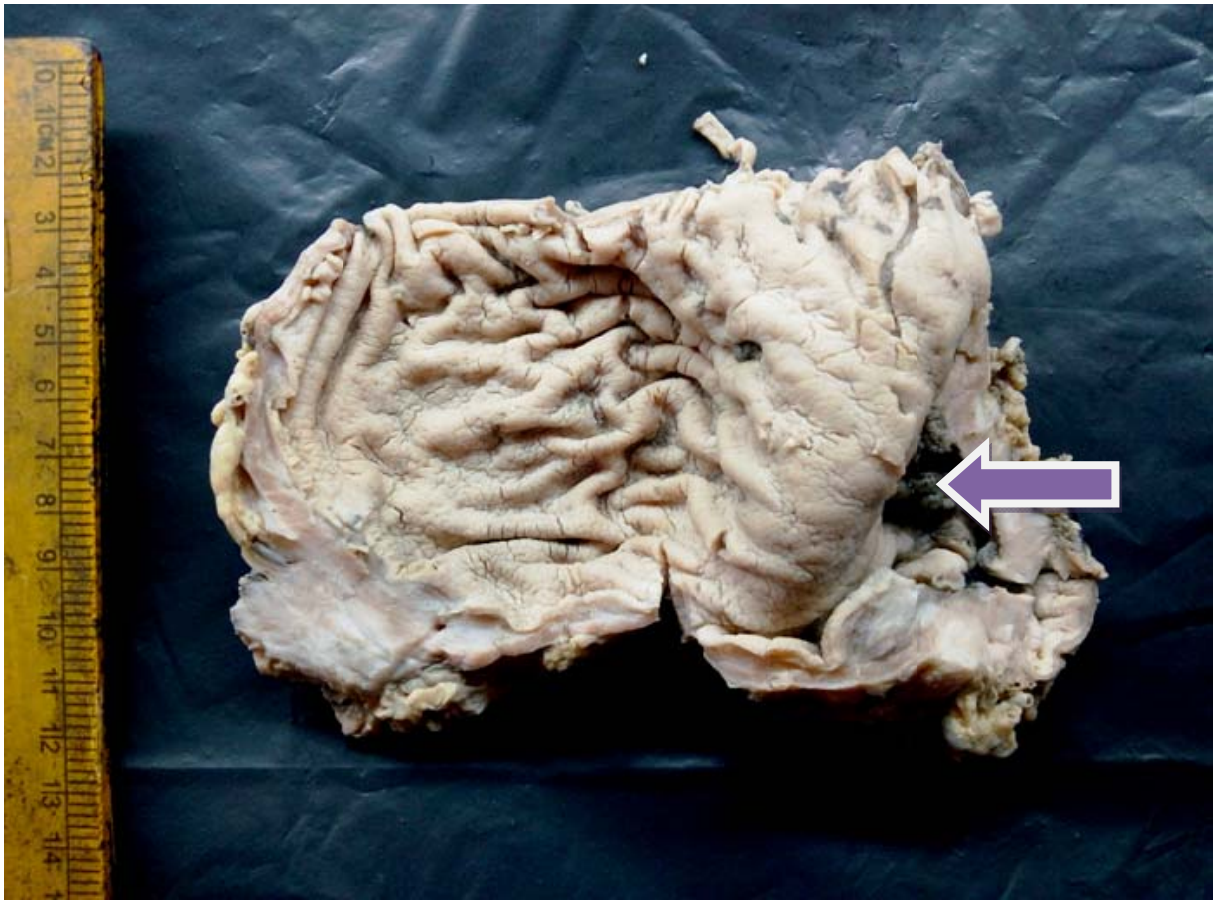


Figure 1: Gastric Carcinoma- Ulcerative

Figure 2: Gastric Carcinoma- Proliferative

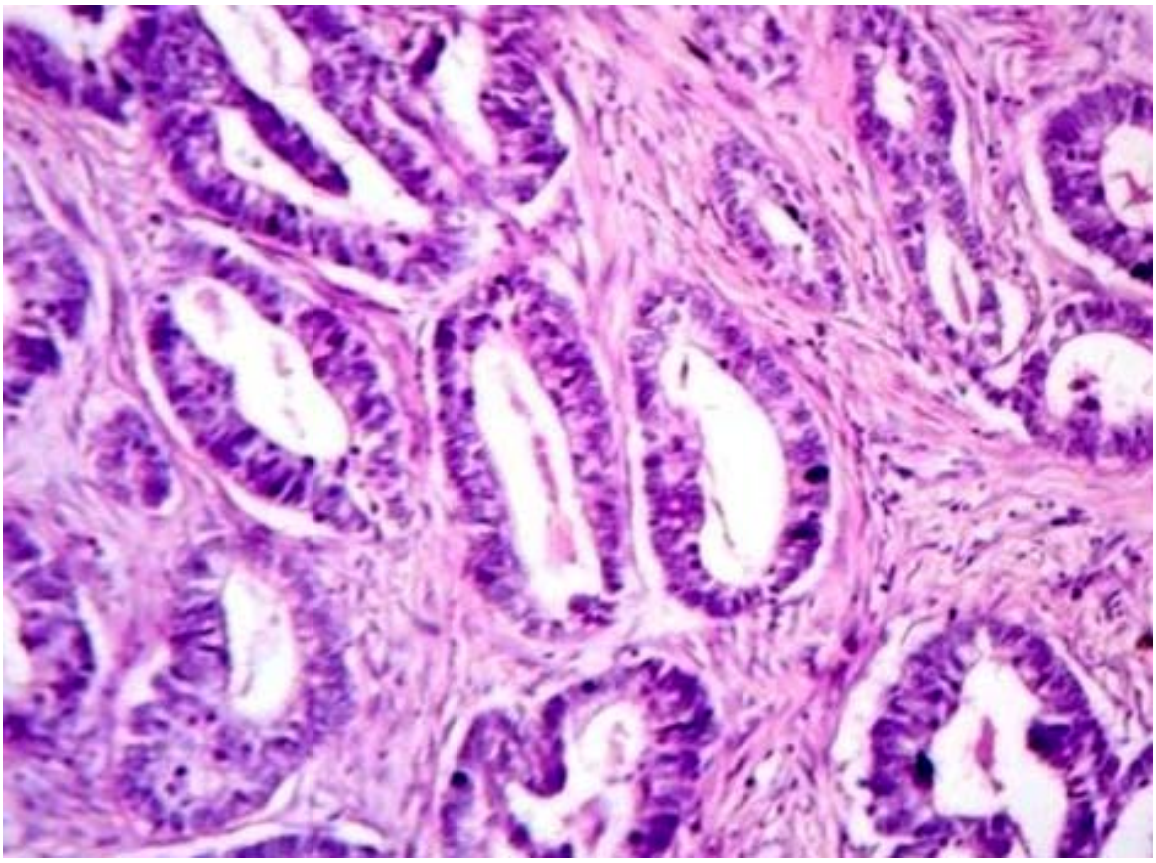


Figure 3: Well differentiated grade showing well formed glands lined by malignant cells, 40x, H & E

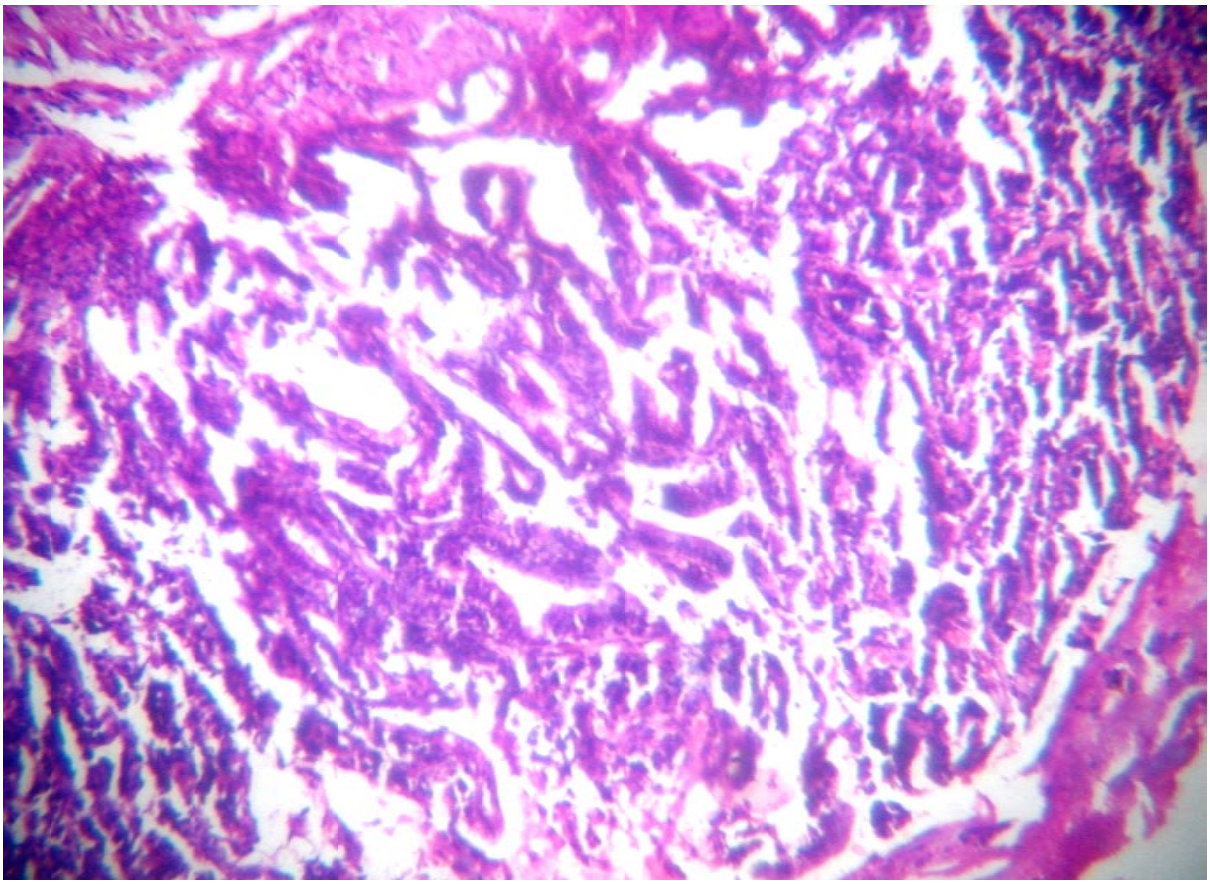


Figure 4: Moderately differentiated grade showing cells arranged in groups and glands, 40x ,H & E

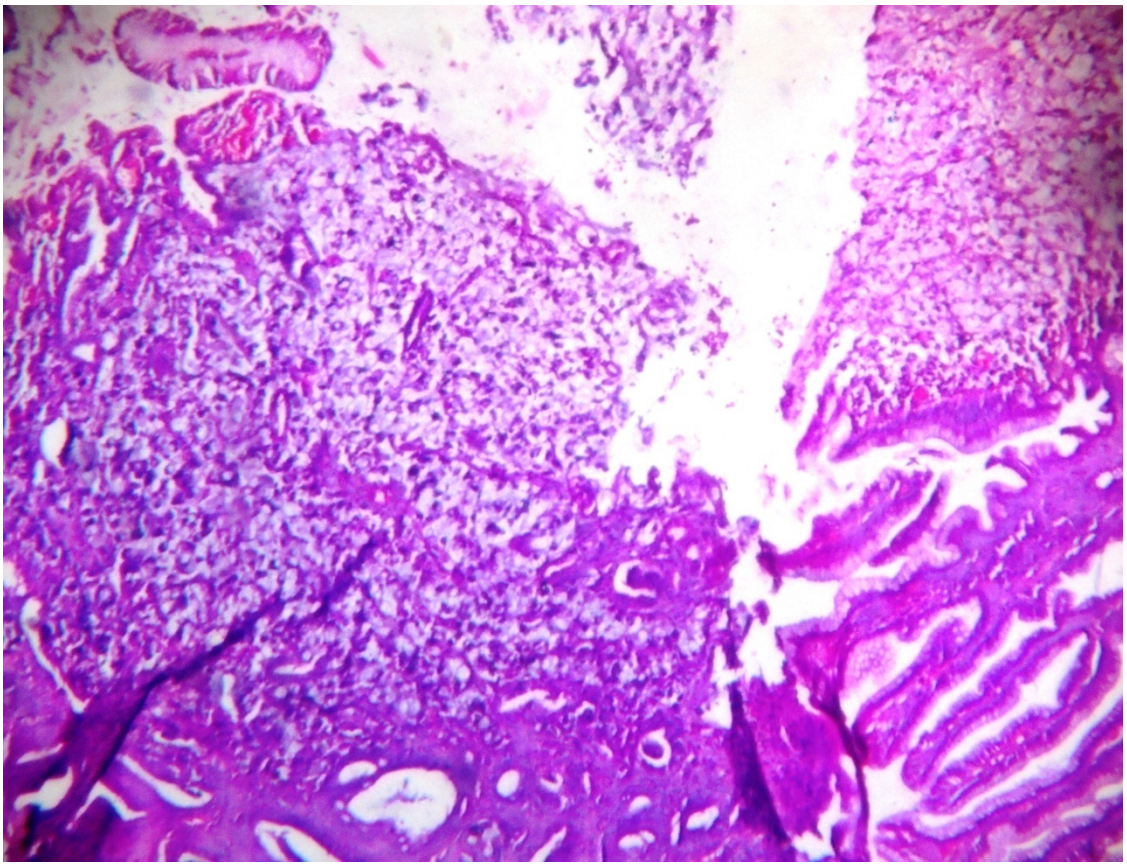


Figure 5: Poorly differentiated grade with cells arranged in sheets and filled with mucin 10x, H &E

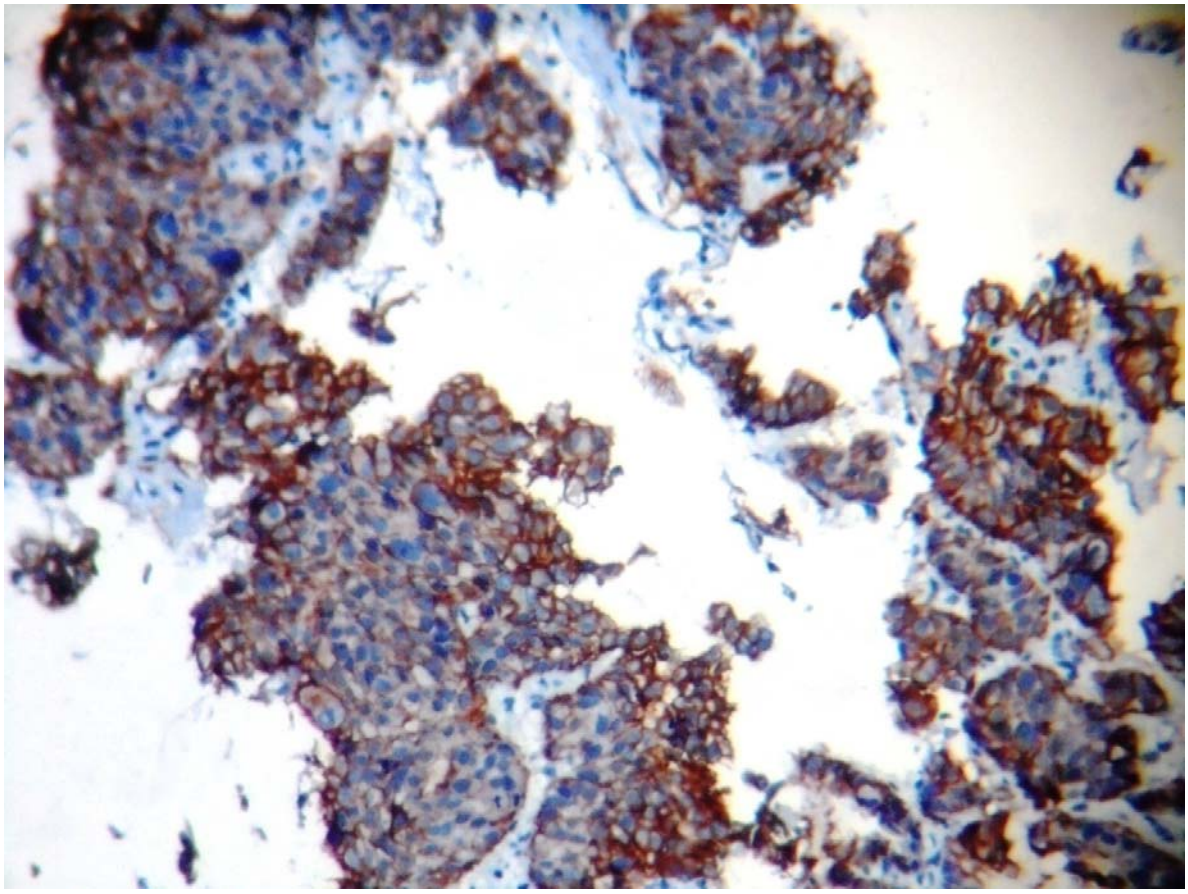


Figure 5.HER-2/Neu Score 3+,

Strong intense completemembranous staining in alltumour cells, 40x,

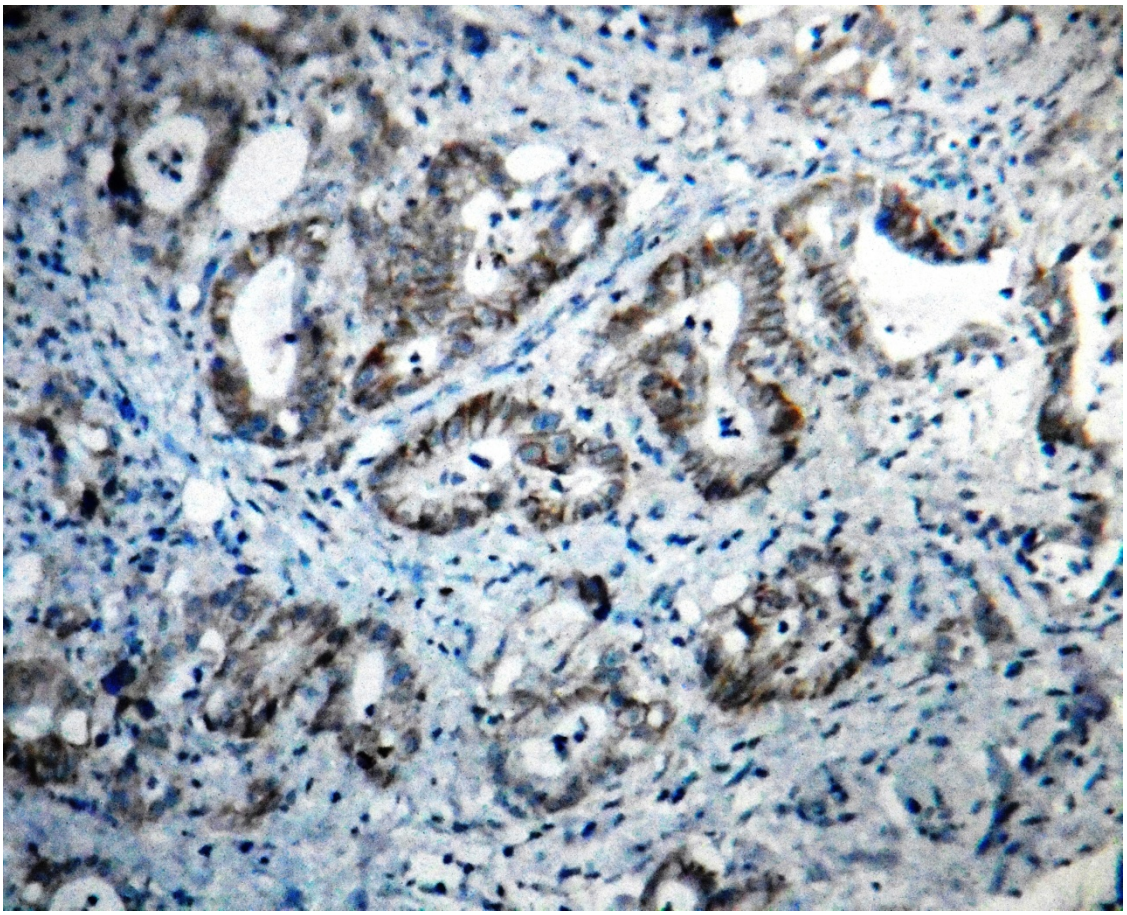


Figure 6- HER-2/Neu Score 2+,

Moderate intense complete staining in > 10% of tumour cells

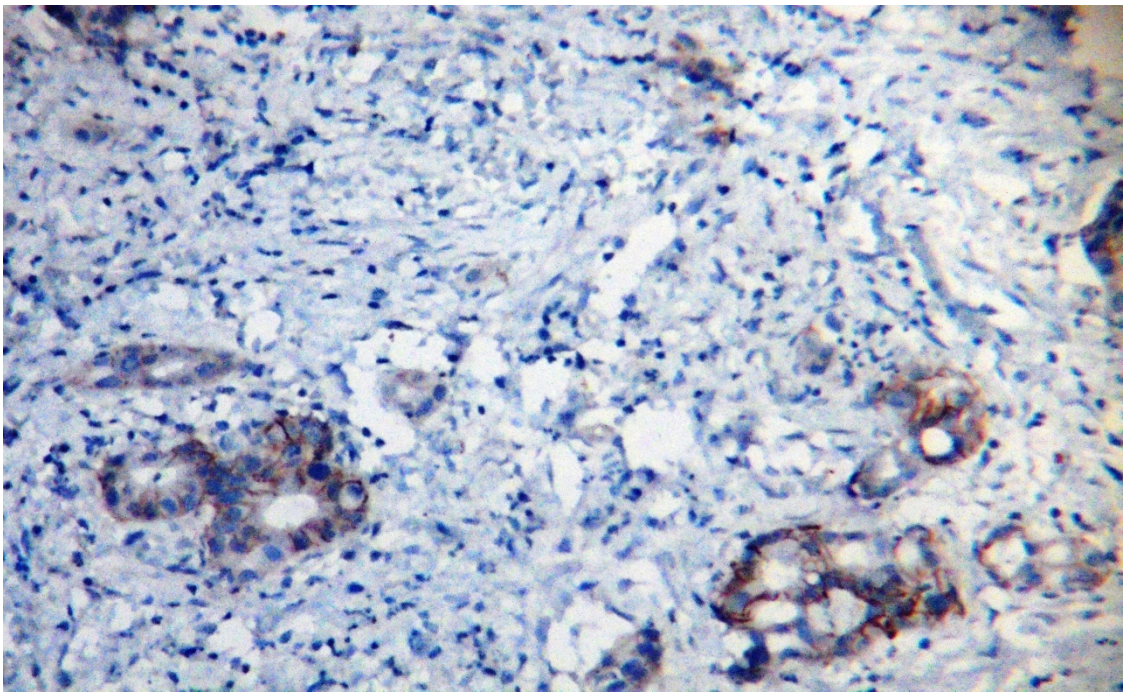


Figure 7 : HER-2/Neu Score 1+,

Incomplete membranous staining in < 10 % of tumour cells,40x

Correlation of HER -2 with Various Clinicopathological Factors:

Table 10: Association of age of patient with HER -2 expression

			HER - 2		
			Positive	**Negative**	**Total**
Agerange	< 50 yrs	Count	6	17	23
		% within HER - 2	24.0%	22.7%	23.0%
	>=50 yrs	Count	19	58	77
		% within HER - 2	76.0%	77.3%	77.0%
Total		Count	25	75	100
		% within HER - 2	100.0%	100.0%	100.0%

Chi Squre test :P Value -0.891(> 0.05)

The percentage of patients with age < 50 yrs showing HER-2/Neu over expression is 26.1% and in those with age more than 50 yrs, overexpression of Her -2 seen in 25 %. There was no significant difference in the age at presentation between the two groups (Table 10 & Chart 10)

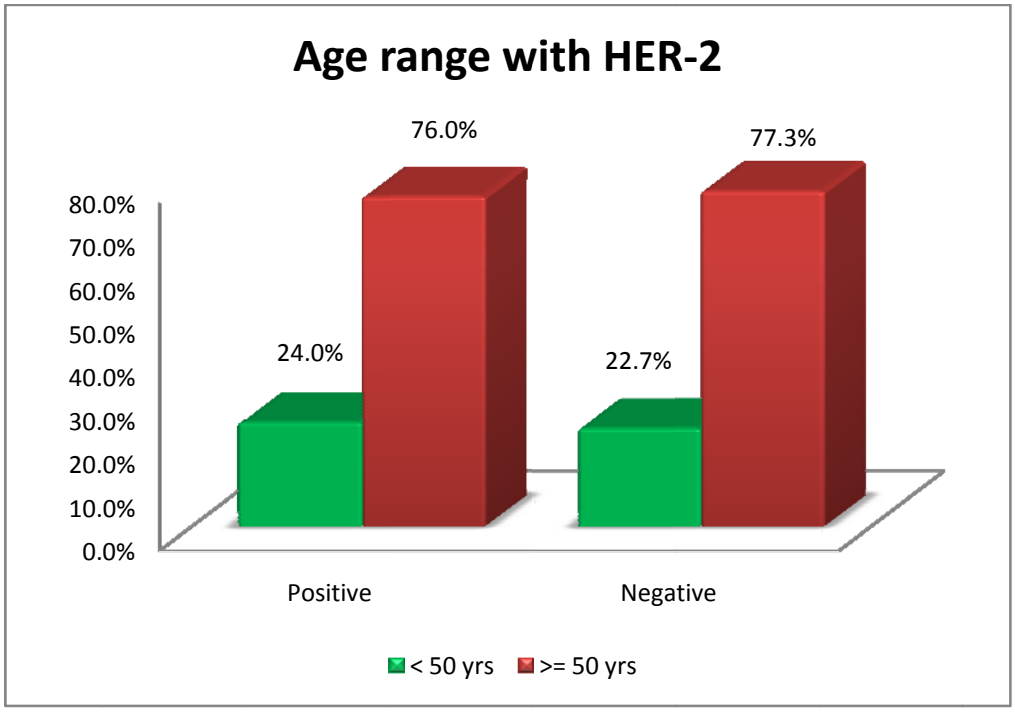


Chart 10: Association of age of patient with HER -2 expression

Table 11: Association of Gender with HER-2/Neu Expression

			HER - 2		
			Positive	**Negative**	**Total**
GENDER	Male	Count	15	53	68
		% within HER - 2	60.0%	70.7%	68.0%
	Female	Count	10	22	32
		% within HER - 2	40.0%	29.3%	32.0%
Total		Count	25	75	100
		% within HER - 2	100.0%	100.0%	100.0%

Chi square test :P value - 0.322 (>0.05)

Of the cases showing HER-2/Neu over expression, 60% were

males and 40% were females and there was no significant difference in

sex wise distribution. (Table 11 & Chart 11)

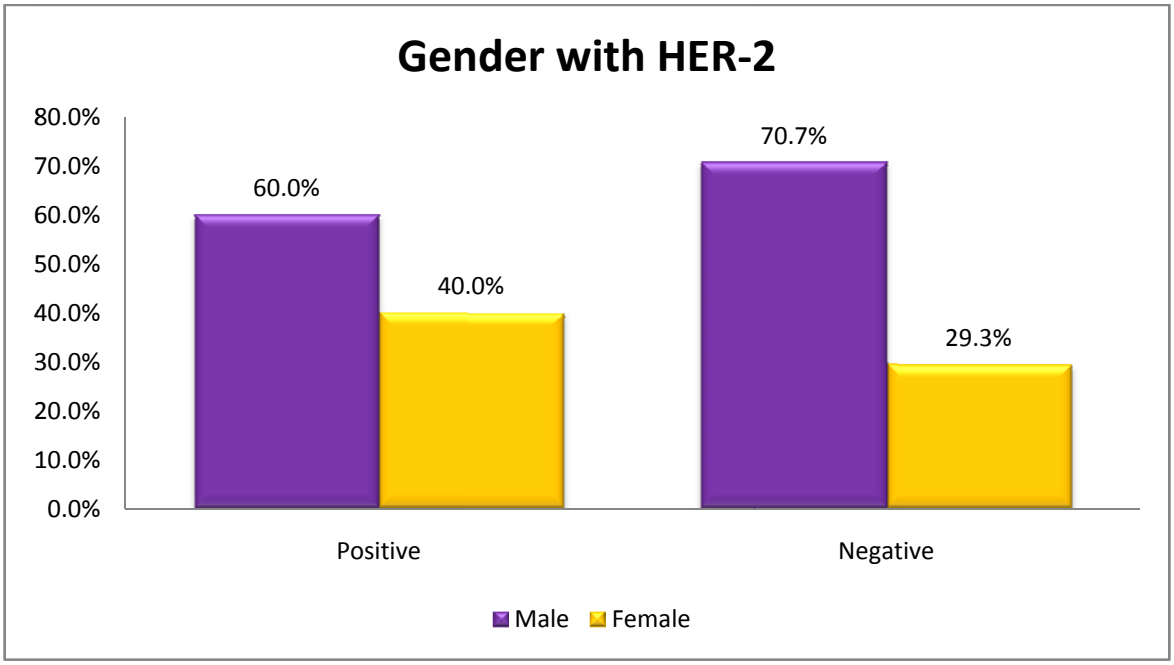


Chart 11: Association of Gender with HER-2/Neu Expression

Table 12: Association of Site with HER-2/Neu Expression

			HER - 2		
			Positive	**Negative**	**Total**
SITE	Cadria	Count	1	5	6
		% within HER - 2	4.0%	6.7%	6.0%
	Body	Count	4	23	27
		% within HER - 2	16.0%	30.7%	27.0%
	Fundus	Count	1	6	7
		% within HER - 2	4.0%	8.0%	7.0%
	PuloroAntrum	Count	19	41	60
		% within HER - 2	76.0%	54.7%	60.0%
Total		Count	25	75	100
		% within HER - 2	100.0%	100.0%	100.0%

P value : 0.312 (> 0.05)

Among the cases showing HER-2/Neu over expression, 76% were from pyloroantral region, 4% were from cardiac region,16% from body and 4% of cases with fundus involvementshowed HER-2/Neu over expression. Association of site of tumour withHER-2/Neu over expression was not statistically significant. (Table12&Chart 12).

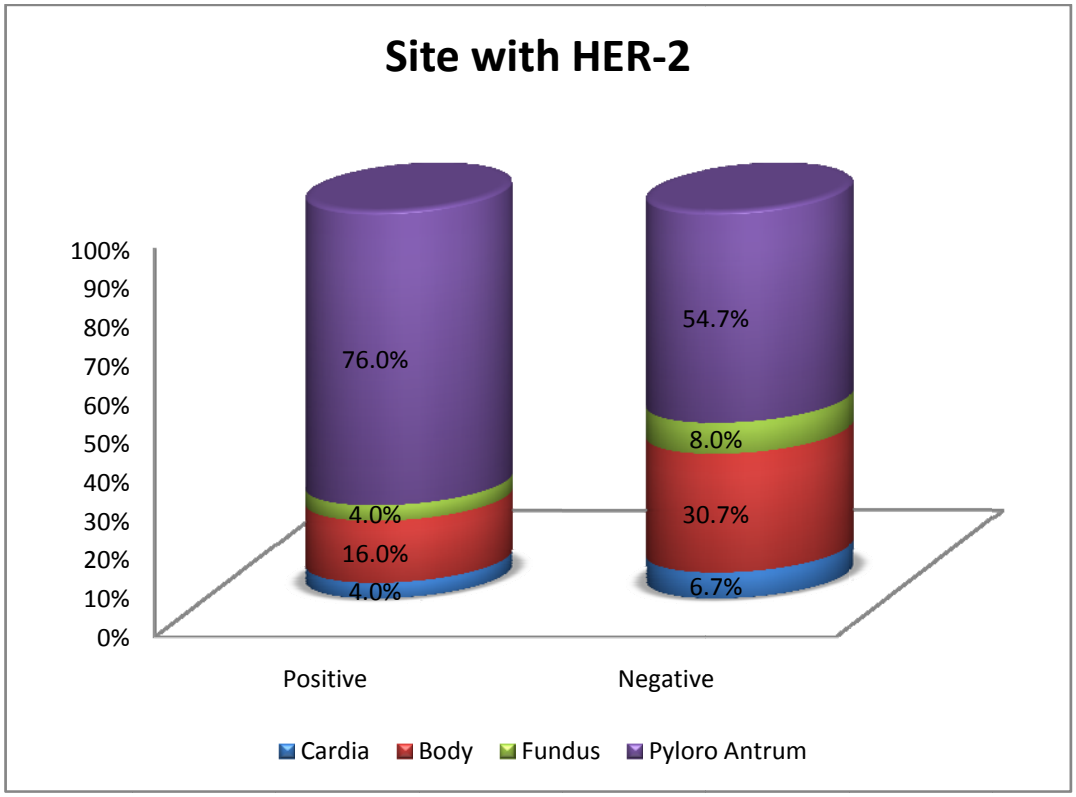


Chart 12: Association of Site with HER-2/Neu Expression

Table 13: Association of type of specimen with HER-2/Neu Expression

			HER - 2		
			Positive	**Negative**	**Total**
TYPE	Endoscopic biopsies	Count	20	53	73
		% within HER - 2	80.0%	70.7%	73.0%
	Gastrectomy	Count	5	22	27
		% within HER - 2	20.0%	29.3%	27.0%
Total		Count	25	75	100
		% within HER - 2	100.0%	100.0%	100.0%

P value : 0.363 (> 0.05)

Among Her -2 positive cases, 80% are from endoscopic biopsies and 20% from gastrectomy specimen. Association of type of specimen withHER-2/Neu over expression was not statistically significant. (Table13 &Chart 13)

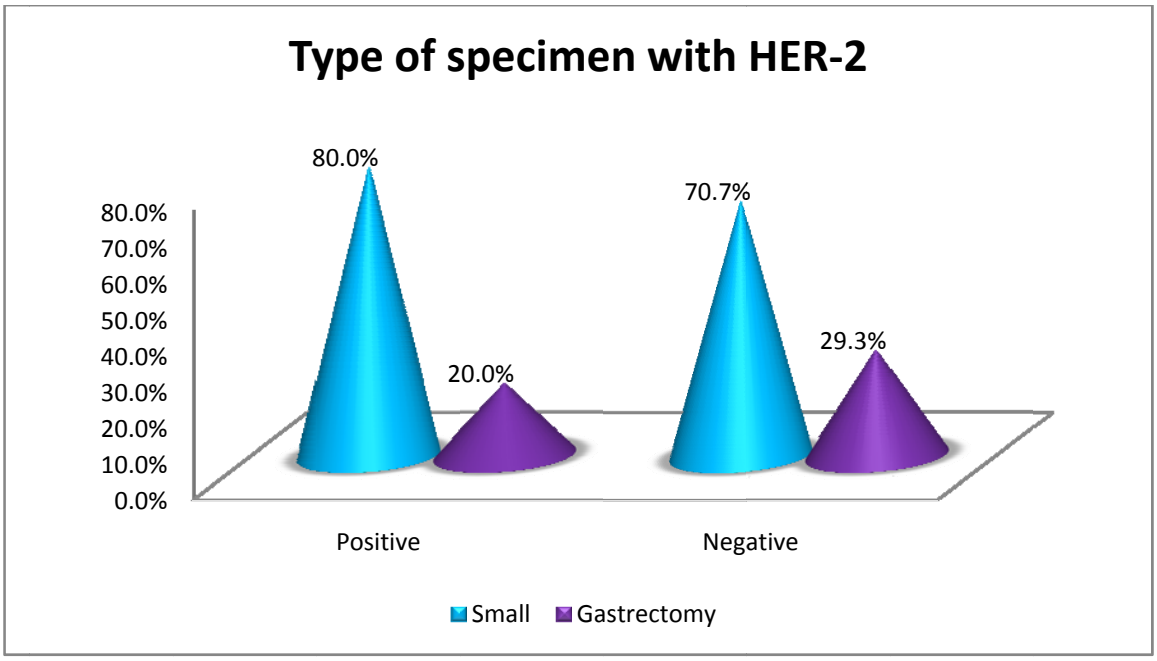


Table 13: Association of type of biopsy with HER-2/Neu Expression

Table 14: Association of clinical features with HER-2/Neu

			HER - 2		
			Positive	**Negative**	**Total**
CLINICAL FEATURE	Abdominal pain	Count	8	21	29
		% within HER - 2	32.0%	28.0%	29.0%
	Abdominal Pain & Obstruction	Count	2	19	21
		% within HER - 2	8.0%	25.3%	21.0%
	All the three	Count	4	7	11
		% within HER - 2	16.0%	9.3%	11.0%
	Abdominal Pain & Loss of Weight &Apetite	Count	6	16	22
		% within HER - 2	24.0%	21.3%	22.0%
	Obstruction	Count	2	5	7
		% within HER - 2	8.0%	6.7%	7.0%
	Obstruction & Loss of Weight &Apetite	Count	1	4	5
		% within HER - 2	4.0%	5.3%	5.0%
	Loss of Weight &Apetite	Count	2	3	5
		% within HER - 2	8.0%	4.0%	5.0%
Total		Count	25	75	100
		% within HER - 2	100.0%	100.0%	100.0%

P value : 0.634 (> 0.05)

Among Her – 2 positive cases most common symptom is only abdominal pain (32%) and least common is combination of obstruction and loss of weight(4%). No statistical significant between two groups

Chart 14: Association of clinical features with HER-2/Neu

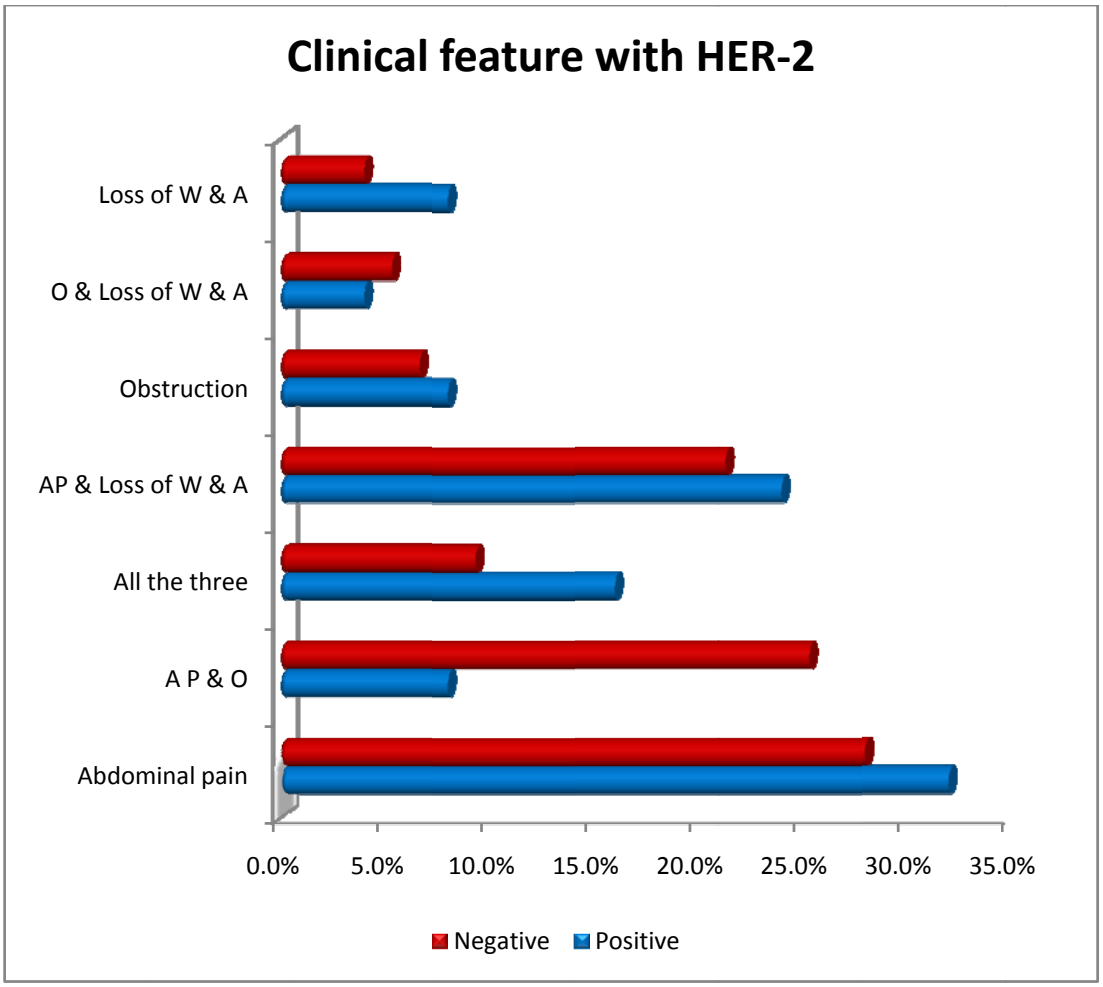


Table 14: Association of Endoscopic Appearance with HER-2/NeuExpression

			HER - 2		
			Positive	**Negative**	**Total**
ENDOSCOPY	Ulcer	Count	20	53	73
		% within HER - 2	80.0%	70.7%	73.0%
	Growth	Count	5	22	27
		% within HER - 2	20.0%	29.3%	27.0%
Total		Count	25	75	100
		% within HER - 2	100.0%	100.0%	100.0%

P Value: 0.363

Among Her – 2 positive cases ,ulcer in endoscopy accounts 80% and growth appearance in endoscopy accounts 20%. No statistical significant between two groups

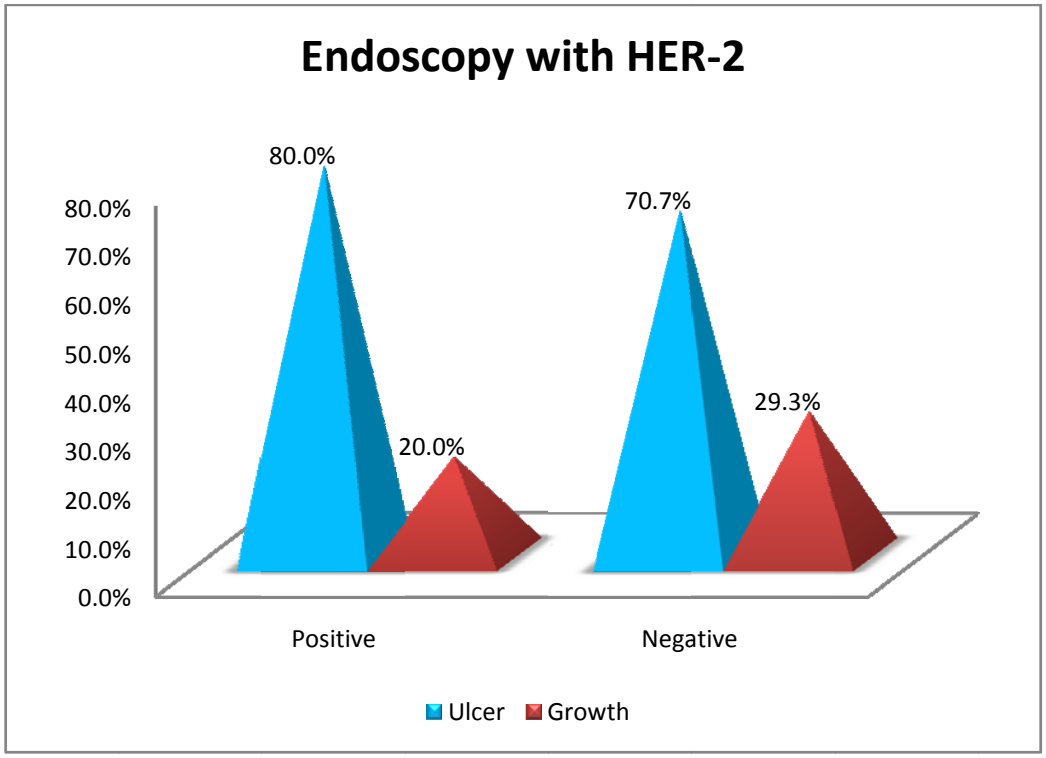


Chart 14: Association of Endoscopic Appearance with HER-2/NeuExpression

Table 15: Association of Gross Appearance with HER-2/Neu

			HER - 2		
			Positive	**Negative**	**Total**
GROSS	Ulcerative	Count	12	33	45
		% within HER - 2	48.0%	44.0%	45.0%
	Proliferative	Count	13	37	50
		% within HER - 2	52.0%	49.3%	50.0%
	Polypoidal	Count	0	2	2
		% within HER - 2	0.0%	2.7%	2.0%
	Infiltrative	Count	0	3	3
		% within HER - 2	0.0%	4.0%	3.0%
Total		Count	25	75	100
		% within HER - 2	100.0%	100.0%	100.0%

P value: 0.624 (>0.05%)

Among the cases showing HER-2/Neu over expression, 48% were ulcerative type, 52% were proliferative type and none were polypoidal and infiltrative type. No statistically significant associationwas found between gross appearance and HER-2/Neu over expression.(Table15 & Chart 15)

Chart 15: Association of Gross Appearance with HER-2/neu

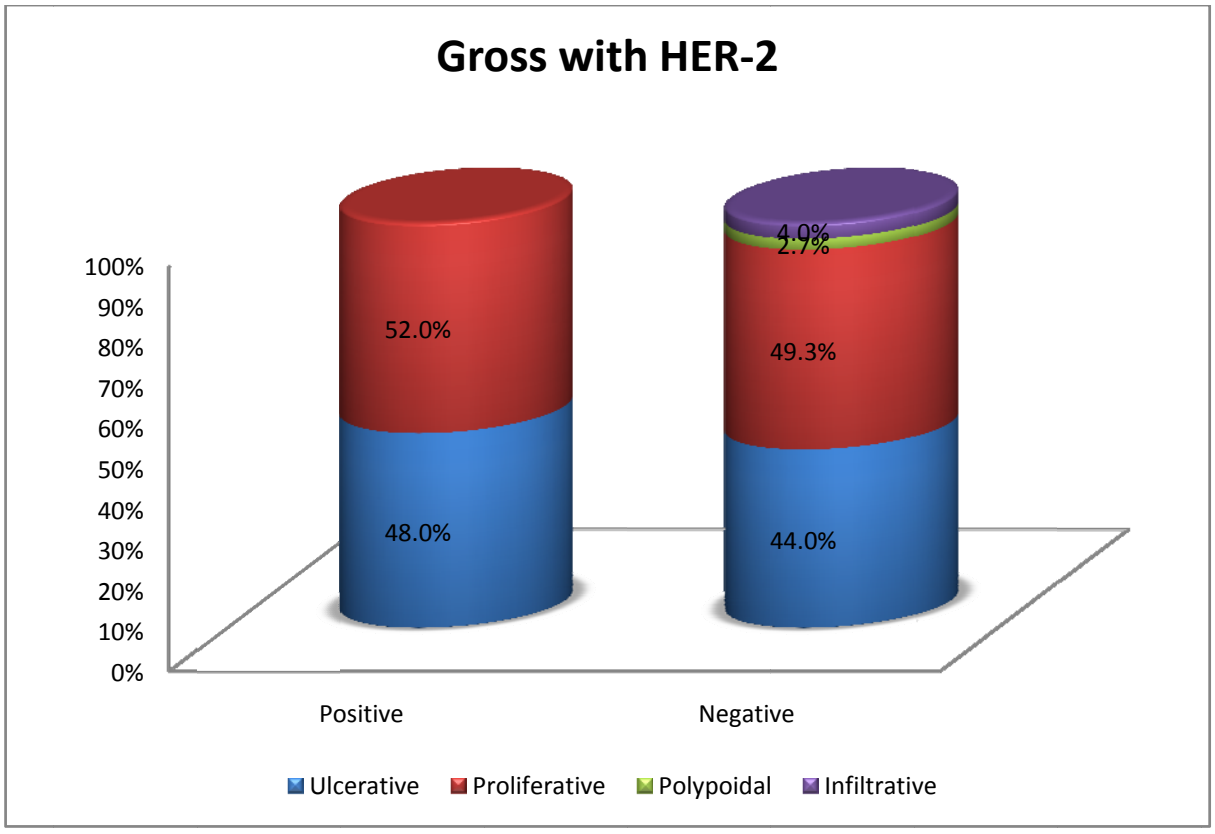


Table 16: Association of Tumour Grade with HER-2/Neu Expression

			HER - 2		
			Positive	**Negative**	**Total**
HPE	Well differentiated	Count	7	10	17
		% within HER - 2	28.0%	13.3%	17.0%
	Moderately differentiated	Count	13	29	42
		% within HER - 2	52.0%	38.7%	42.0%
	Poorly differentiated	Count	5	36	41
		% within HER - 2	20.0%	48.0%	41.0%
Total		Count	25	75	100
		% within HER - 2	100.0%	100.0%	100.0%

P value: **0.034 (<0.05)**

Positivity for HER-2/Neu was seem to be more with moderately differentiated cases (52%) than well differentiated (28%) or poorly differentiated (20%) cases and **the association was statistically significant. (P value < 0.05)** (Table 16 & Chart 16)

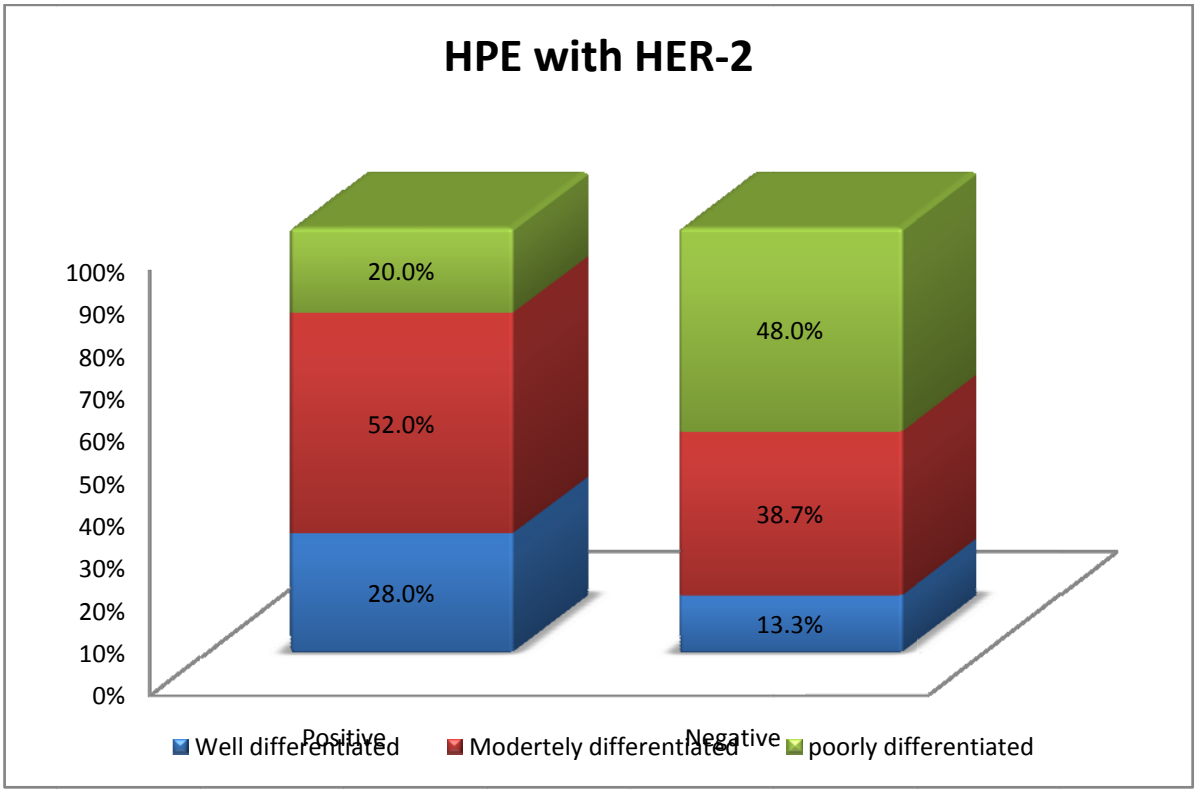


Table 16: Association of Tumour Grade with HER-2/Neu Expression

DISCUSSION

Gastric cancer had been described as early as 1500 BC in manuscripts from ancient Egypt called Ebers Papyrus[1] . Gastric cancer is the fourth most common cancer and the second leading cause of death due to cancer worldwide after lung cancer[2].

Gastric cancer is more common in male than in female in the ratio of 2:1. It is a disease of elderly with higher incidence around 65yrs. For last few years there is decline in incidence rate in the western countries [8]. In Asia it is still one ofthe most common malignancies accounting for 18% of all malignancies.In countries like Japan and Korea it accounts for 56% of malignancies [7].

The poor prognosis of gastric adenocarcinoma is due to its late presentation, nonspecific symptoms like dyspepsia in early stage and limitations in treatment options. Molecular markers are vital in determining the disease progression and hence disease outcome, survival and prognosis. An association between clinicopathological features and molecular markers of gastric adeno carcinoma would give a clue toward the relationship between them and hence provide us an extra tool to combat the high mortality due to these carcinomas.

Though many studies have been conducted in gastric carcinoma all over the world for the expression of HER-2/Neu and their prognostic significance, the results are still contradictory. Some found a statistically significant association of these markers with prognosis and survival, while others found no such association.

In this study, immunohistochemical evaluation was done in 100gastric carcinoma cases; attempt was made to correlate the expressionof HER-2/Neu with various clinicopathological factors .

The age of the patients ranged from 27 years to 72 years, with a mean age of 60.3years. The age group showing the greatest incidence of gastric carcinoma was 55 to 65 years. This is correlated with Zhang HK et al , who observed a mean age of 52 years with the age group ranging between 25 and 75 years.

In the current study, the incidence of gastric carcinoma in males were 68% and females were 28% . Nobuyuki Igarashi et al who noted an incidence of 74.1% and 25.9% in males and females respectively has been correlated with our study.

The most common site of gastric carcinoma in this study was pyloroantral region (60%), which is similar to the study by C Fondevilla et al showing occurrence of 51%of cases in the pyloroantral region[(44)].

In this study moderately differentiated tumours were morecommon than other grades accounting for 42% of cases, which is correlated with Fondevila et al study (49%)[(44%)].

The most common clinical feature in this study is abdominal pain only (29%) which is correlated with Wanebo HJ et al showing 30 % of cases with abdominal pain only.

The most common gross appearance seen in our study population is proliferative pattern (50%) and least one is polypoidal with incidence of 2%

Her -2 Overexpression is seen in 25% of cases correlated with

Tanner et al who got 36.6% results and Yano et al study in which 27 % of cases were positive for Her -2 receptor[(51)]

Correlation of HER-2/Neu Expression with Various

Clinicopathological Factors

H R Raziee et al (2007)[(15)] studied 100 cases of gastric tumours & found a significant association of HER-2/Neu over expression with well differentiated grade, and no association between age, gender, location of tumour and depth of infiltration.

Zhiyong Liam et al (2008) studied 100 cases and found no significant association of over expression of HER-2/Neu withany clinicopathological factors.

Similarly ,S. D. Xie et al (2009)[60] and Xie Li Zhang et al (2009) were not able to demonstrate association with any other known clinicopathological and prognostic factors.

In this study, ***a statistically significant association was obtained between histological grade and HER-2/Neu overexpression***.

Positivity for HER-2/Neu was seem to be more with moderately differentiated cases (52%) than well differentiated (28%) or poorlydifferentiated (20%) cases with P value < 0.05. This is correlated with Razee et al[15] , who found that Her -2 over expression in noted in Moderately differentiated (67%) and well differentiated tumours (20%).

All other variables compared like age, sex, clinical features, location of tumour , gross and endoscopic appearance were found to be statistically non significant.

SUMMARY

- Gastric carcinoma had a peak incidence in the 55 to 65 years agegroup. The oldest age of presentation was 80 years and theyoungest age of presentation was 27 years.
- Incidence was more in males (68%) than females (32%).
- Among the 100 cases 27were gastrectomy specimens and73 were endoscopic biopsies.
- The most common site of occurrence was pyloroantral region(60%).
- Proliferative type was the most common morphological type seen with incidence of 50%
- Moderately differentiated histological grade was the most common grade constituting for 42% of gastric carcinoma cases.
- The most common clinical feature is abdominal pain only accounting 29% of cases.
- HER-2/Neu over expression was seen in 25% of cases.
- A significant association was found between HER-2/Neu over expression and histologic grading of gastric adenocarcinoma.
- No association was found between HER-2/Neu expression and age,sex,site of tumour, gross appearance, endoscopic appearance and clinical features

CONCLUSION

Many gastric adeno carcinoma patients in Government Kilpauk Medical College Hospital were older than 5o years of age with male preponderance which is similar to several other studies conducted throughout the world.

The most common Clinical feature is abdominal pain and the common site involved being Pyloroantral region. Morphlogic subtype among them is done and proliferative type is found to be the most common. The most common histologic grade is moderate differentiation. 25% of gastric adenocarcinoma cases showed Her-2 receptor over expression. Statistical significance was found out between Her -2 receptor over expression and histological grade.

Delays in diagnosis and limitation of therapeutic options contribute to poor prognosis of gastric adeno carcinoma. Hence, the contribution of these genetic markers like Her -2 receptor towards prediction of progression and prognosis along with newer therapeutic modalities could be of immense benefit in gastric cancer patients.

ANNEXURE – I

PROFORMA

Case number : Name :

HPE number : Age :

IP number : Sex :

Clinical features :

Clinical diagnosis :

Endoscopy :

Previous HPE report:

Nature of specimen : Total gastrectomy/Subtotal gastrectomy/Endoscopic biopsy

Gross appearance:

Tumour site :

MICROSCOPY:

Histological grade : G1 / G2 / G3

IMMUNOHISTOCHEMISTRY

HER-2/Neu: Intensity &Percentage of cells showing staining

ANNEXURE II

TNM STAGING OF GASTRIC TUMOURS

T – Primary Tumour

TX - Primary tumour cannot be assessed

T0 - No evidence of primary tumour

Tis - Carcinoma in situ

T1 - Tumour invades lamina propria or submucosa

- T1a-Tumour invades lamina propria or muscularis mucosa

- T1b- Tumour invades submucosa

T2 - Tumour invades muscularispropria

T3 - Tumour penetrates subserosa without invasion of serosa

T4 - Tumour invades serosa or adjacent structures

- T4a- Tumour invades serosa

- T4b- Tumour invades adjacent structures

N – Regional Lymph Nodes

NX - Regional lymph nodes cannot be assessed

N0 - No regional lymph node metastasis

N1 - Metastasis in 1 to 2 regional lymph nodes

N2 - Metastasis in 3 to 6 regional lymph nodes

N3 - Metastasis in more than 7 regional lymph nodes

M – Distant Metastasis

MX - Distant metastasis cannot be assessed

M0 - No distant metastasis

M1 - Distant metastasis

STAGE GROUPING

Stage 0 Tis N0 M0

Stage IA T1 N0 M0

Stage IB T1 N1 M0
T2 N0 M0

Stage IIAT1 N2 M0
T2 N1 M0
T3 N0 M0

Stage IIB T1 N3 M0
T2 N2 M0
T3 N1 M0

Stage IIIA T2 N3 M0
T3 N2 M0
T4a N1 M0

Stage IIIB T2 N3 M0
T3 N2 M0
T4a N1 M0

Stage IIICT4a N2 M0
T4b N0 M0
T4b N1 M0

Stage IV Any T Any N M1

ANNEXURE III

IMMUNOHISTOCHEMISTRY PROCEDURE

1. 4µ thick sections were cut from formalin fixed paraffin embeddedtissue samples and transferred to gelatin-chrome alum coated slides.
2. The slides were incubated at 58ºC for overnight.
3. The sections were deparaffinized in xylene for 15 minutes x 2 changes.
4. The sections were dehydrated with absolute alcohol for 5 minutes x 2changes.
5. The sections were washed in tap water for 10 minutes.
6. The slides were then immersed in distilled water for 5 minutes.
7. Heat induced antigen retrieval was done with microwave oven inappropriate temperature with citrate buffer for 20 to 25 minutes.
8. The slides were then cooled to room temperature and washed inrunning tap water for 5 minutes.
9. The slides were then rinsed in distilled water for 5 minutes.
10. Wash with appropriate wash buffer (phosphate buffer) for 5 minutes x2 changes.
11. Apply peroxidase block over the sections for 10 minutes.
12. Wash the slides in phosphate buffer for 5 minutes x 2 changes.
13. Cover the sections with power block for 15 minutes.

14. The sections were drained (without washing) and appropriate primaryantibody was applied over the sections and incubated for 1 hour.

15. The slides were washed in phosphate buffer for 5 minutes x 2changes.

16. The slides were covered with Super Enhancer for 30 minutes.

17. The slides were washed in phosphate buffer for 5 minutes x 2changes.

18. The slides were covered with SS Label for 30 minutes.

19. Wash in phosphate buffer for 5 minutes x 2 changes.

20. DAB substrate was prepared by diluting 1 drop of DAB chromogen to1 ml of DAB buffer.

21. DAB substrate solution was applied on the sections for 8 minutes.

22. Wash with phosphate buffer solution for 5 minutes x 2 changes.

23. The slides were washed well in running tap water for 5 minutes.

24. The sections were counterstained with Hematoxylin stain for 2seconds (1 dip).

25. The slides were washed in running tap water for 3 minutes.

26. The slides are air dried, cleared with xylene and mounted with DPX.

ANNEXURE IV

SCORING SYSTEM FOR THE IMMUNOHISTOCHEMICAL MARKER HER-2 RECEPTOR

0 : no discernible staining or background type staining;

1+ : discontinuous membrane staining;

2+ : membrane staining with moderate intensity

3+ : strong and complete plasma membrane staining.

More than 10% of the cells are required to meet the criteria for HER2 analysis. 3+ cases are classified as over expression

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INSTITUTIONAL ETHICS COMMITTEE
GOVT.KILPAUK MEDICAL COLLEGE,
CHENNAI-10
Ref.No.4721/ME-1/Ethics/2016 Dt: 11.08.2016
CERTIFICATE OF APPROVAL

The Institutional Ethical Committee of Govt. Kilpauk Medical College, Chennai reviewed and discussed the application for approval **"Analysis of Human Epidermal growth Factor receptor-2 expression in Gastric adenocarcinoma"** – For Project Work Submitted by Dr.V.Lokesh Kumar,MD, Dept. of Pathology, Govt. KMC, Chennai-10.

The Proposal is APPROVED.

The Institutional Ethical Committee expects to be informed about the progress of the study any Adverse Drug Reaction Occurring in the Course of the study any change in the protocol and patient information / informed consent and asks to be provided a copy of the final report.

DEAN 22/8/16

Ethical Committee
Govt.Kilpauk Medical College,
Chennai

22/8/16.

My Comp<E Colon< ME 1 Sec> Ethical Committee

			MALE-1	ENDOSCPOIC BIOPSY -1	CADRIA-1	ABDOMINAL PAIN-1	ULCER-1	ULCERATIVE -1	GRADE1 -1	POSITIVE1
			FEMALE-0	GASTRECTOMY-2	BODY-2	OBSTRUCTION-2	GROWTH-2	PROLIFERATIVE-2	GRADE2 -2	NEAGATIVE-2
					FUNDUS-3	LOSS W & A-3		POLYPOIDAL-3	GRADE3-3	
					PYLORO ANTRUM-4			INFILTRATIVE-4		
S.NO	**BIOPSY NO**	**AGE**	**GENDER**	**TYPE**	**SITE**	**CLINICAL FEATURE**	**ENDOSCOPY**	**GROSS**	**HPE**	**HER -2**
1	2318/14	65	1	2	4	1,2,3	1	1	2	1
2	2321/14	54	1	2	4	1,2,3	1	2	1	1
3	2333/14	35	1	1	3	1	2	2	3	2
4	2338/14	52	2	2	1	1,2	1	1	3	2
5	2369/14	59	1	1	2	1	1	1	1	2
6	2422/14	59	1	1	4	1,2.3	1	3	2	2
7	2442/14	58	1	1	4	1,2,3	1	1	3	2
8	2523/14	27	2	2	4	1,2	2	2	2	2
9	2526/14	56	1	1	2	2	1	1	3	2
10	2535/14	51	1	1	4	1,2	1	1	2	2
11	2539/14	46	1	1	4	1,2,3	1	4	2	2
12	2739/14	51	2	1	1	1	1	2	2	2
13	2882/14	50	1	1	3	1,2	1	4	3	2
14	2913/14	59	2	1	4	1,2	1	1	2	1
15	2928/14	58	1	1	4	1	1	1	1	1
16	23/15	61	2	1	4	1	1	1	2	1
17	31/15	54	1	1	4	1	1	1	1	1
18	40/15	40	2	1	4	1	1	1	2	1
19	70/15	55	1	1	4	1,3	1	1	3	2
20	95/15	69	2	2	2	1,3	2	2	2	2
21	102/15	66	2	2	2	1	1	2	3	2
22	120/15	63	1	2	4	1,2	1	1	3	2
23	188/15	59	1	2	3	1,2,3	1	2	2	2
24	397/15	50	1	1	4	1,3	1	1	1	2
25	502/15	29	1	1	1	1	1	2	2	2
26	557/15	51	2	2	4	1,2,3	1	1	2	2
27	574/15	62	1	1	4	1,2	2	2	2	2
28	588/15	54	1	1	3	1	1	2	1	2
29	708/15	53	1	1	4	1,2,3	1	2	2	2
30	797/15	39	1	1	2	1	1	2	1	1
31	816/15	52	1	1	2	2	1	2	2	2
32	822/15	51	1	1	2	1,2	1	1	3	2
33	850/15	50	2	2	4	1,2	2	1	3	2
34	922/15	59	2	1	4	3	1	2	2	1
35	929/15	67	1	1	4	1,2	2	2	3	2
36	1141/15	41	1	1	4	1	2	2	2	1
37	1192/15	61	1	1	4	1	1	1	1	1
38	1194/15	62	1	1	4	2	1	1	2	2
39	1286/15	58	1	1	4	2	2	2	1	1
40	1290/15	59	2	2	4	2,3	1	2	2	1
41	1359/15	42	1	1	2	1	1	1	1	2
42	1366/15	52	1	1	4	3	1	2	2	1
43	1469/15	57	1	1	2	2,3	1	1	3	2
44	1560/15	58	1	1	4	1,3	2	2	2	2
45	1649/15	67	2	1	4	1,3	2	2	2	2
46	1847/15	37	1	1	4	1,3	2	2	2	2
47	1881/15	58	1	1	2	3	1	1	2	2
48	1924/15	36	1	1	4	1,2	2	1	3	2
49	1925/15	46	1	2	4	1	1	2	2	2
50	1995/15	49	2	1	2	1,3	1	4	3	2
51	1997/15	72	1	2	4	1	1	2	1	2
52	2015/15	61	1	1	4	1,2	1	1	3	2
53	2050/15	68	1	1	4	1,2	1	2	2	1
54	2068/15	35	1	1	2	1,2	1	2	3	2
55	2122/15	67	2	2	2	2	1	1	3	2
56	2139/15	64	2	2	4	1,3	1	2	3	2
57	2192/15	59	1	2	4	1,3	1	2	3	2
58	2279/15	58	1	1	4	1,3	1	2	3	2
59	2397/15	57	1	1	2	1	1	1	3	2
60	2399/15	38	2	1	3	1,2,3	2	2	3	1
61	2413/15	71	1	2	4	1	1	2	3	2
62	2640/15	63	2	1	4	1	1	2	2	2
63	2648/15	56	1	2	4	1,3	2	2	3	1
64	2732/15	57	1	1	2	2,3	2	1	2	2
65	2774/15	57	1	1	4	2	2	2	2	2
66	2784/15	27	1	2	2	1	2	2	3	2
67	008/16	73	1	1	2	1	1	1	3	2
68	27/16	69	2	1	2	1	1	2	3	2
69	37/16	58	1	1	4	1,2	1	1	2	2
70	75/16	80	2	1	1	1,3	1	1	2	2
71	82/16	67	1	1	4	1,2	2	2	3	2
72	92/16	39	1	1	4	1,2	1	1	3	2
73	104/16	63	1	1	4	1	2	2	1	2
74	112/16	61	1	2	4	2,3	2	2	2	2
75	114/16	55	2	2	2	2,3	1	1	3	2
76	155/16	40	2	1	4	1,3	1	1	2	1
77	178/16	50	1	1	2	1,3	1	1	3	1
78	216/16	34	1	1	4	1	1	1	1	2
79	250/16	59	2	1	4	1,2,3	2	3	1	2

80	265/16	54	1	1	4	1,2	1	2	2	2
81	288/16	56	1	1	1	1	2	1	2	2
82	293/16	55	2	1	2	1	1	2	2	2
83	324/16	37	1	1	2	3	2	1	3	2
84	353/16	52	1	1	2	1	1	2	1	1
85	384/16	49	1	1	4	1,3	1	1	3	1
86	394/16	57	2	1	2	1,3	1	1	1	2
87	402/16	45	1	1	4	1,3	1	1	2	2
88	409/16	63	2	2	4	1,3	2	1	2	2
89	410/16	62	1	2	4	1,3	1	2	3	2
90	419/16	61	1	1	1	1,2,3	1	1	2	1
91	433/16	60	2	1	2	1,3	1	1	1	2
92	440/16	57	2	1	2	1,3	2	2	3	1
93	446/16	42	1	2	3	1,2	2	2	3	2
94	474/16	59	1	2	3	1,2	1	2	3	2
95	485/16	56	1	1	4	1	1	1	3	2
96	511/16	70	2	2	2	1	1	2	3	2
97	540/16	72	2	1	4	1,3	1	2	2	1
98	595/16	61	2	2	4	2	1	1	2	1
99	613/16	42	2	1	4	3	1	2	3	2
100	902/16	54	1	1	4	1,2	2	2	3	2