Evaluation of HER-2/neu status in Gastric Carcinoma

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DECLARATION

I solemnly declare that this dissertation titled "**Evaluation of HER-2/neu status in Gastric Carcinoma"** is done by me in the Department of Medical Oncology, Madras Medical College & Rajiv Gandhi Government General Hospital, Chennai under the guidance and supervision of **Prof. K.Kalaichelvi, MD., DM.,** Professor & Head of the Department, Department of Medical Oncology, Madras Medical College & Rajiv Gandhi Government General Hospital, Chennai. This dissertation is submitted to the Tamil Nadu Dr.MGR Medical University, Chennai in partial fulfillment of the university requirements for the award of the degree of DM.Medical Oncology.

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CERTIFICATE

This is to certify that the Dissertation entitled, **"Evaluation of HER-2/neu status in Gastric Carcinoma"** is the bonafide record work done by **Dr. K.B. AKILA**, under our guidance and supervision in the Department of Medical Oncology, Rajiv Gandhi Government General Hospital, Madras Medical College, Chennai, submitted as partial fulfillment for the requirements of D.M. Degree examination Branch VII MEDICAL ONCOLOGY, AUGUST 2012, under The Dr.M.G.R. Medical University, Chennai.

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CONTENTS

INTRODUCTION

Gastric carcinoma is the fourth most commonly diagnosed cancer in the world and is the second leading cause of cancer related death. In the West, the incidence is spontaneously declining for unknown reasons. In Asia it is still one of the commonest cancers accounting to around 13% of all malignancies. In countries like Japan and Korea in Eastern Asia, it accounts for 56% of all malignancies. Early diagnosis is challenging because of absence of symptoms in this stage of disease. More than 50% of patients with gastric cancer present in advanced, unresectable stages, making cure impossible. Two-thirds of these patients who undergo radical surgery will experience. Systemic treatment is the only option for the patients presenting in advanced stages. The median survival after diagnosis of metastatic disease is approximately 10-11 months with currently available therapies. Despite the introduction of multimodality treatment, neoadjuvant chemotherapy, postoperative chemoradiation and new chemotherapeutic regimens, there is only a minimal impact on the relapse free survival and overall survival of these patients. Many single agents and combination chemotherapeutic agents are active in the treatment of metastatic disease. Objective response rates ranging from 10% to 30% for single-agent and 30% to 60% for combination regimens have been reported. Although a large number of

chemotherapy regimens are available, there is still no internationally accepted standard of care. Survival of patients presenting with advanced gastric cancer is still only 4%-10% at five years. Newer therapies are urgently needed for their better outcome. Recently, understanding of the molecular basis of cancer has contributed to the development of rationally designed molecular targeted therapies, which interfere with signalling cascades involved in cell differentiation, proliferation, and survival, of which EGFR plays an important role. Trastuzumab is one of the targeted drugs against EGFR2 (commonly known as HER-2/neu) and has recently shown to increase survival in patients with metastatic gastric carcinoma when given along with combination chemotherapy.

Recent evidence suggests that patients diagnosed with metastatic gastric cancer should have the HER-2/neu status of their tumors to get benefit from treatment with Trastuzumab in combination with chemotherapy in case of HER-2/neu positivity. We conducted this study to analyse the prevalence and significance of HER-2/neu over expression in patients with gastric carcinoma.

REVIEW OF LITERATURE

Over 870,000 cases of stomach cancer diagnosed each year. [1,2] It is more common in Eastern Asia where it accounts for more than 50% of all malignancies. It is rare in European countries and America. In India Gastric cancer is the fifth most common malignancy in males and seventh most common in females.[3] The highest incidence in India is in Mizoram where it accounts for nearly 30% of all newly detected cancers. The reason for this increase is thought to be genetic and dietary factors although the exact reason is not understood. In the Madras Metropolitan Tumor Registry, carcinoma of stomach ranks second from top among males and fifth among females in crude incidence rate.

In Asian population most of the malignancies are in the distal stomach where as in the West it is more in the proximal stomach.[4] At the time of diagnosis, gastric cancers are localized and surgically resectable in approximately one half of the patients. Regional nodal metastases or direct infiltration of surrounding organs or structures are frequently encountered and preclude cure in many patients. Although relapse after complete surgical resection occurs most commonly in the tumor bed and nodal regions, systemic pattern of relapse is also known to occur commonly in gastric cancer.[5] The overall 5-year survival rate of

all people with stomach cancer is only about 28% irrespective of the stage at presentation.

ETIOLOGICAL FACTORS

Etiological factors for gastric cancer include smoked or salted foods, foods contaminated with aflatoxin, low intake of fruits and vegetables, low socioeconomic status, and possibly a decreased use of refrigeration.[6] Possible occupational relationships include coal mining and rubber or asbestos industry.

PRE MALIGNANT LESIONS

Precursor neoplastic conditions include pernicious anemia, achlorhydria, atrophic gastritis, gastric ulcers, and adenomatous polyps.[8] Between 5% and 10% of individuals with pernicious anemia subsequently develop malignancy. Prior partial gastrectomy for benign gastric or duodenal ulcer disease produces an increased risk of subsequent malignancy in the gastric remnant with latency periods of 20 years or more. Helicobacter pylori infection has been shown to predispose to a three- to six fold increased risk of gastric cancer, but the precise role of this bacterium in the etiology of gastric cancer remains unknown.[7] H.pylori is associated mainly with distal and intestinal-type of gastric cancers.

GENETIC SUSCEPTIBILITY

Some individuals are at increased risk of developing gastric cancer, because of dominantly inherited cancer predisposition syndromes, such as Familial adenomatous polyposis, Lynch syndrome,[9] and Li-Fraumeni syndrome.[10] Patients with Peutz-Jeghers are also at risk for developing gastric cancers. [11]

Germline mutations in the E-cadherin gene (CDH1) account for 30-40% of Heriditary Diffuse Gastric Cancer.[12] In these patients, the lifetime risk of developing a gastric cancer is about 67% in men and 83% in women.

SITE OF TUMOR

The site of origin of tumor within the stomach has changed over recent decades, with more proximal lesions now being diagnosed and treated. The largest percentage of gastric cancers still arises within the antrum or distal stomach (around 40%), are least common in the body of the stomach (around 25%), and are of intermediate frequency in the fundus and esophagogastric junction (around 35%).

PATHOLOGY

Two distinct histologic types of gastric cancer, the "intestinal type" and "diffuse type", have been described by Lauren.[13] The diffuse type of gastric cancer is undifferentiated and is characterized by the loss of E-cadherin expression; an adhesion protein that helps maintains cellular organization. The well differentiated intestinal type is sporadic and highly associated with environmental exposures, especially H. pylori infection.[14] There are also biologic differences between these subtypes of gastric cancer that may guide treatment strategies. Overexpression of HER2 is associated with intestinal-type gastric carcinomas more frequently than diffuse type due to unknown reasons. [15]

PROGNOSTIC FACTORS

The most meaningful prognostic indicators relate to extent of the disease which is otherwise the stage of the disease. The prognostic factors are as follows-

1. Presence of peritoneal disease.
2. Number and location of lymph nodes.
3. Adjacent organ infiltration.

Prognosis is generally worse with higher grade and diffuse-type carcinomas, which usually present with higher pathologic stages of disease but none of these are independent prognostic variables. The 5-year survival rates for gastric cancer, by stage are as follows:

Stage IA	71%
Stage IB	57%
Stage IIA	45%
Stage IIB	33%
Stage IIIA	20%
Stage IIIB	14%
Stage IIIC	9%
Stage IV	4%

These are well known prognostic factors. Recently evidence also exists that overexpression of HER-2/neu in gastric cancer is a new, independent prognostic factor for overall survival.[16,17]

PREDICTIVE FACTORS

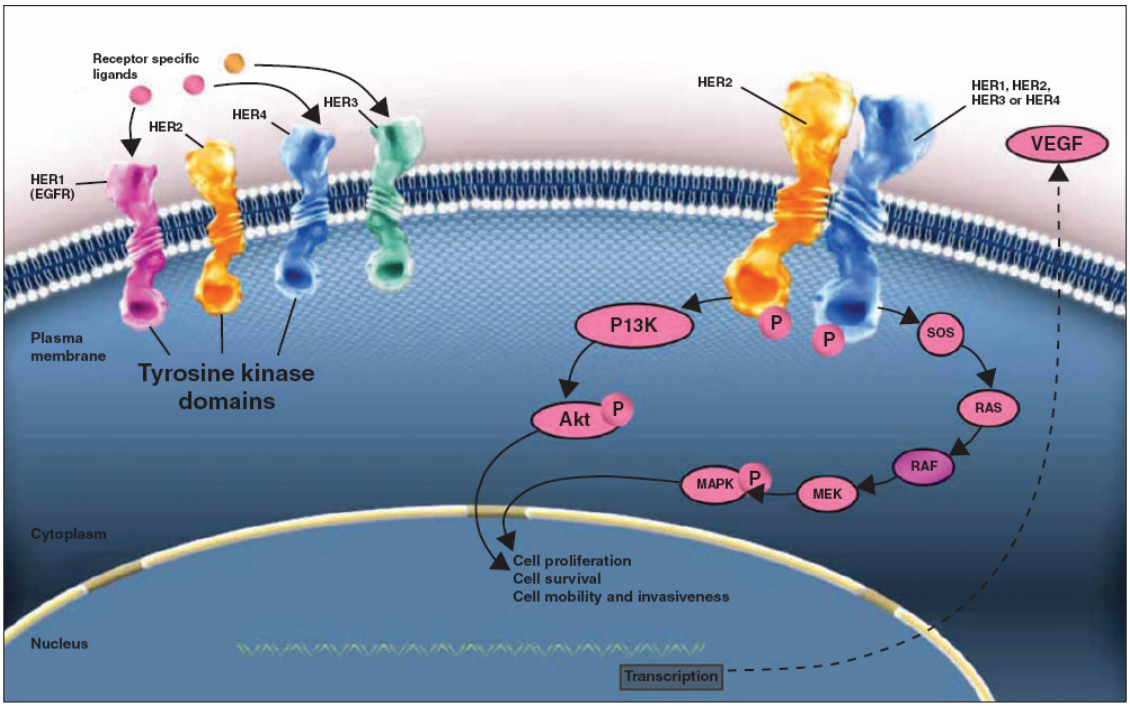
There has not been any predictive marker for survival in gastric cancer patients until molecular studies were done. HER-2/ neu gene is one of the molecular markers which is usually overexpressed in breast cancer patients. Recently molecular studies have shown that HER-2/ neu

gene is also overexpressed in 20-25% of patients with gastric cancer and treatment with HER-2/ neu antibody has shown to improve survival in HER-2/ neu overexpressed patients when given along with chemotherapy. This is the only known predictive factor in carcinoma stomach. Its role as prognostic factor is yet to be determined. [18]

EPIDERMAL GROWTH FACTOR RECEPTORS

Within a multi-cellular organism, regulation and organization require biological signals to be transmitted from one cell to another, across cell membranes. Such is the case with growth factors, which originates at one site in an organism and needs to be distributed throughout the organism to many cell types in order to exert their pleiotropic effects. Systems have evolved to allow a soluble signals like growth factor to be conveyed from the extracellular space in to the cytoplasm and nucleus of a cell, thus directing protein synthesis, cellular growth, and proliferation. Growth factors appear to induce aggregation of their receptors, leading to activation of these receptors and signal propagation across the membranes. If unregulated, however, these growth signals have the potential to promote inappropriate proliferation. Receptor aggregation in the absence of signal represents unregulated

Signal Transduction by the HER Family Promotes Proliferation, Survival, and Invasiveness



growth factor receptor activity which is postulated to occur in human neoplasia.

Erb B receptors are expressed in various tissues of epithelial, mesenchymal and neuronal origin, where they are involved in controlling diverse biological processes such as proliferation, differentiation, migration and apoptosis. Their expression is deregulated in many types of human cancer. Owing to the importance of Erb B proteins in cellular transformation and development, a lot of attention has been focused on this family of receptor tyrosine kinases. [19]

The EGFR family of trans membrane receptor tyrosine kinases is composed of four members: HER- 1 also known as erb B 1, HER-2 also known as erb B2 , HER- 3 also known as erb b3, and HER -4 also termed erb B4. The molecular structure of the receptor consists of an extracellular binding domain, a short transmembrane domain and an intracellular domain with tyrosine kinase(TK) activity except for HER-3 data. [20]

Driven by the binding specificities of the bivalent, epidermal growth factor related peptide ligands and the complement receptor available on the cell, Erb B receptor form homodimeric and heterodimeric complexes. All pair wise combinations of the four

receptors can be induced by ligands generating a potential for signal diversification. Alternatively receptor over expression promotes spontaneous receptor dimerization in the absence of a ligand and constitute receptor activation.[21]

Binding of the ligand, neuregulin, to HER-3 allows for hetero dimerization with HER-2 and subsequent transphosphorylation. Activated HER-3 is capable of activating downstream pathways, and in particular, is a potent activator of phosphatidylinositol-3- kinase (PI3K)/Akt pathways. HER-2/neu receptor must homodimerize (ligand-independent dimerization) or heterodimerize with another member of the EGFR family, such as HER-3 or HER-4 (ligand-dependent dimerization) to undergo activation, autophosphorylation, and triggering of the downstream signaling cascade. Crystal structure studies of HER-2 receptor have revealed fixed conformation resembling ligand activated state, capable of interacting with other EGFR family members in the absence of direct ligand binding. HER-2 can enhance signalling by forming a heterodimer with HER-3, where this has been reported to be the most mitogenic dimer. [21]

ErbB2 receptor heterodimerization also results in signal diversification, activation of STAT transcription factor is also triggered by ErbB2 / Erb B4 induced heterodimerisation**.**

SIGNALING KINETICS-

The cell employs several mechanism to re-establish its default setting after ligand induced RTK activation. These mechanisms include receptor dephosphorylation by tyrosine phosphates, receptor desensitisation by phosphorylation of specific serine and threonine residues and final receptor downregulation by internalisation and subsequent lysosomal degradation of the receptor.

HER-2/neu over expression also leads to increased activity of HIF (hypoxia inducible factor) and subsequently increased VEGF (Vascular Endothelial Growth Factor) expression in these cells. Overexpression of HER-2/neu receptor also led to the increase in the basal level of VEGF secretion in these cells. Subsequent exposure to hergulin led to further induction of VEGF secretion .These findings indicate that the HER-2/neu system is an important target for direct and indirect therapy for cancer and clinical trials of this approach of inhibiting HER-2/neu are currently ongoing.

HER-2/neu TESTING IN GASTRIC CANCER IS NECESSARY DUE TO THE FOLLOWING REASONS

- HER-2/neu gene amplification and HER-2/neu protein over expression have been observed in various solid tumors other than breast, including gastric carcinomas. [22-27]
- The rate of HER-2/neu positivity in advanced gastric cancer is comparable to that seen in locally advanced and metastatic breast cancer using validated methodology, the large sample set from the ToGA trial revealed a HER-2/neu positivity rate of 22% in advanced Gastric carcinoma. [28]
- HER-2/neu has predictive value in gastric cancer. [18]

As in breast cancer, accurate HER-2/neu testing is essential to identify patients who may benefit from treatment with HER-2/neu targeted therapy

THE METHODS BY WHICH HER2 STATUS CAN BE ASSESSED

1. IHC- Immunohistochemistry.
2. FISH – Fluroscent in situ hybridisation
3. CISH – Chromogenicn in situ hybridisation
4. SISH –Silver in situ hybridisation

Though so many methods are there only IHC and FISH are validated and IHC is the most common method use to assess HER-2/neu status.[29]

Guidance for determining HER2 status in gastric cancer.

- IHC and FISH test kit reagents are identical to the breast cancer HER-2/neu assay systems in terms of reagents, antibodies and probes.
- Bright-field techniques (IHC) should be used as the primary testing modality as they allow identification of small tumour foci with HER-2/neu overexpression within heterogeneous tumour tissue
- Accurate HER-2/neu testing in gastric cancer is dependent on adherence to the modified IHC scoring system described by Hofmann et al 2008. [30]
- Highly experienced pathologists are required for accurate determination of HER-2/neu status and nuances between breast and gastric tissues.

The methods by which IHC can be done are

1. Hercep test (Dako cytomation)
2. CONFIRM anti her2 (Ventana)

IHC can be performed on the tissues obtained after radical surgery or with even a small tissue obtained by endoscopic biopsy. The interpretation of HER-2/neu in gastric cancer by IHC is being standardized and a consensus on reporting HER-2/neu positivity by IHC has been arrived at recently. While interpreting the points to be followed are:

- Tumour cells showing either complete, basolateral or lateral membrane staining should be scored.
- Cytoplasmic staining should never be included when interpreting results.
- Normal epithelial cells should never be scored
- Artefacts may give rise to false positive interpretation

Modified scoring system by Hoffmann et al 2008 is adhered to when IHC is done.

The parameters used for IHC scoring in gastric carcinoma are as follows-

1. Intensity of reactivity- such as “absent”, “faint”, (weak), “moderate”, or “ strong.”
2. Pattern of membrane staining- complete membrane staining was not necessary; instead even basolateral and lateral staining are considered positive.

3. Number of positive cells- at least 10% of the cells in surgical specimen stained or single cluster of cells in biopsy specimen are considered positive if adequate intensity is present.

Scoring criteria in surgical specimen

Score 0	No reactivity or membranous reactivity in less than 10% of the cells.
Score 1+	Faint membranous reactivity in less than or equal to10% of the cells, cells are reactive only in part of their membrane.
Score 2+	Weak to moderate complete ,basolateral or lateral membranous reactivity in more than 10% of the cells.
Score 3+	Strong complete,basolateral or lateral membranous reactivity in more than 10% of the cells.

Scoring criteria in Biopsy specimen

Score 0	No reactivity or membranous reactivity in any of the tumor cell.
Score 1+	Tumor cell cluster with faint or barely perceptible membranous reactivity irrespective of the percentage of cells stained.
Score 2+	Tumor cell cluster with Weak to moderate complete, basolateral or lateral membranous reactivity irrespective of percentage of tumor cells stained.
Score 3+	Tumor cell cluster with strong complete ,basolateral or lateral membranous reactivity irrespective of percentage of tumor cells stained.

HER2 Overexpression assessment by IHC

Score 0	Negative
Score 1+	Negative
Score 2+	Equivocal
Score 3+	Positive

Interpretation of IHC

Incomplete membrane positivity

In gastric cancer, lateral membranous staining with linear staining at contact sites between 2 cells or basolateral membranous staining creating a U-shaped staining pattern is observed. This is due to the higher frequency of glandular formations with the lumen in gastric cancer (intestinal type) wherein basolateral (non luminal) membranes are stained. The basolateral staining pattern is probably secondary to the absence of growth factor receptors in the luminal part of the cell. This incomplete membrane positivity would be considered negative in IHC HER-2/neu scoring in breast cancer, but should be considered positive in gastric cancer. The requirement for complete membranous staining is thus omitted in gastric cancer. [31]

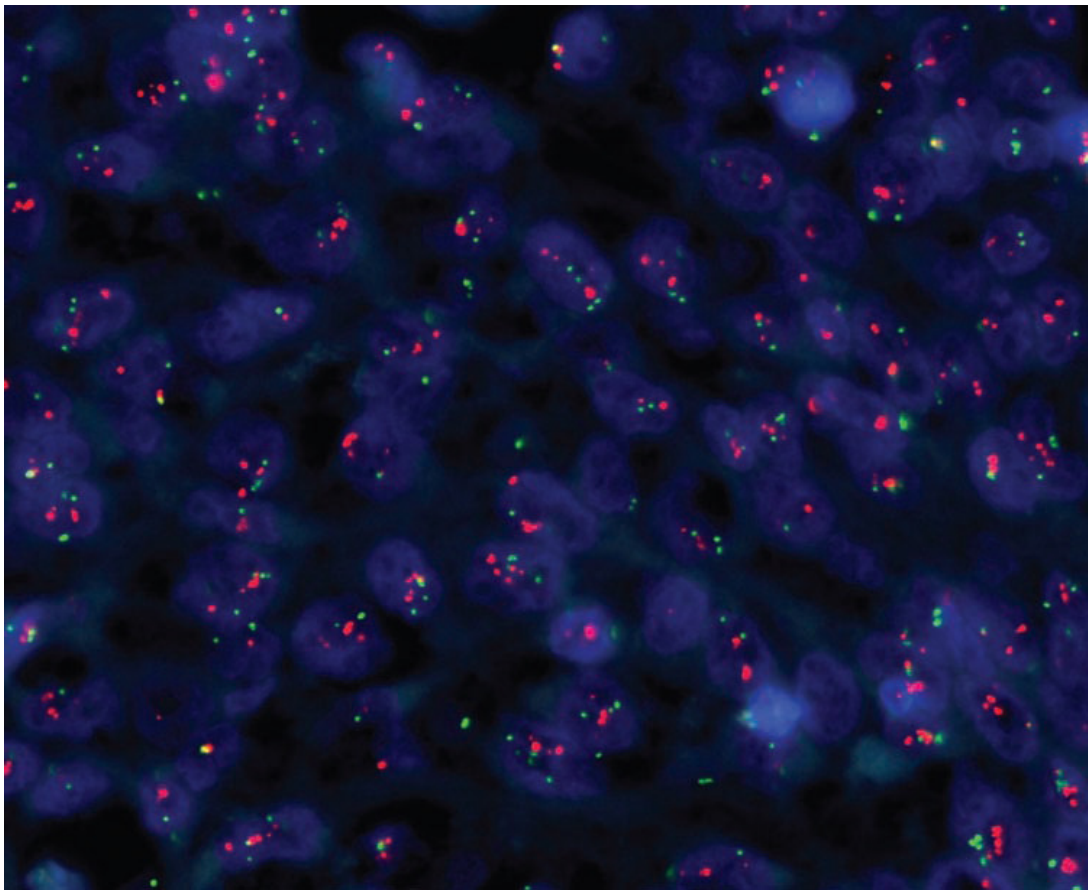
Heterogeneity

In contrast to breast cancer where IHC for HER-2/neu is usually homogenous, tumour heterogeneity is more common in gastric cancer. Heterogeneous staining can sometimes be seen within one gland. The most important reason for heterogeneous staining, however, is that up to one third of gastric cancers are of mixed intestinal/diffuse type. Strong staining is often seen in areas of an intestinal type of differentiation, while areas of diffuse types of differentiation are negative. [34] Signet ring cell carcinomas are almost always negative. An identical heterogeneous pattern of staining is identified at the DNA level (amplification). Because of heterogeneity, the 10% cut-off level for positivity, which is required in breast cancer, is omitted in gastric cancer. Positivity in gastric cancer specimens is thus independent of the percentage of stained cells and it is sufficient to have a cohesive group of cells displaying HER-2/neu positivity. The heterogeneity of HER-2/neu overexpression / amplication is difficult to explain from the biologic point of view the mechanisms leading to silencing HER-2/neu expression in an area of a tumor with homogeneous HER-2/neu amplification, is at present unknown.[32]

FISH

Gene amplification assessment is done by FISH. It is commonly done when IHC results are 2+ for confirmation of HER-2/neu positivity.

HER-2/neu overexpression by FISH



A HER-2/neu:CEP17 (centromeric probe 17) ratio of >2.2 is now used to define HER-2/neu positivity (amplification). Ratios of 1.8–2.2 to define equivocal and <1.8 are used to denote negative categories. FISH is not routinely used for testing, because it is a difficult, cumbersome and expensive technique that requires trained personnel which is not available in every pathology laboratory. Moreover, fluorescence fades upon storage for a long time thus making it difficult to preserve the slides for further reference if needed. In addition, the fluorescent probes in the kits have a limited half life. Detailed morphological features of the tumor are also usually difficult to observe due to the required protein digestion and the fluorescent mode, and heterogeneity can be missed since spots are evaluated at ×100 magnification using oil immersion. [33]

During endoscopy, as many biopsies as possible must be taken from the area suspicious for cancer. It is recommended to take at least 6 biopsies to increase the results Biopsies as well as surgical specimens can be used for HER2 testing with similar success rates. Indeed, the percentage of positivity is not statistically different for biopsies (22.8%) and surgical samples (20.0%) in the ToGA trial.[34]

The concordance between IHC and FISH is more than 95%. The other methods which can be used for testing HER-2/neu are CISH and

SISH. Prediction of HER-2/neu status by imaging mass spectrometry is based on the molecular alteration brought about by its over expression. This will enable us to detect unique molecular and genetic alterations that are independent of site of tumor and may be targeted with drugs. [35]

CONCORDANCE BETWEEN PRIMARY AND METASTATIC SITES

When HER-2/neu status was assessed by IHC and FISH on samples obtained from metastatic sites of gastric carcinoma and paired primary tumors, to establish whether treatment decision with anti HER-2/neu can be taken by assessing it in the metastatic tissue if easily accessible, it was found that there was 95% concordance of receptor expression between primary and metastatic site. Thus in patients in whom metastatic sites are easily accessible HER-2/neu status can be studied from these lesions and treatment started accordingly.[36]

Expression of TOP II ALPHA gene

When patients who were HER-2/neu positive were tested for TOP II alpha, two thirds of them showed co-amplification of TOP II gene. This suggests that majority of the patient who are HER-2/neu positive will respond to Top II inhibiting drugs. This is in line with clinical

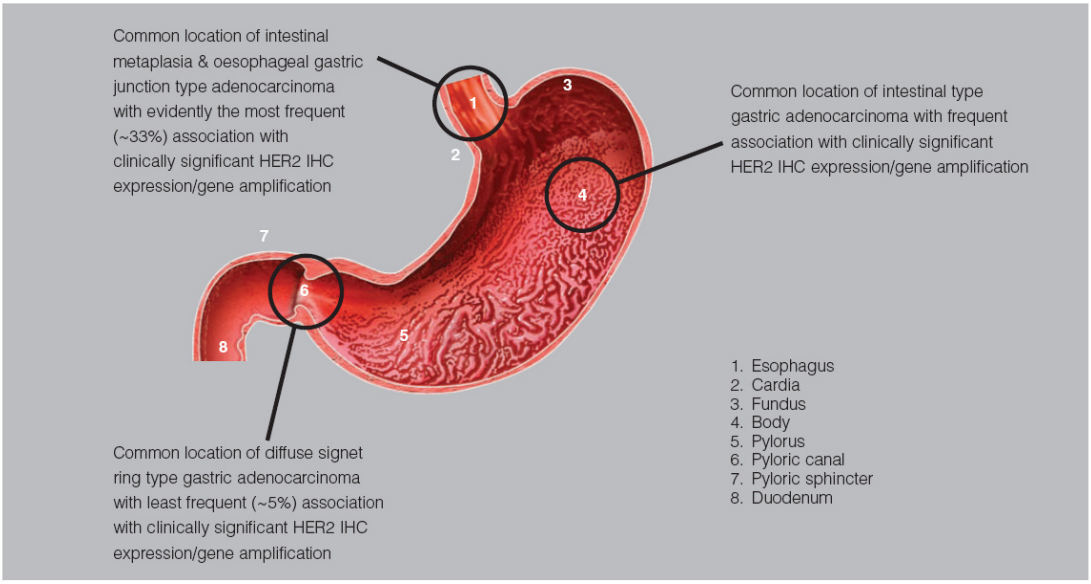
findings and topII inhibitors Adriamicin and Etoposide are widely used cytotoxic drugs in treating gastric cancer.[37,38]

CLINCO PATHOLOGIC CHARACTERISTICS ASSOCIATED WITH HER-2/neu OVEREXPRESSION

Clinicopathological data was obtained from patients who had undergone surgical resection for histologically proven gastric cancer representing the entire population of surgically operable gastric cancer patients at different institutions who had given consent for research by the results were analysed statistically against available clinicopathological criteria-gender, age, tumour size, tumour stage, histological grade, vascular invasion, perineural invasion, lymphatic invasion and survival time. Survival was measured from the date of diagnosis until the date from death from gastric cancer, or was censored until the date of the last follow-up for non-gastric cancer related deaths and survivors). No significant relation was found between clinicopathologic variables (sex and age of the patients, and tumor diameter, differentiation, location) [39,40]

HER-2/neu status was correlated with the depth of invasion, TNM stage, lymph node and distant metastasis ($P<0.05$). No significant relation

Common Sites of HER-2/neu overexpression



was found between clini-copathologic variables (sex and age of the patients, and tumor diameter, differentiation, location) [41]

HER-2/neu positivity differed significantly by histological subtype (intestinal 34%, diffuse 6%, mixed 20%) [42] and according to the site of the tumor (32% GEJ and 18% gastric localization). A higher rate of HER-2/neu positivity was seen in GEJ tumors than in gastric cancer samples (34% vs 20%). The reasons quoted for high positivity were the association of this oncogene with a specific histologic tumor type and that certain characteristics (e.g. HER-2/neu overexpression and intestinal phenotype) may be expressed together preferentially. In the Finnish study, amplification of HER-2/neu was strongly associated with poor carcinoma-specific survival, particularly evident in the subgroup of intestinal type of cancers (P =0.0019), which is usually considered to associate with more favorable prognosis than the diffuse type of gastric adenocarcinoma.

Independent risk factors of nodal metastasis include the depth of submucosal invasion, tumor diameter greater than 3.0-3.5 cm, the presence of lymphovascular permeation, depressed or ulcerated lesions, and undifferentiated histology. There was no correlation between HER-2/neu and these factors.

The role of HER-2/neu as a prognostic factor in gastric cancer has been controversial because some of the initial studies failed to find its association with prognosis.

TREATMENT OF GASTRIC CANCER:-

Surgery is the main modality of treatment. Radical surgery is not feasible as the disease is locally advanced with adjacent organ infiltration most of the times. In patients who undergo radical surgery recurrence can be reduced by giving adjuvant chemotherapy.

The goal is improvement in overall survival and to prevent recurrence. Meta-analysis of adjuvant chemotherapy by Wagner et al [(43)] included 13 trials with a total of 1,990 patients. The absolute risk reduction using chemotherapy was estimated to be 4%. The median survival was improved from 4.3 months for best supportive care to approximately 11 months for chemotherapy. There was also a modest improvement in time to progression from 7 months for patients receiving chemotherapy and 2.5 months for patients receiving best supportive care. Wagner et al concluded that the evidence supporting initiating chemotherapy for patients with advanced incurable gastric cancer was convincing.

Mari et al[44] recently reported the results of a meta-analysis of 20 individual clinical trials. A total of 3,650 patients were involved. Seven of these trials included the use of fluorouracil plus an anthracycline. They concluded that there was a small survival benefit.

Moertel et al [45] reported the results of a small randomized trial of radiation therapy (3,750 cGy in 24 fractions) plus 5-FU (15 mg/kg in three doses) versus surgery alone arm. A statistical assessment was done on the local, distant relapse, and overall survival of these patients. These data suggest that the primary effect of postoperative chemoradiation treatment was a reduction in local recurrence rates without apparent differences in distant relapse rates and survival.

Platinum compounds are an important part of treatment of gastric cancer. The use of cisplatin, in both previously treated and untreated patients, showed a response rate of approximately 15%. With 5 flurouracil overall response rates were 10% to 20%, with a median duration of response, or time to progression, of approximately 4 months. A third class of cytotoxic agents with activity in gastric cancer is the Taxanes. Docetaxel has been more extensively studied. In a recent review, Cosimo [46] reviewed trials in which docetaxel was used as single agent and the response rate was 19.1%.

Importantly, in those best supportive care trials that reported 2-year survival only patients who received chemotherapy survived. The 2-year survival rate was 5% to 14% for patients receiving combination chemotherapy versus 0% for patients receiving best supportive care. The results of these studies and supportive evidence from more recent trials indicate that fit patients with advanced incurable gastric cancer who can tolerate potential toxicities have a modest but real benefit with chemotherapy in survival when compared to best supportive care.

Targeting HER-2 in gastric cancer

The Her-2/neu gene encodes a 185-kDa transmembrane glycoprotein, possessing intrinsic protein tyrosine kinase activity.[51] HER-2/neu overexpression is increasingly recognized as a frequent molecular abnormality, driven as in gastric cancer by gene amplification. It has been solidly correlated to poor outcomes and a more aggressive disease. Additionally, preclinical data with invitro and invivo models are showing significant antitumor efficacy of anti-HER-2/neu therapies (particularly monoclonal antibodies directed towards the protein). As a result, several clinical trials are exploring in different settings and with diverse designs the potential of anti- HER-2/neu therapies in patients with gastric cancer.

TRASTUZUMAB [47]

Trastuzumab is a recombinant humanized anti-HER-2/neu monoclonal antibody directed against the HER-2/neu extracelluar domain. The antibody was humanized to minimize the immunogenicity associated with murine monoclonal antibody and to enhance endogenous immune antitumor effects. Its exact mechanism of action is not completely known, however extracellular and intracellular actions have been postulated.

Extracellular Mechanisms

1. Blocks HER-2/neu receptor cleavage and inhibits dimerization consequently reducing HER-2/neu signaling.

2. Increases receptor destruction by endocytosis. Trastuzumab seems to induce HER-2/neu down regulation and subsequent degradation in HER-2/neu over-expressing cancer cells.

Intracellular Mechanisms:

1. Inhibits intracellular signaling pathways, such as phosphoinositide 3-kinase (PI3K) signaling.

2. Has anti-angiogenesis effect through decreasing vascular endothelial growth factor (VEGF) produced by tumor cells; it may

indirectly modulate proangiogenic and antiangiogenic factors, and also has synergistic activity with chemotherapy.

3. By inducing cyclin-dependent (CDK) inhibitor p27 Facilitates G1 phase arrest.

4. Has Cytotoxic and Cytostatic activity due to immune system recruitment by antibody-dependent cell-mediated cytotoxicity (ADCC).

Trastuzumab was used along with chemotherapy in clinical trials and has proven to increase the survival of patients with metastatic gastric cancer. ToGA (Trastuzumab for Gastric Cancer) was a phase 3, open-label, international, randomised controlled trial. Patients eligible for inclusion had gastric or gastro – oesophageal junction cancer and if their tumours showed gene amplification by fluorescence insitu hybridisation or overexpression of HER-2/neu protein by immunohistochemistry. Participants were randomly assigned in a 1:1 ratio to receive a chemotherapy regimen consisting of cisplatin plus capecitabine or 5 fluorouracil plus cisplatin alone or chemotherapy in combination with intravenous trastuzumab given every 3 weeks for six cycles.

Capecitabine was given at a dose of 1000 mg/sq.m orally twice a day for 14 days followed by a 1-week rest, or fluorouracil 800 mg/sq.m

per day was given by continuous intravenous infusion on days 1-5 of each cycle. Cisplatin at a dose of 80 mg/sq.m on day 1 was given by intravenous infusion. Trastuzumab was given on day 1 of the first cycle by intravenous infusion at a dose of 8 mg/kg, followed by 6 mg/kg every 3 weeks until, unacceptable toxicity, disease progression or withdrawal of consent.

Median overall survival in patients assigned to trastuzumab plus chemotherapy was 13.8 months compared with 11.1[(48)] months in those assigned to chemotherapy alone, corresponding to reduction in the death rate by 26%. Confirmatory analysis that included all 594 randomised patients provided consistent results. The survival benefit was most evident in patients with high levels of HER-2/neu over -expression ,that is whose tumor was FISH+ or IHC 3+ ,with median overall survival of 16.0 vs 11.8 months for patients with high and low levels of HER-2/neu expression. “For gastric cancer, this was probably one of the largest differences we have seen in overall survival with the addition of just one agent in the recent decades.

The adverse event profile was similar in both the groups. There was no difference between both groups in frequency of grade 3 or 4 adverse events apart from diarrhoea. Cardiac adverse events was seen

only in 6% of patients with no difference between the trastuzumab plus chemotherapy and chemotherapy alone groups.

Establishing the HER-2/neu status in advanced gastric cancers and in OG junction cancers has become a routine diagnostic histopathological investigation. After TOGA trial combination of Trastuzumab with Cisplatin and 5FU doublet is now a standard treatment options for gastric carcinoma patients with HER-2/neu positivity.. FDA has approved Trastuzumab use in patients with Her-2/neu positive carcinoma stomach patients. In addition to Herceptin, Lapatinib is also being investigated in patients with HER-2/neu positive gastric cancer. LOGIC, a phase III clinical trial in which Lapatinib is being used along with Oxaliplatin and Capecitabine is ongoing. sIn a Phase II trial it showed a median survival of 5 months with an overall response rate of 9%. The TYTAN trial is an ongoing trial evaluating Lapatinib with Paclitaxel in second line setting.

At present, the most common method to assess HER-2/neu overexpression is IHC, which is a technique available in most pathology laboratories to detect protein expression levels. Since the costs for trastuzumab therapy are high and side effects are minimal, accurate selection of patients for this therapy is useful in prolonging survival for desired patients.

AIM OF THE STUDY

The aim of this study:

1) To assess HER-2/neu overexpression in gastric carcinoma patients
2) To assess the correlation between HER-2/neu overexpression and clinico-pathologic characteristics.

MATERIALS AND METHODS

The present study is a Prospective study conducted in the Department of Medical Oncology, Madras Medical College and Rajiv Gandhi Government General Hospital for a period of one year between January 2011 and December 2011. Informed written consent was obtained from all patients prior to the start of the study. Institutional Ethics committee approval was obtained at the start of the study.

Inclusion criteria:

1. Patients aged 18-65 years with gastric carcinoma proven by histopathology.
2. Adenocarcinoma by histology.

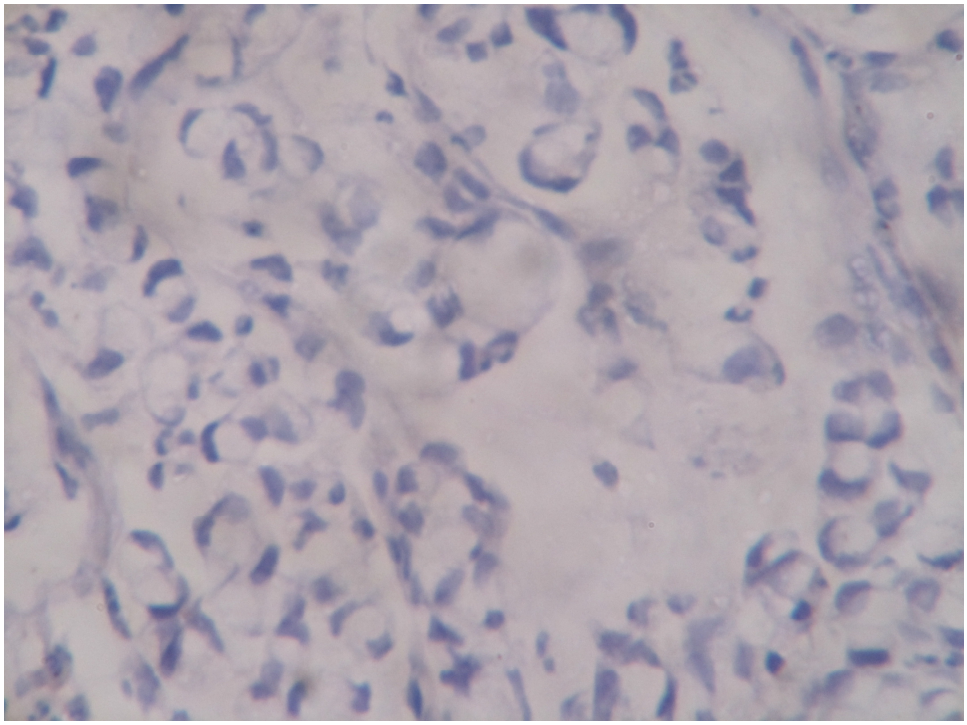
Exclusion criteria:

Histologies other than adneocarcinoma.

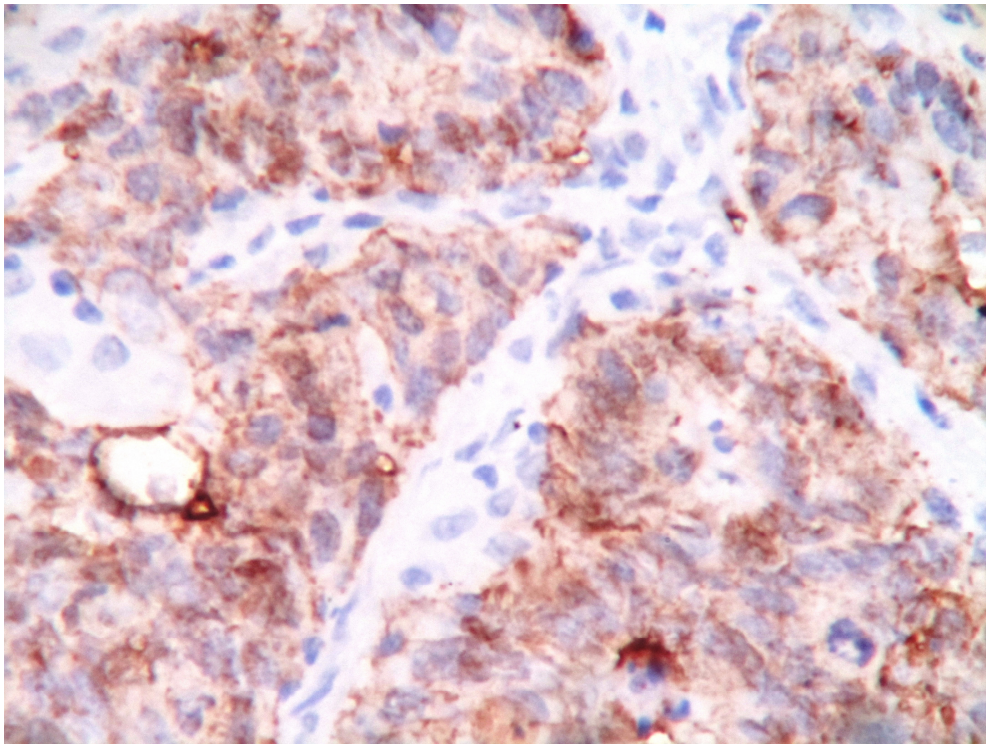
METHODS

All selected patients underwent diagnostic upper gastrointestinal endoscopy. A CT scan of abdomen was done for all patients as staging procedure. The AJCC TNM cancer staging 7th edition was used for

IHC - NEGATIVE



IHC – SCORE 1+



staging. Of the 50 samples evaluated, 25 were endoscopic specimens and 25 were post gastrectomy specimens.

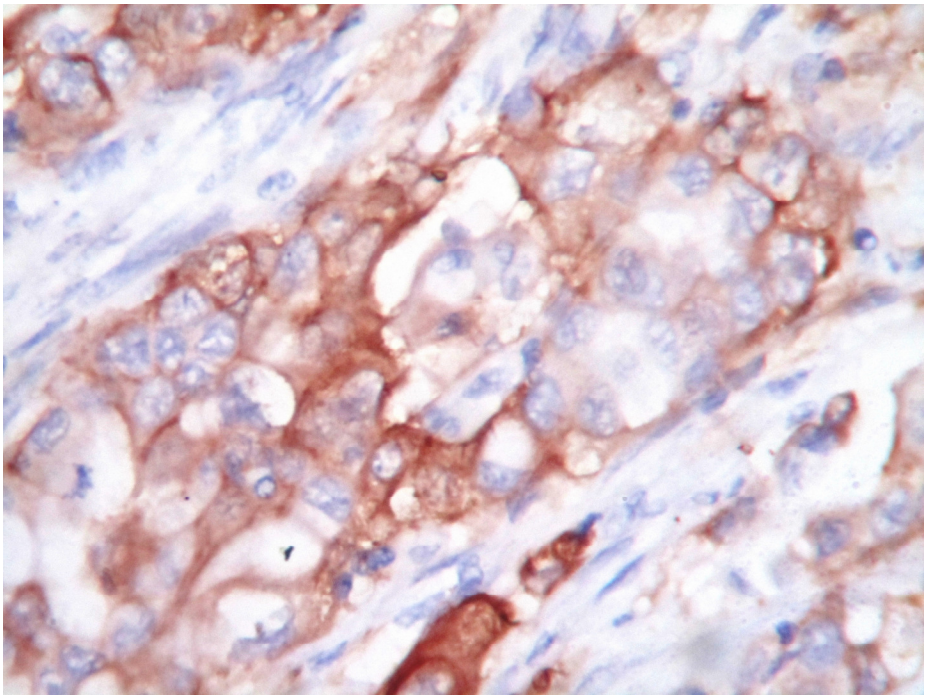
IHC EVALUATION

IHC evaluation of HER-2/neu was done in paraffin embedded tissue samples using Super Sensitive polymer HPR system based on non-biotic polymeric technology. Four micron thick sections from formalin fixed paraffin embedded tissue samples were transferred on to gelatin coated slides. Antigen retrieval was done using the heating method (microwave).The antigen was bound with mouse monoclonal antibody (Biogenex) against HER-2/neu and then detected by addition of secondary antibody conjugated with horse radish peroxidase-polymer and Diaminobenzidine subtrate.

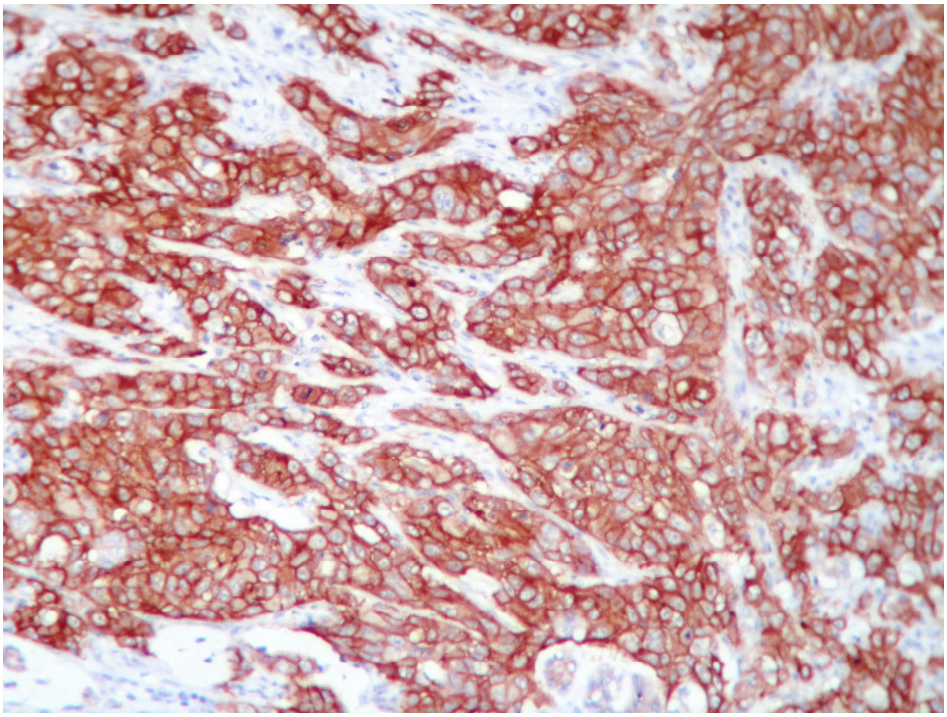
STEPS IN IHC

1. Four micron thin sections cut from formalin fixed paraffin embedded tissue samples and transferred to gelatin chrome alum coated slides.
2. Slides incubated at fifty eight degrees overnight.
3. Sections deparrafined in xylene twice for fifteen minutes each time.

IHC – SCORE 2+



IHC – SCORE 3+



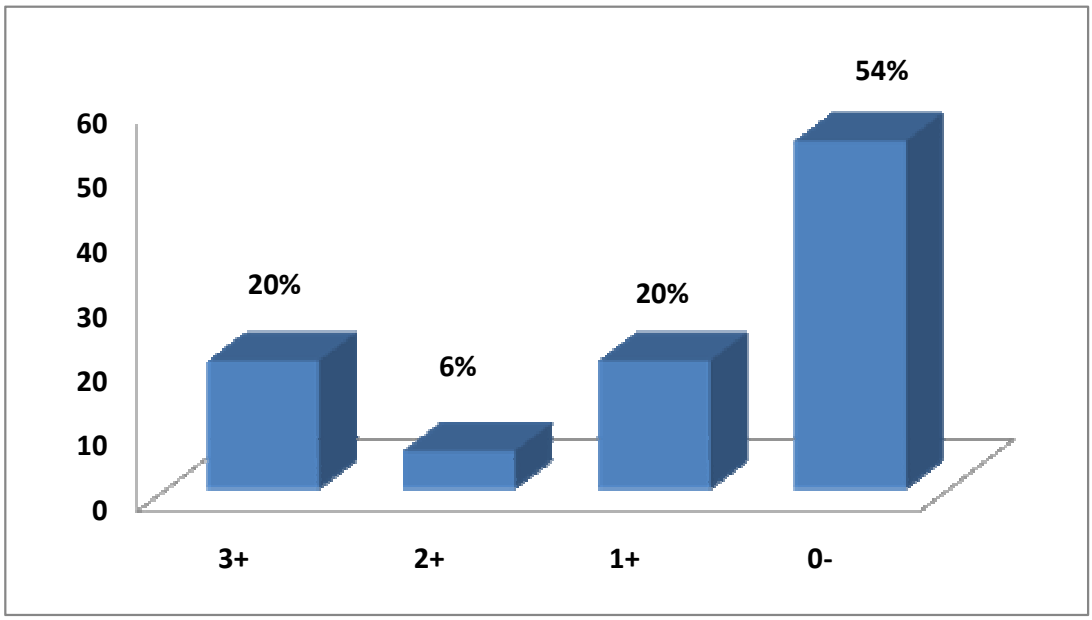
4. Sections washed dehydrated with absolute alcohol for five minutes twice.
5. Sections washed with tap water for ten minutes.
6. Slides immersed in distilled water for five minutes.
7. Heat induced antigen retrieval done with microwave oven in appropriate temperature with citrate buffer for twenty to twenty five minutes.
8. Slides rinsed in distilled water for five minutes.
9. Slides cooled to room temperature and washed with running tap water for five minutes.
10. Washed with phosphate buffer twice for five minutes each time.
11. Application of peroxidase block over section for ten minutes.
12. Slides washed in phosphate buffer twice for five minutes each time.
13. Sections covered with power block for fifteen minutes.
14. Sections drained without washing and primary antibody applied over sections and incubated for one hour at room temperature.
15. Slides covered with super enhancer for thirty minutes.
16. Slides washed in phosphate buffer twice for five minutes.
17. Slides covered with super sensitive label for thirty minutes.

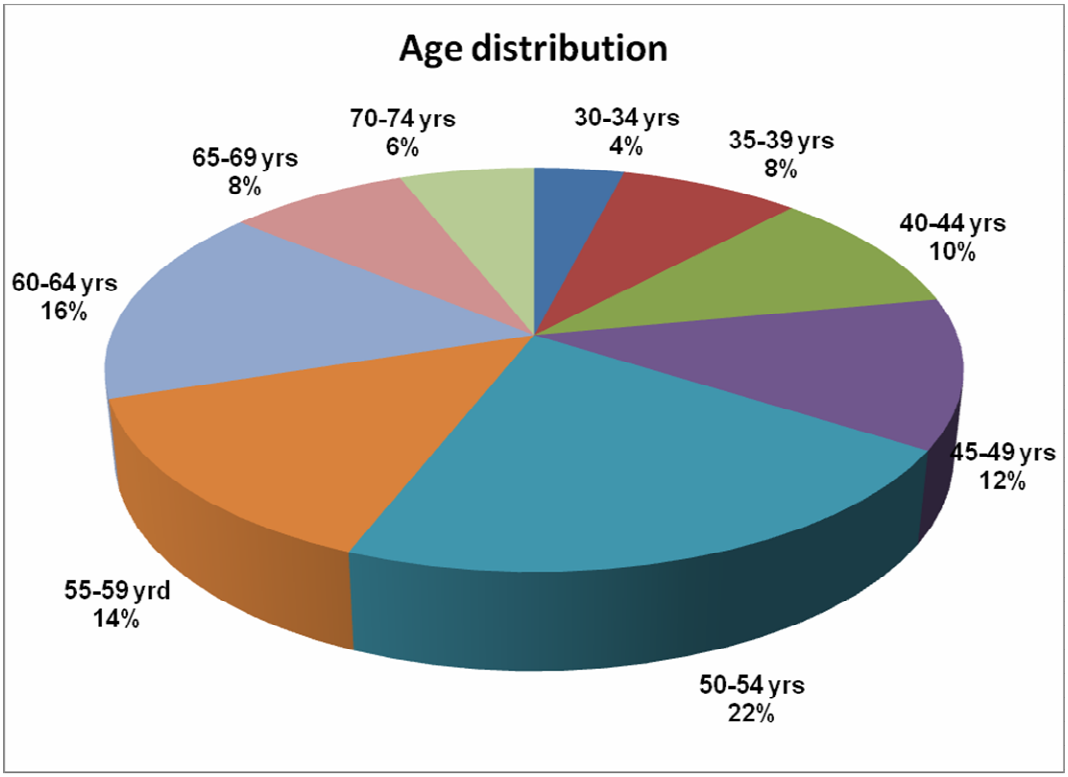
18. Slides washed with phosphate buffer twice for five minutes.
19. Diaminobenzidine substrate freshly prepared by diluting one drop of DAB chromogen with one milliliter of DAB buffer.
20. DAB substrate solution applied on to sections for eight minutes.
21. Washed with phosphate buffer twice for five minutes each time.
22. Slides washed in running tap water for five minutes.
23. Sections counter stained with hematoxylin stain for two seconds.
24. Slides washed in running tap water for three minutes.
25. Slides air dried, cleared with xylene and mounted with DPX.

Reporting was done using Hoffman Score

Results were tabulated in an excel sheet and data was analysed using SPSS Version 17.0 and chi-square 2 tail test was used and 'P' value of less than 0.05 was considered to be statistically significant.

IHC Score Distribution





RESULTS AND ANALYSIS OF OBSERVED DATA

A total of 50 patients were included in this study. Various clinicopathologic characteristic features like age, sex, histopathological type and grade were analysed for association with Her-2/neu gene overexpression.

IHC SCORE

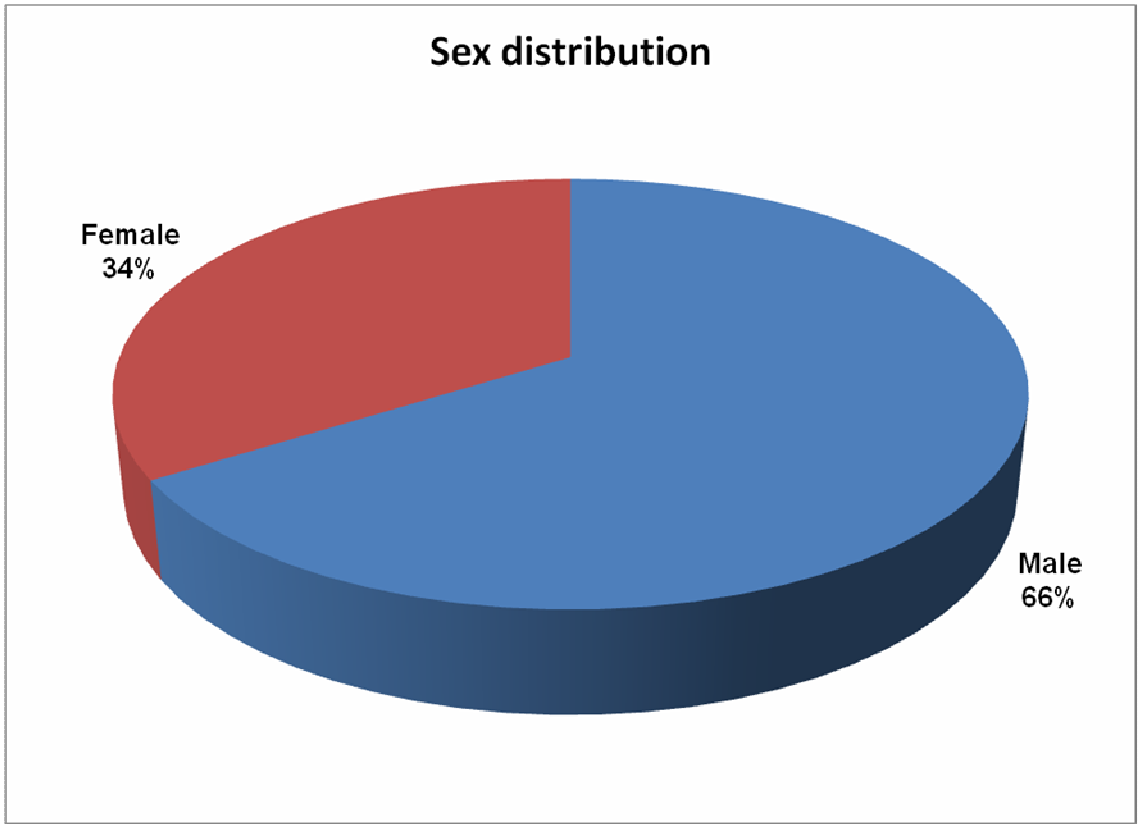
Out of 50 patients, 10 (20%) were 3+, 3 patients (6%) had 2+, 10 patients (20%) had 1+ and 27 patients (54%) had 0 score by IHC.

AGE DISTRIBUTION

Most of the patients were above 50 years of age (n=33,66%) and 22 patients below 50 years of age (34%). The mean age of the study population was 52 $\pm$ 10 years (range 32-72 years). There was no difference in the age at presentation in HER-2/neu positive and negative patients.(p=0.941)

AGE DISTRIBUTION OF PATIENTS

	HER 2	N	Mean	Std. Dev	P-Value
Age (years)	Negative	40	52.43	10.507	0.941
	Positive	10	52.70	10.328	

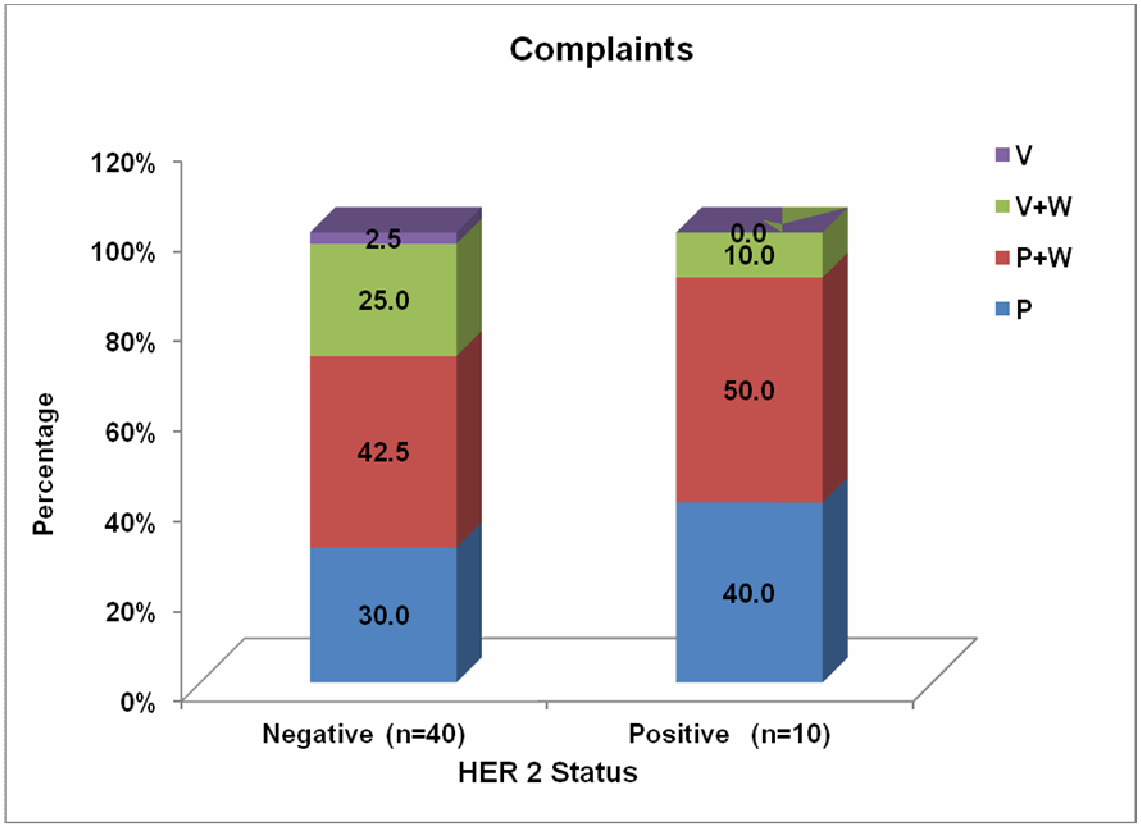

Sex distribution
Female
34%
Male
66%

SEX DISTRIBUTION

In the present study, 33 were males (66%) and 17 were females (34%.). The Male: Female ratio was 2:1. When further analysis was done we found that in both HER-2/neu positive and negative patients, males contributed to two thirds of the disease population and females contributed to one third of patients. This indicates that there was no sex prediliction for HER-2/neu gene.

SEX DISTRIBUTION OF PATIENTS

Gender	HER 2				Total		P-Value
	Negative		Positive				
	N	%	N	%	N	%	
Male	26	65.0	7	70.0	33	66.0	0.997
Female	14	35.0	3	30.0	17	34.0	
Total	40	100.0	10	100.0	50	100.0	

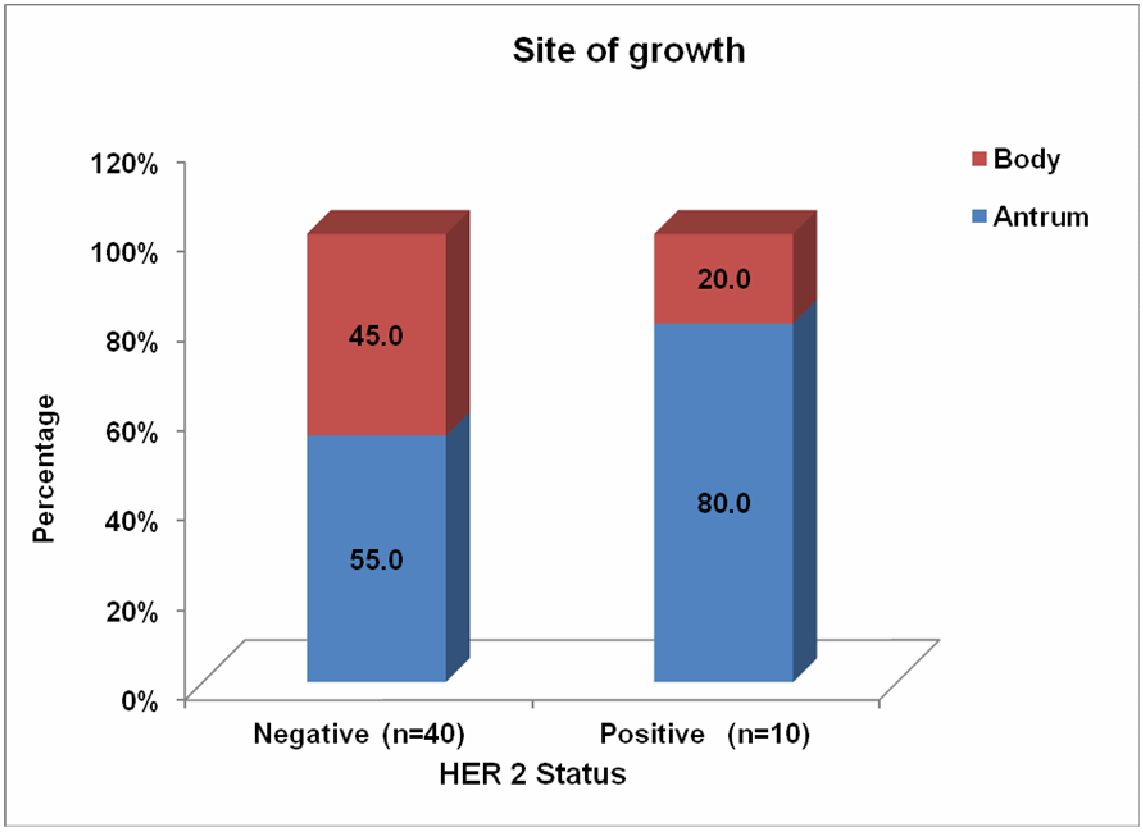

Complaints
120%
100%
80%
60%
40%
20%
0%
Percentage
2.5
25.0
42.5
30.0
0.0
10.0
50.0
40.0
V
V+W
P+W
P
Negative (n=40)
Positive (n=10)
HER 2 Status

SYMTOMATOLOGY

Analysis was done to determine whether there was any difference in the chief complaints of the patient between HER-2/neu positive and negative patients. The duration of symptoms was between three to six months irrespective of the HER-2/neu status. The most common complaint was abdominal pain and associated weight loss in 22 patients (44%), 16 patients (32%) had presented to us with abdominal pain alone as their chief complaint and 11 (22%) patients had presented with vomiting and weight loss. One patient (2%) had presented with vomiting alone as the chief symptom, he was found to have features of acute intestinal obstruction and underwent surgery immediately. This patient was negative for HER-2/neu overexpression. HER-2/neu gene positive patients did not have any specific symptom due to its overexpression.

SYMPTOMS

Complaint	HER 2				Total		P-Value
	Negative		Positive				
	N	%	N	%	N	%	
Pain	12	30.0	4	40.0	16	32.0	0.701
Pain, Weight loss	17	42.5	5	50.0	22	44.0	
Vomiting , Weight loss	10	25.0	1	10.0	11	22.0	
Vomiting	1	2.5	0	0.0	1	2.0	
Total	40	100.0	10	100.0	50	100.0	

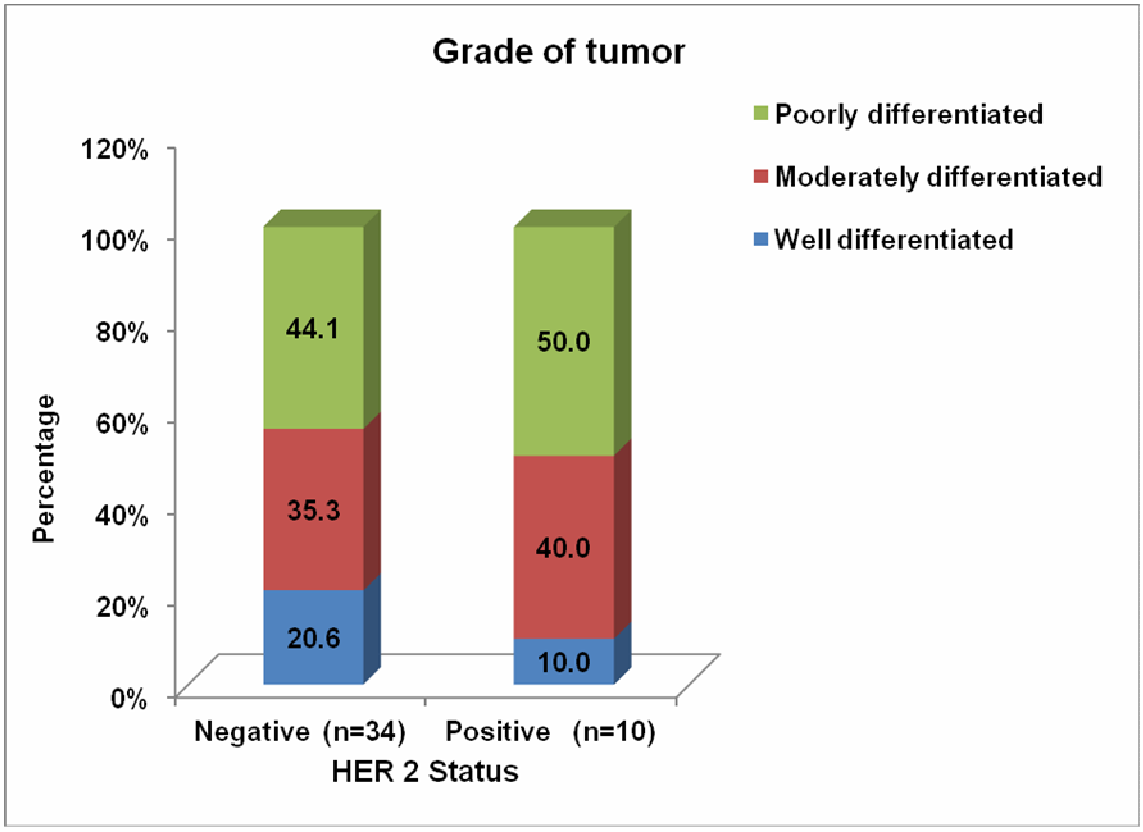

Site of growth
120%
100%
80%
60%
40%
20%
0%
Percentage
45.0
55.0
20.0
80.0
Body
Antrum
Negative (n=40)
Positive (n=10)
HER 2 Status

ENDOSCOPY

All patients underwent endoscopy. .At endoscopy, 30(60%) patients had antral involvement and 20 (40%) had lesion in the body of the stomach .Though HER-2/neu positive patients had prediliction towards antral region when compared to the negative population, the P value was not significant (P=0.279). All (100%) patients had proliferative type of growth.

SITE OF LESION IN ENDOSCOPY

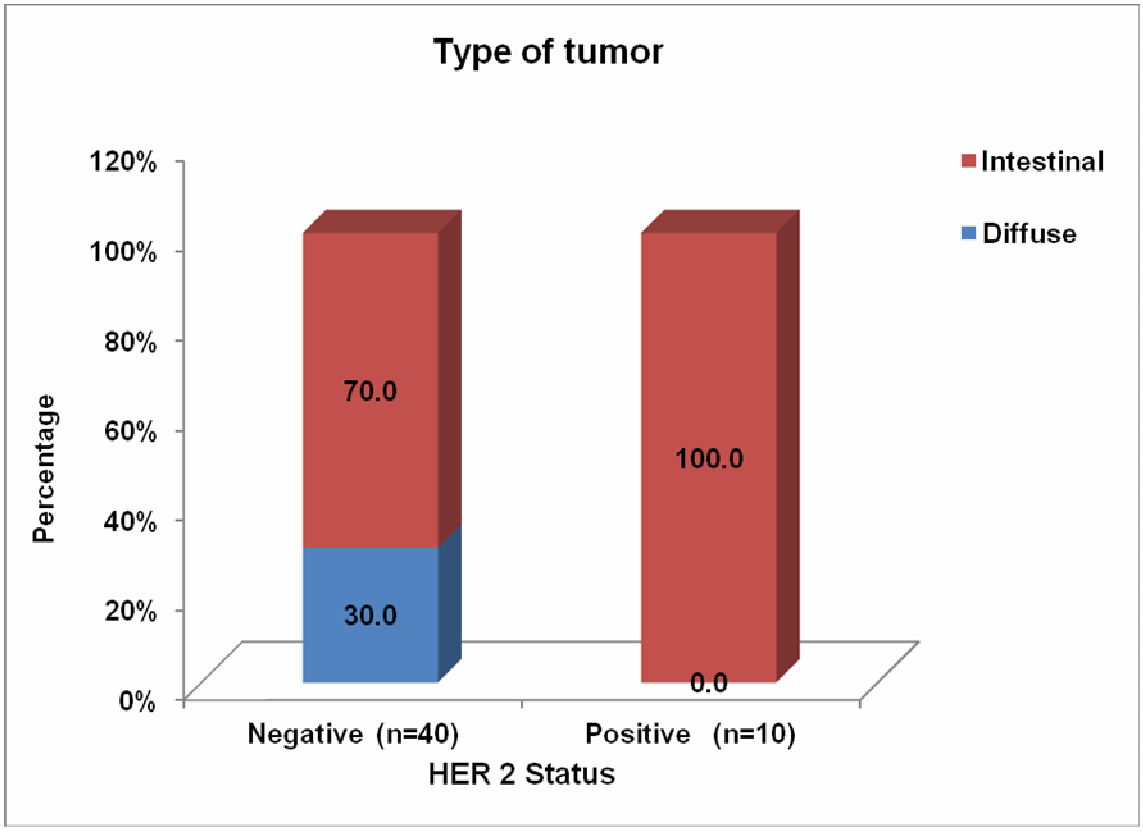
Endoscopy site	HER 2				Total		P-Value
	Negative		Positive				
	N	%	N	%	N	%	
Antrum	22	55.0	8	80.0	30	60.0	0.279
Body	18	45.0	2	20.0	20	40.0	
Total	40	100.0	10	100.0	50	100.0	


Grade of tumor
Poorly differentiated
Moderately differentiated
Well differentiated
120%
100%
80%
60%
40%
20%
0%
Percentage
44.1
35.3
20.6
50.0
40.0
10.0
Negative (n=34)
Positive (n=10)
HER 2 Status

GRADE OF THE TUMOR

Analysis was done to determine whether HER-2/neu positivity was associated with the grade of tumor. Fifty two percent of the patients had poorly differentiated carcinoma, 30% had moderately differentiated carcinoma and 18% had well differentiated carcinoma. In HER-2/neu positive subgroup 50% had poorly differentiated grade, 40% with moderately differentiated grade and only 10 % were well differentiated grade. In HER-2/neu negative group 52% had poorly differentiated grade, 28% had moderately differentiated grade and 20% had well differentiated grade. There was no significant difference in grade of tumor between HER-2/neu positive and negative tumors.(P=0.647)

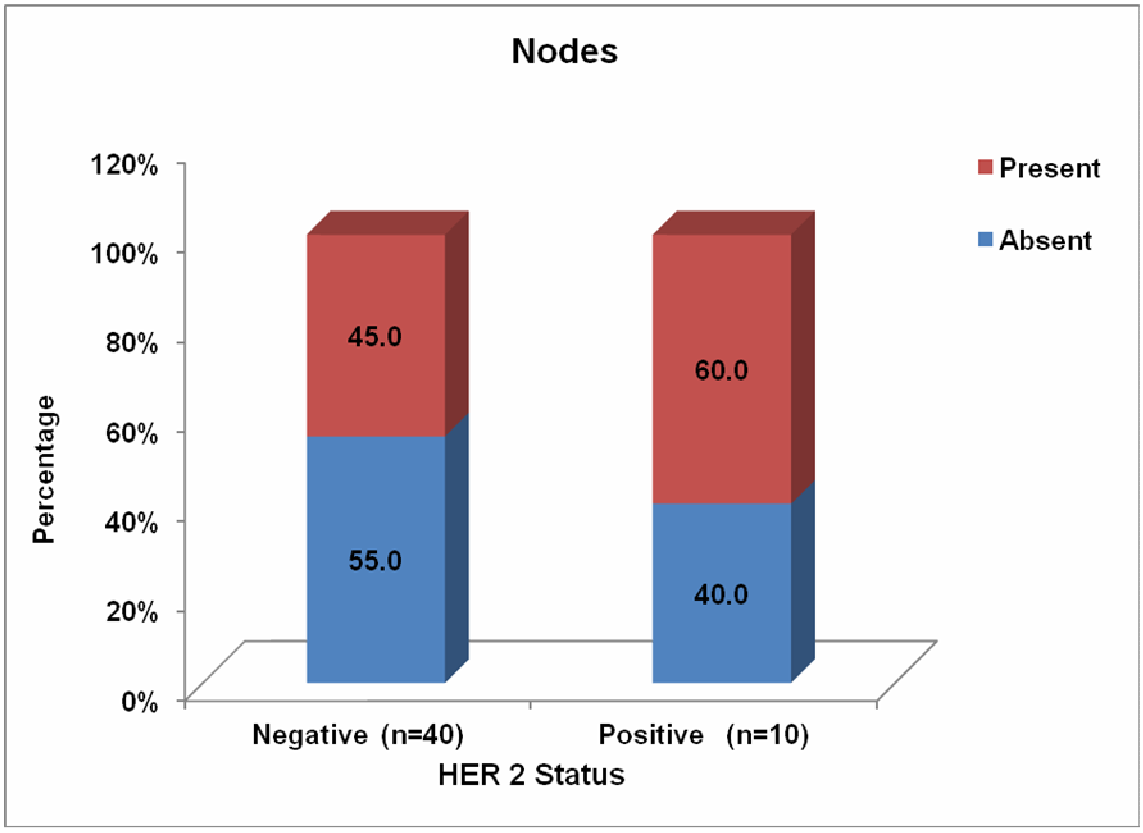
HPE grade	**HER 2**				**Total**		**P-Value**
	Negative		**Positive**				
	N	**%**	**N**	**%**	**N**	**%**	
Well differentiated	8	20.0	1	10.0	9	18.0	0.647
Moderately differentiated	11	27.5	4	40.0	15	30.0	
Poorly differentiated	21	52.5	5	50.0	26	52.0	
Total	40	100.0	10	100.0	50	100.0	


Type of tumor
120%
100%
80%
60%
40%
20%
0%
Percentage
70.0
30.0
100.0
0.0
Intestinal
Diffuse
Negative (n=40)
Positive (n=10)
HER 2 Status

LAURENS TYPE OF TUMOR

Analysis was done to find out the association between HER-2/neu positivity and any particular pathological type in Laurens classification. It was observed that 38 patients (76%) had intestinal type of cancer and 12 patients (24%) had diffuse type of cancer. All 10 patients with HER-2/neu positivity had only intestinal type of cancer. In the HER-2/neu negative group, 28 patients(70%) had intestinal type of cancer and 12 patients(30%) had diffuse type of cancer. Although HER-2/neu positivity was strongly associated with intestinal type of tumor, it did not differ significantly from the Her 2 negative group.(P=0.116)

I / D	HER 2				Total		P-Value
	Negative		Positive				
	N	%	N	%	N	%	
Diffuse	12	30.0	0	0.0	12	24.0	0.116
Intestinal	28	70.0	10	100.0	38	76.0	
Total	40	100.0	10	100.0	50	100.0	


Nodes
120%
100%
80%
60%
40%
20%
0%
Percentage
45.0
55.0
60.0
40.0
Present
Absent
Negative (n=40)
Positive (n=10)
HER 2 Status

CT ABDOMEN

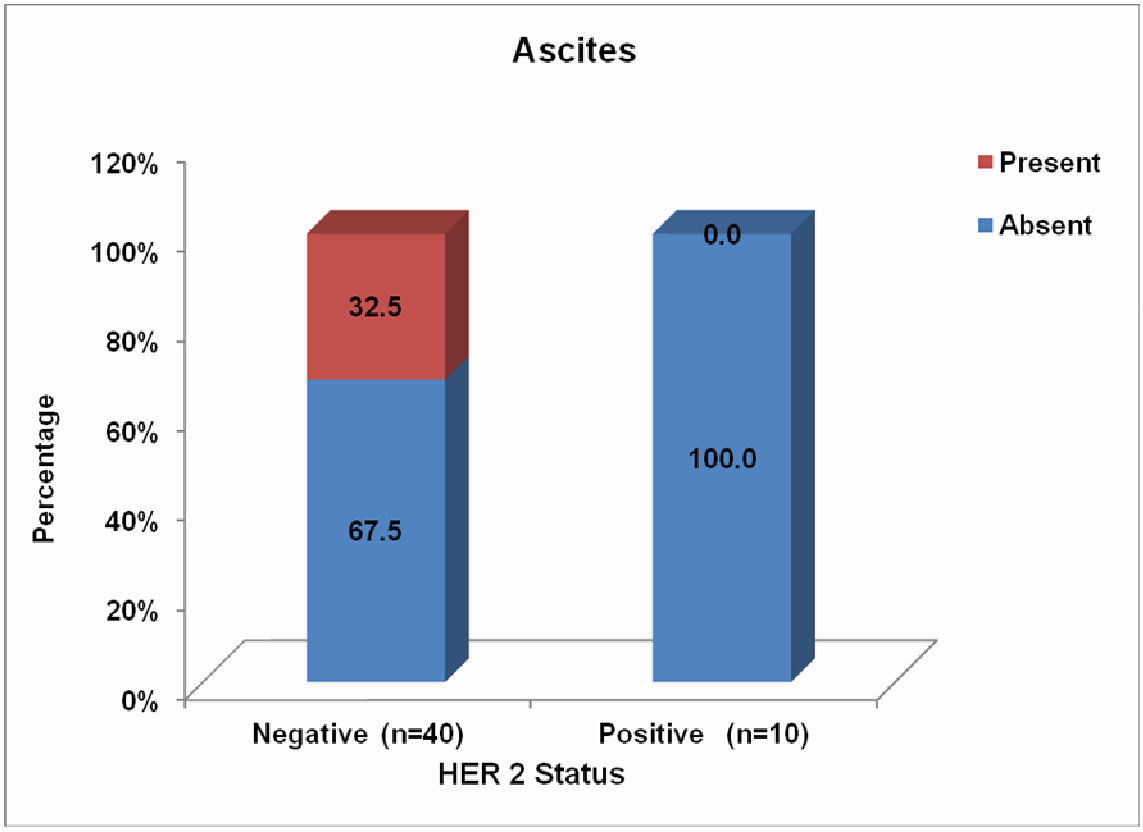
NODAL ASSESSMENT BY IMAGING

All patients underwent CT abdomen as part of staging workup and to assess the feasibility of surgery. The Pyloric, Gastroduodenal, Gastroepiploic, Splenic, Pancreaticoduodenal, Peripancreatic,Right gastric,Left gastric, hepatoduodenal, celiac and common hepatic nodes were considered as regional nodes. Presence of Portal, Para aortic, Retroperitoneal, Retropancreatic and Mesentric nodes was considered to be metastatic disease.

On imaging, 26 patients (52%) had regional nodes and 24 patients (48%) did not have nodal involvement. Nodes were present in 40% of HER-2/neu negative patients. Though 60 % of HER-2/neu positive patients had nodes, the difference between them was not statistically significant.(p=0.620)

PRESENCE OF NODE BY IMAGING

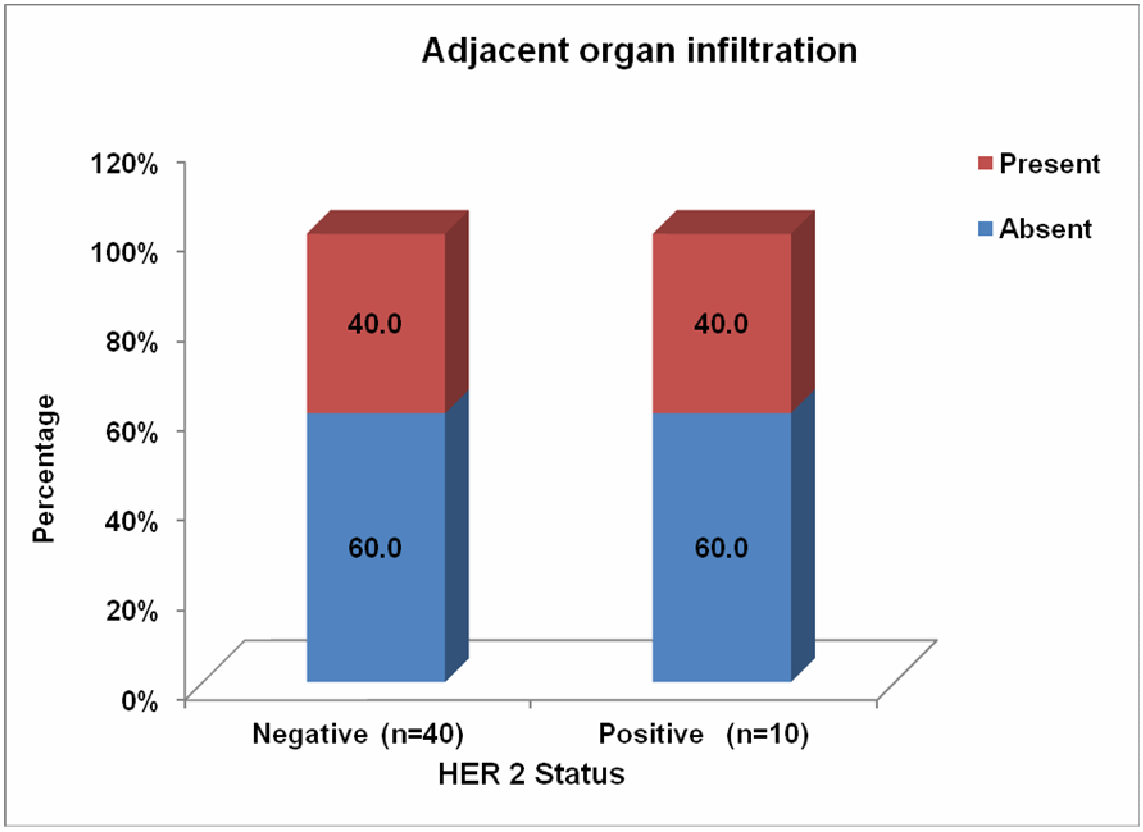
CT Nodes	HER 2				Total		P-Value
	Negative		Positive				
	N	%	N	%	N	%	
Absent	22	55.0	4	40.0	26	52.0	0.620
Present	18	45.0	6	60.0	24	48.0	
Total	40	100.0	10	100.0	50	100.0	


Ascites
120%
100%
80%
60%
40%
20%
0%
Percentage
32.5
67.5
0.0
100.0
Present
Absent
Negative (n=40)
Positive (n=10)
HER 2 Status

ASCITES

Correlation between presence of HER-2/neu positivity and presence or absence of ascites was analysed. Ascites was present in 13 patients (26%) and absent in 37 patients(74%). Patients with ascites were inoperable at diagnosis itself and were considered only for palliative treatment. None the patients with ascites had HER-2/neu positivity.

CT ascites	HER 2				Total		P-Value
	Negative		Positive				
	N	%	N	%	N	%	
Absent	27	67.5	10	100.0	37	74.0	0.091
Present	13	32.5	0	0.0	13	26.0	
Total	40	100.0	10	100.0	50	100.0	

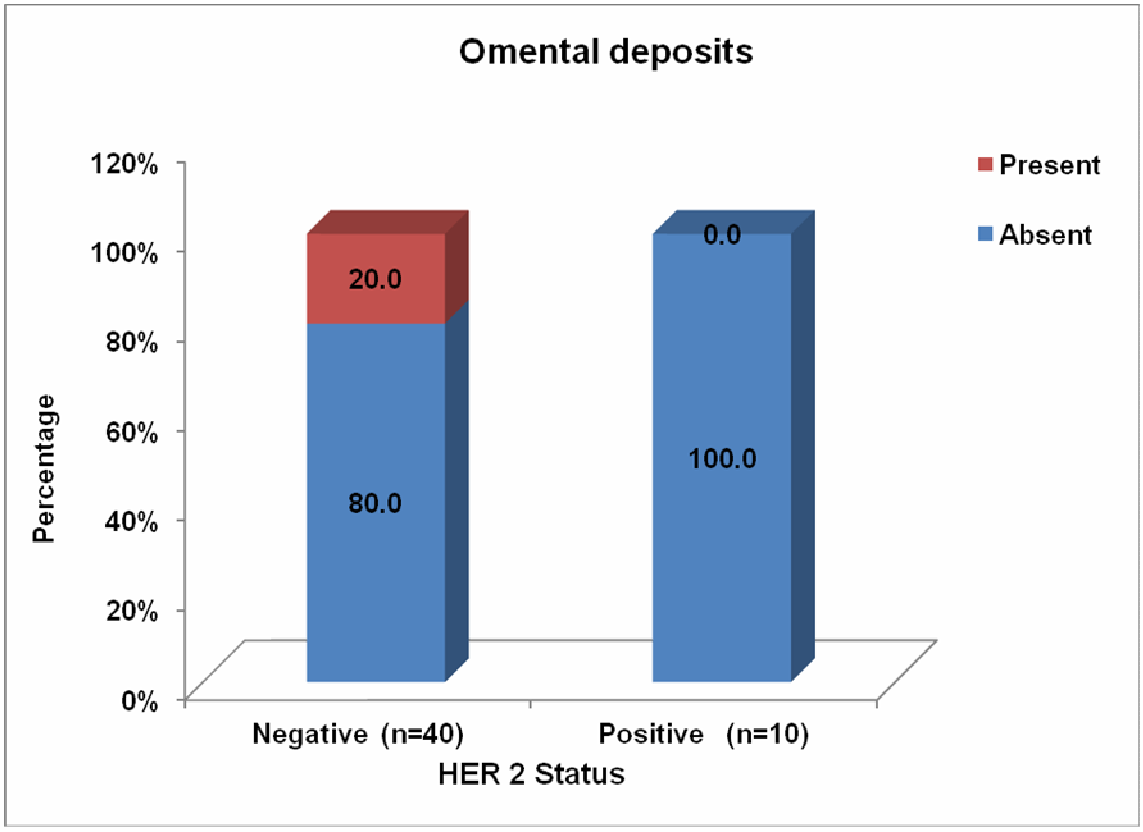

Adjacent organ infiltration
120%
100%
80%
60%
40%
20%
0%
Percentage
40.0
40.0
60.0
60.0
Present
Absent
Negative (n=40)
Positive (n=10)
HER 2 Status

ADJACENT ORGAN INVOLVEMENT

Twenty patients(40%) had adjacent organ infiltration . Most patients had posterior infiltration in to pancreas. The other organs involved were duodenum , transverse colon, and splenic flexure. Forty percent of patients in both HER-2/neu positive and negative group had adjacent organ infiltration. There was no correlation between HER-2/neu positivity and T stage of the disease.

ADJACENT ORGAN INVOLVEMENT BY IMAGING

CT adjacent organ infiltration	HER 2				Total		P-Value
	Negative		Positive				
	N	%	N	%		%	
Absent	24	60.0	6	60.0	30	60.0	1.000
Present	16	40.0	4	40.0	20	40.0	
Total	40	100.0	10	100.0	50	100.0	

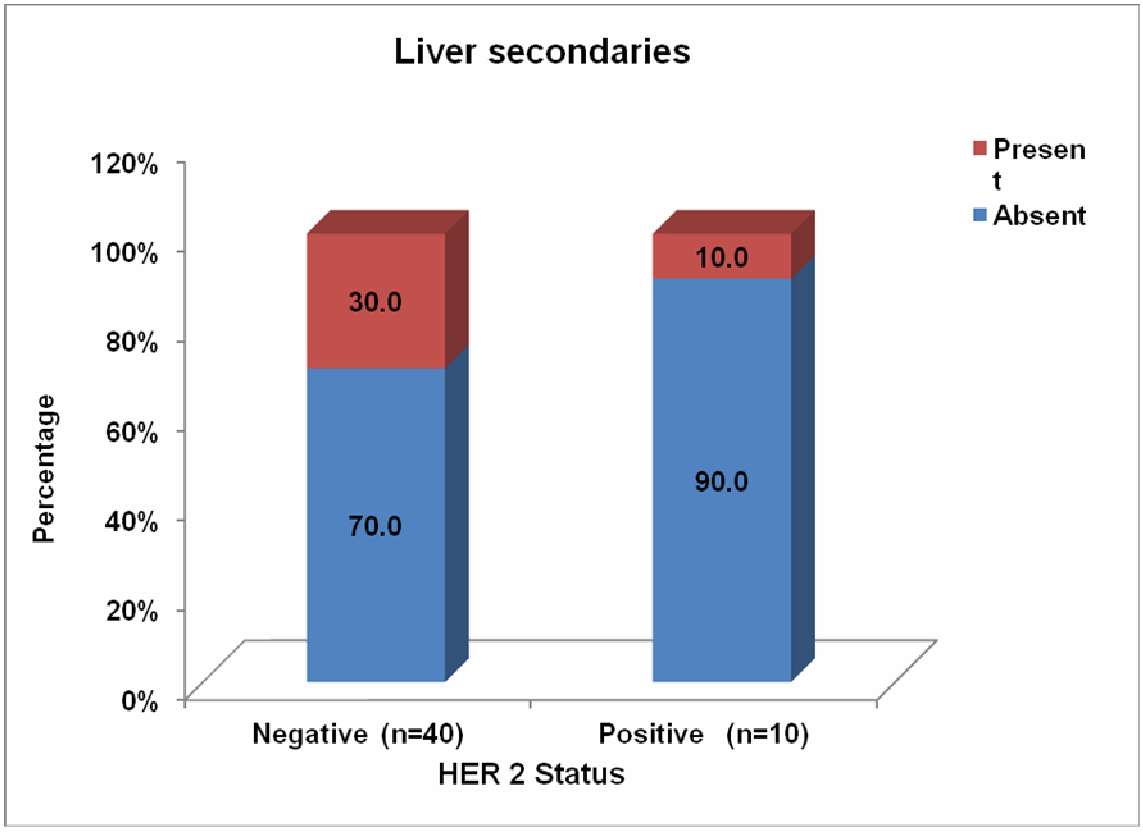

Omental deposits
120%
100%
80%
60%
40%
20%
0%
Percentage
20.0
80.0
0.0
100.0
Present
Absent
Negative (n=40)
Positive (n=10)
HER 2 Status

OMENTAL DEPOSITS

Out of fifty patients, eight patients (16%) had Omental deposits on imaging and 42 patients (84%) did not have omental deposits. none of the HER-2/neu positive patients had omental deposit.

OMENTAL DEPOSIT BY IMAGING

CT Omental deposits	HER 2				Total		P-Value
	Negative		Positive				
	N	%	N	%	N	%	
Absent	32	80.0	10	100.0	42	84.0	
Present	8	20.0	0	0.0	8	16.0	0.289
Total	40	100.0	10	100.0	50	100.0	

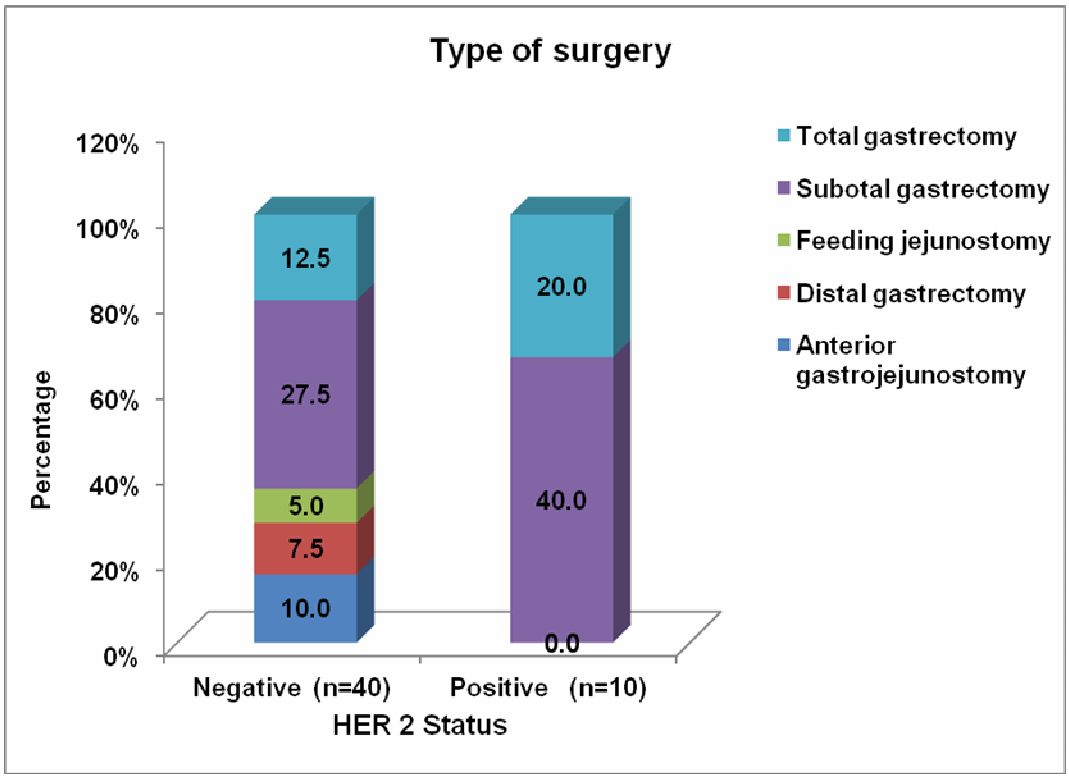

Liver secondaries
120%
100%
80%
60%
40%
20%
0%
Percentage
30.0
70.0
10.0
90.0
Present
Absent
Negative (n=40)
Positive (n=10)
HER 2 Status

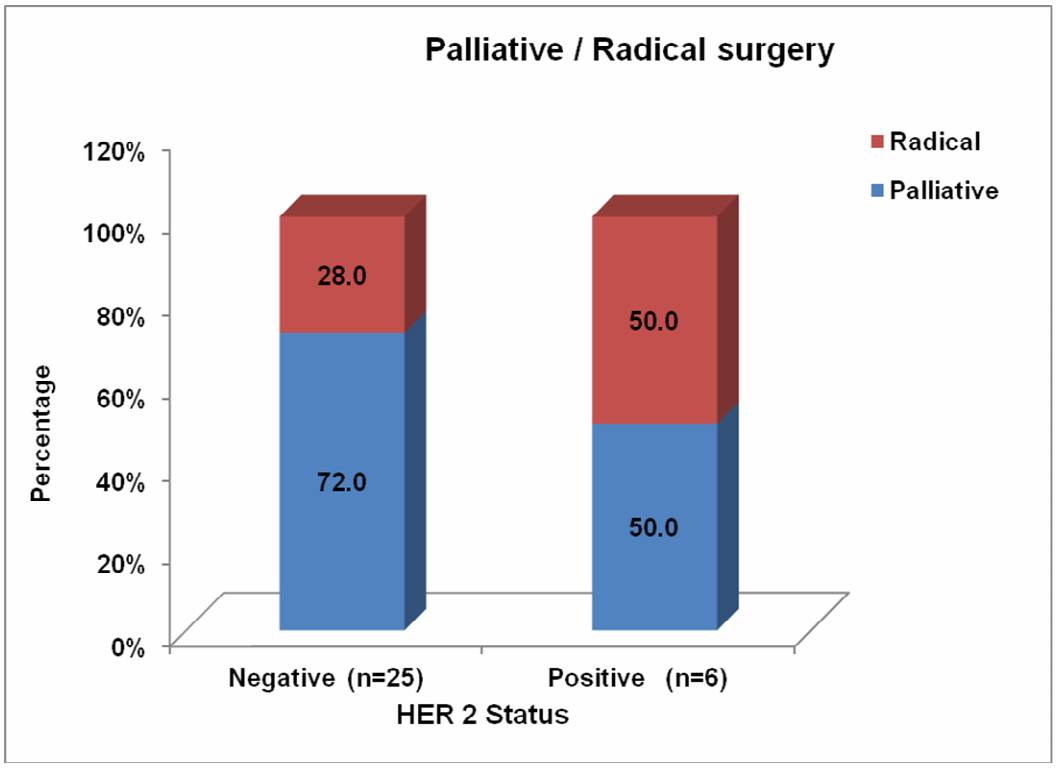
LIVER SECONDARIES BY IMAGING

Liver secondaries were seen in 13 patients (26%). Liver secondaries were seen in 30% of HER-2/neu negative and10% of HER-2/neu positive patients. There was no significant correlation between HER-2/neu and presence of liver secondaries.

LIVER SECONDARIES BY IMAGING

CT liver secondaries	HER 2				Total		P-Value
	Negative		Positive				
	N	%	N	%	N	%	
Absent	28	70.0	9	90.0	37	74.0	0.375
Present	12	30.0	1	10.0	13	26.0	
Total	40	100.0	10	100.0	50	100.0	


Type of surgery
120%
100%
80%
60%
40%
20%
0%
Percentage
12.5
27.5
5.0
7.5
10.0
20.0
40.0
0.0
Negative (n=40)
Positive (n=10)
HER 2 Status
Total gastrectomy
Subotal gastrectomy
Feeding jejunostomy
Distal gastrectomy
Anterior gastrojejunostomy


Palliative / Radical surgery
120%
100%
80%
60%
40%
20%
0%
Percentage
28.0
72.0
50.0
50.0
Negative (n=25)
Positive (n=6)
HER 2 Status
Radical
Palliative

SURGERY

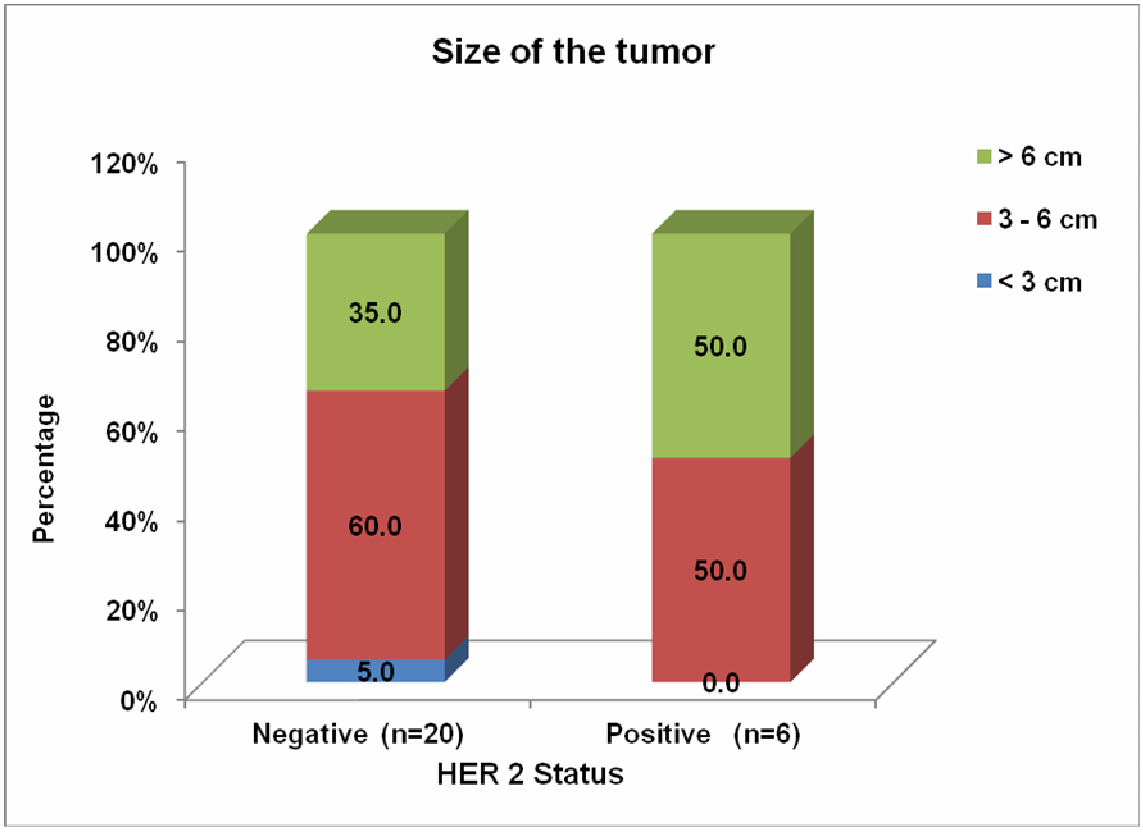
Depending on the stage of the disease and the site of the disease 31 (62%) patients underwent various type of surgeries as follows

Types of Surgery

1. Subtotal gastrectomy
2. Total gastrectomy
3. Anterior gastrojejunostomy
4. Distal gastrectomy
5. Feeding jejunostomy

Radical surgery was done for 28% of HER-2/neu negative and 50% of HER-2/neu positive patients.

Palliative/ Radical	HER 2				Total		P-Value
	Negative		Positive				
	N	%	N	%	N	%	
Palliative	15	72.0	3	50.0	18	67.7	
Radical	4	28.0	3	50.0	7	32.3	0.583
Total	19	100.0	6	100.0	25	100.0	

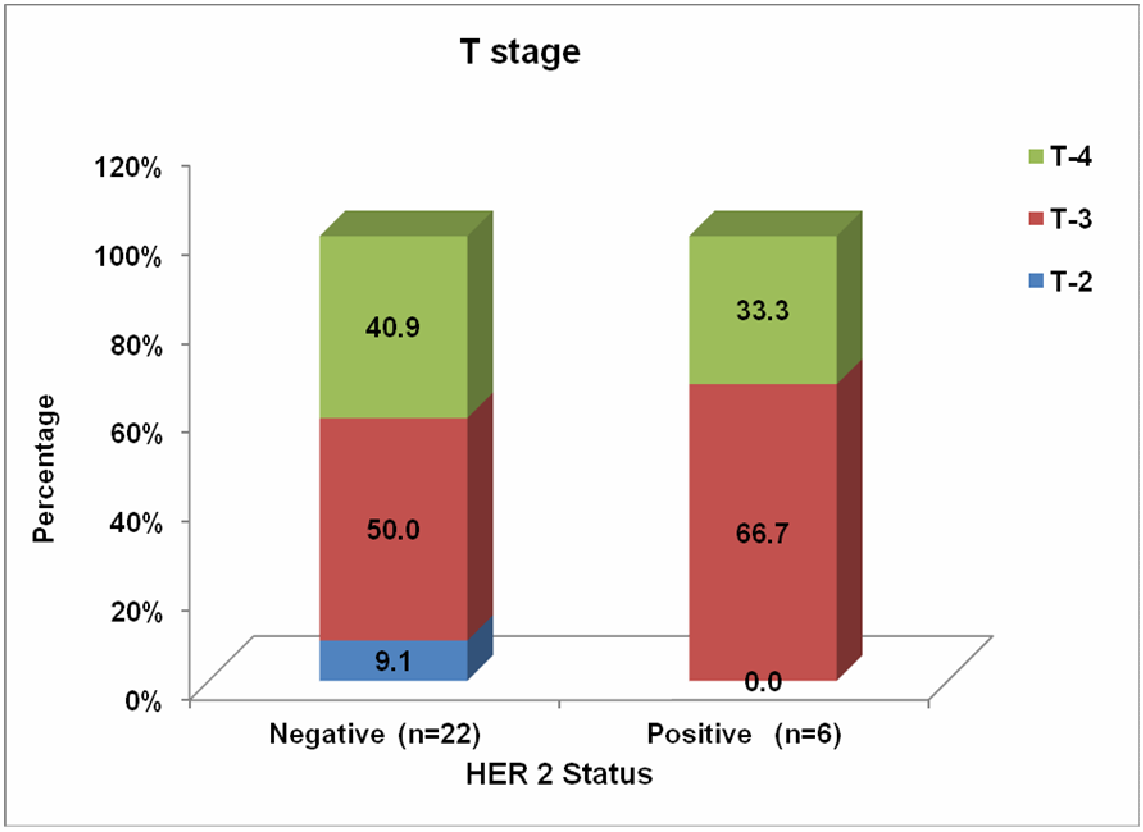

Size of the tumor
120%
100%
80%
60%
40%
20%
0%
Percentage
35.0
60.0
5.0
50.0
50.0
0.0
> 6 cm
3 - 6 cm
< 3 cm
Negative (n=20)
Positive (n=6)
HER 2 Status

SIZE OF THE TUMOR

Analysis was done to see for correlation between size of the tumor and HER-2/neu Overexpression .Tumor size was less than three cm in one patient (4%) who was HER-2/neu negative. Fifteen patients (57%) had tumor size of 3-6 cms. Fifty percent of HER-2/neu positive patients and 60% in HER 2 negative patients had 3 to 6 centimeter tumor. Ten patients(39%) had tumor more than 6 centimeter. Thirty five percent of HER-2/neu negative and 5o% of HER-2/neu positive population had tumor size >6 centimeter. Though HER-2/neu positive patients had larger tumor size, it was not statistically significant.(p=0.650)

SIZE OF THE TUMOR

Histopathology size	HER 2				Total		P-Value
	Negative		Positive				
	N	%	N	%	N	%	
< 3 cm	1	5.0	0	0.0	1	3.8	0.650
3 - 6 cm	11	60.0	3	50.0	14	57.7	
> 6 cm	7	35.0	3	50.0	10	38.5	
Total	20	100.0	6	100.0	25	100.0	

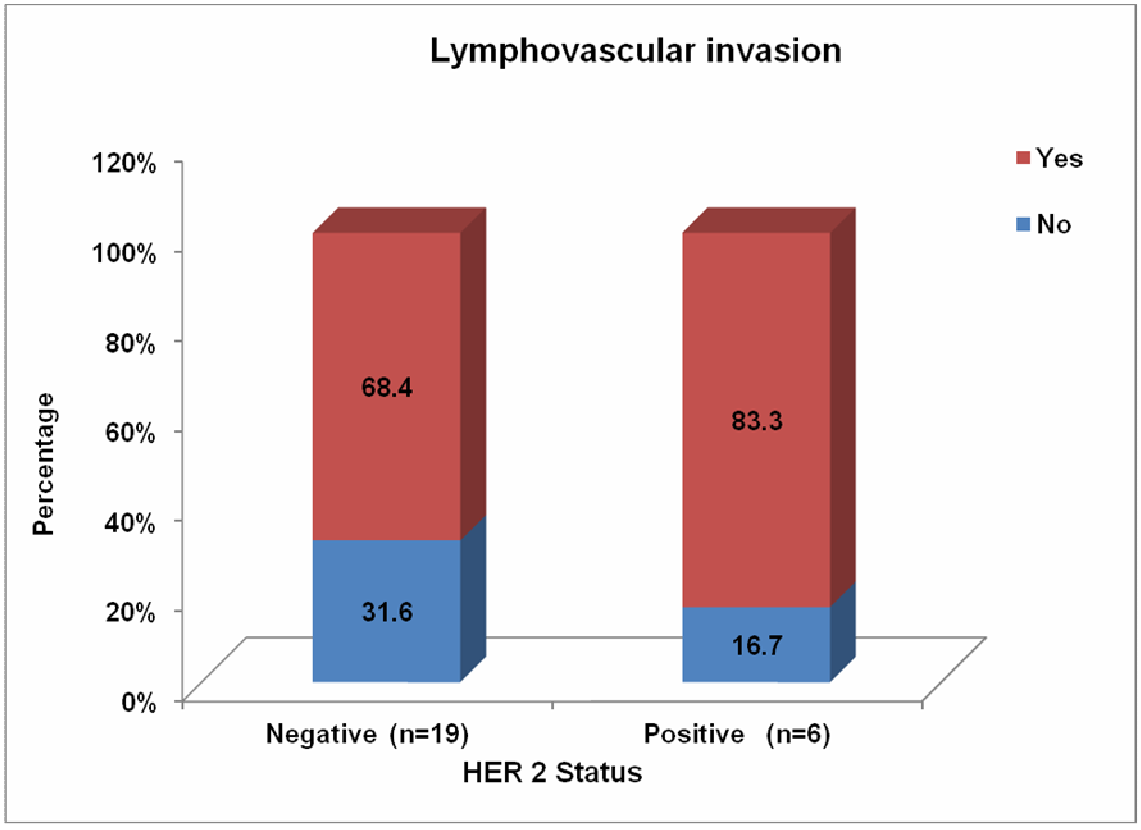

T stage
120%
100%
80%
60%
40%
20%
0%
Percentage
40.9
50.0
9.1
33.3
66.7
0.0
T-4
T-3
T-2
Negative (n=22)
Positive (n=6)
HER 2 Status

'T' STAGE

Tumor stage was assessed using the AJCC TNM 7th edition. Two patients (8%) had T2 disease and they were found to be HER-2/neu negative. Fifteen patients (54%) had T3 disease . T3 disease was seen in 50% in HER-2/neu negative and 66% in HER-2/neu positive patients. Out of 11 patients (38%) who had T4 disease, 34% were HER-2/neu positive and 41% were HER-2/neu negative.

'T' STAGE BY HPE

T-Stage	HER 2				Total		P-Value
	Negative		Positive				
	N	%	N	%	N	%	
T-2	2	9.1	0	0.0	2	8	0.651
T-3	11	50.0	4	66.7	15	54	
T-4	9	40.9	2	33.3	11	38	
Total	22	100.0	6	100.0	28	100.0	

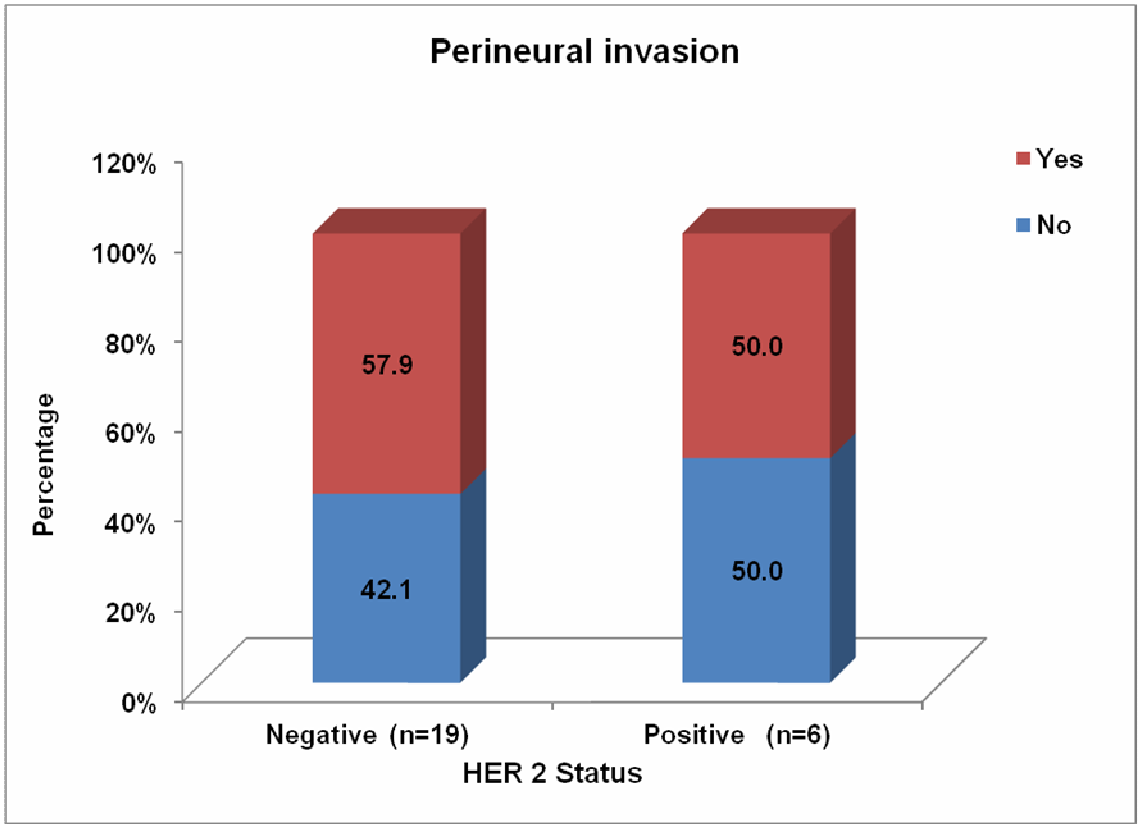

Lymphovascular invasion
120%
100%
80%
60%
40%
20%
0%
Percentage
68.4
31.6
83.3
16.7
Yes
No
Negative (n=19)
Positive (n=6)
HER 2 Status

LYMPHOVASCULAR INVASION

It was observed that 18 out of 25 patients who underwent radical surgery (72%) had lymphovascular invasion. Lymphovascular invasion was seen in 83% of HER-2/neu positive and 69% of HER-2/neu negative patients.There was no statistically significant difference between both groups.(P=0.851)

LYMPHOVASCULAR INVASION

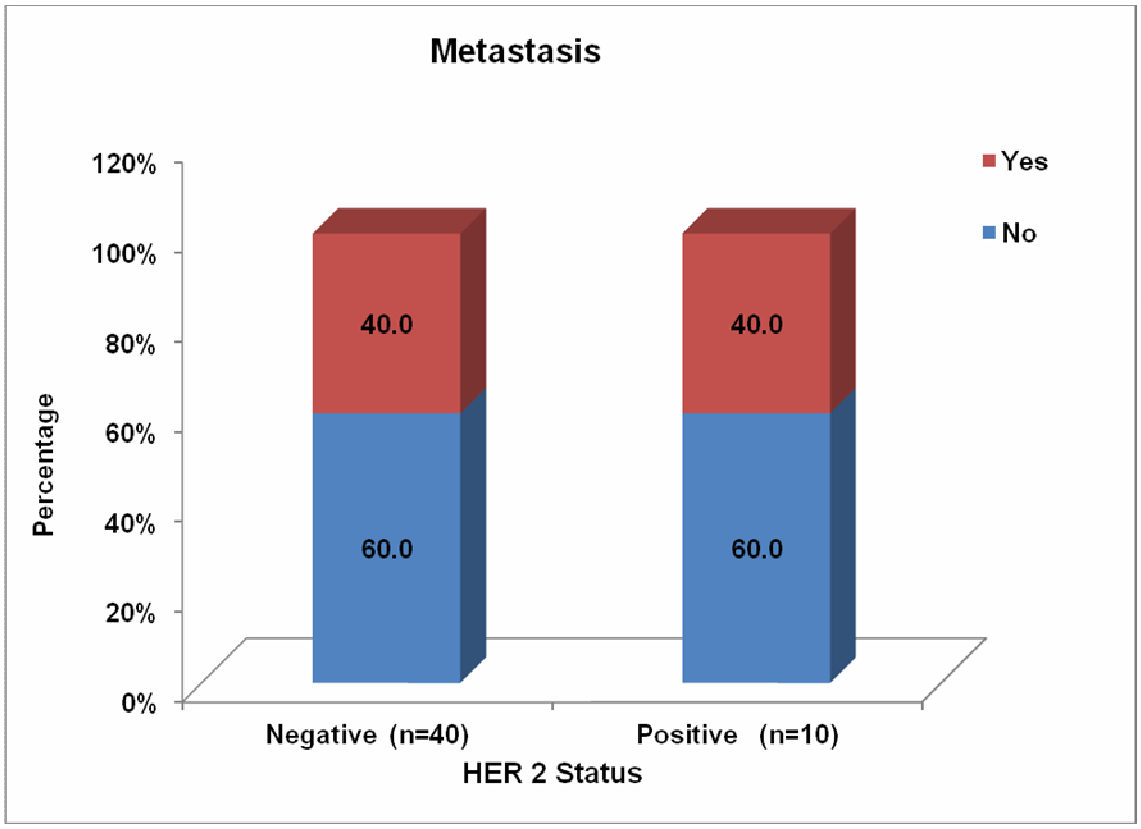
Lymphovascular invasion	HER 2				Total		P-Value
	Negative		Positive				
	N	%	N	%	N	%	
No	6	31.6	1	16.7	7	28.0	0.851
Yes	13	68.4	5	83.3	18	72.0	
Total	19	100.0	6	100.0	25	100.0	


Perineural invasion
120%
100%
80%
60%
40%
20%
0%
Percentage
57.9
42.1
50.0
50.0
Yes
No
Negative (n=19)
Positive (n=6)
HER 2 Status

PERINEURAL INVASION

Analysis for presence or absence of perineural invasion was done in relation to HER-2/neu overexpression. Fourteen out of 25 patients (56%) had perineural invasion and 11 patients (44%) had no perineural invasion .Perineural invasion was found in 50% of HER-2/neu positive group and in 58% of HER-2/neu negative subgroup and there was no significant difference in both the groups.(P=0.997)

Perineural Invasion	HER 2				Total		P-Value
	Negative		Positive				
	N	%	N	%	N	%	
No	8	42.1	3	50.0	11	44.0	0.997
Yes	11	57.9	3	50.0	14	56.0	
Total	19	100.0	6	100.0	25	100.0	


Metastasis
120%
100%
80%
60%
40%
20%
0%
Percentage
40.0
40.0
60.0
60.0
Yes
No
Negative (n=40)
Positive (n=10)
HER 2 Status

METASTASIS

Out of 50 patients, 20 (40%) had metastatic disease at presentation. The most common sites of metastasis were peritoneum, omentum and liver parenchyma. The incidence of metastatic diseases was 40% of patients in both the subgroups and was not significantly different. (P=1)

Metastasis	HER 2				Total		P-Value
	Negative		Positive				
	N	%	N	%	N	%	
No	24	60.0	6	60.0	30	60.0	1.000
Yes	16	40.0	4	40.0	20	40.0	
Total	40	100.0	10	100.0	50	100.0	

DISCUSSION

Gastric carcinoma is one among the top five causes of cancer in both males and females. It is also listed among the top cancer related mortalities. Despite the advancements in the treatment of gastric cancer, none has shown to improve the survival significantly. There was no additional improvement in overall survival with addition of Epirubicin or Docetaxol to Cisplatin and 5FU combinations although the response rate was better. The role of targeted therapy has recently come into play after the establishment of the role of HER-2/neu over-expression in gastric malignancies. Trastuzumab ,an anti HER-2/neu antibody ,in combination with Cisplatin and 5FU in advanced gastric carcinoma patients has shown to improve overall survival in TOGA trial This study was undertaken to find out the prevalence and the clinicopathological correlation in HER-2/neu over –expression in gastric carcinoma patients.

Most of the observations made in this study correlated well with the world literature. The prevalence of HER-2/neu over-expression in this study population was 20%, which correlated well with other studies. (8-27%).

The age of the patients at diagnosis in this study ranged between 35-75 with 66% more than 50 years of age. This was in contradiction to

SEER data in 2010 which states the mean age at diagnosis of gastric cancer is around 70 years. Our patients were diagnosed at much earlier age .According to SEER data only 17% were below 50 years of age .in the present study, 34% presented at an earlier age .The etiological and epidemiological factors leading to such drastic difference at presentation needs to be analysed. There was no difference in age at presentation between HER-2/neu positive and negative patients. This observation was similar to the studies made by YQ and Cai X et al.

There was a strong male preponderance for gastric cancer, with a 2:1 ratio of male: female. This was similar to the data by SEER in 2010 which showed the incidence of gastric carcinoma in males to around 64% and females around 36%. Antral region was commonly involved and this is in concordance with the study by Hermann RE et al.

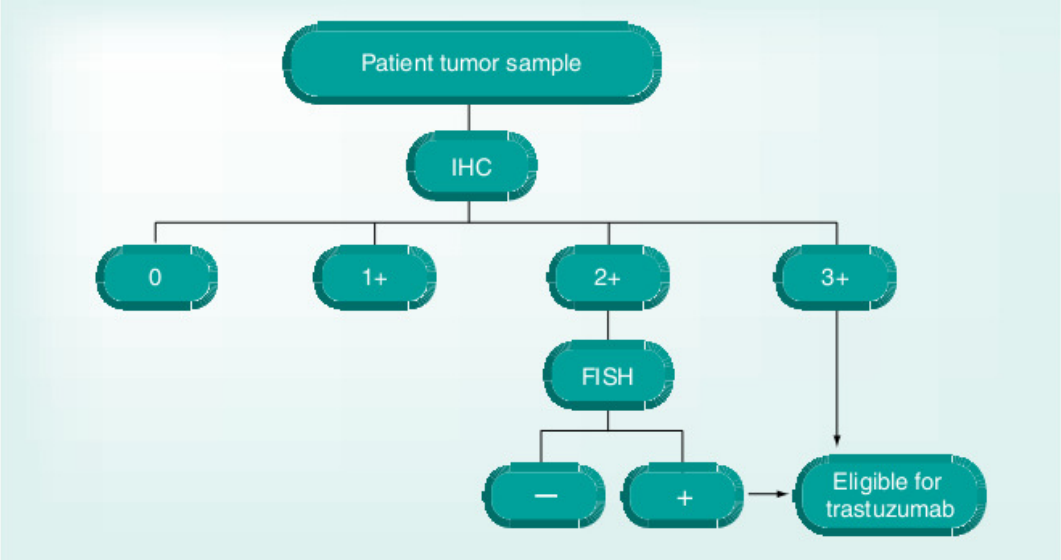
When analysis was done for the significance of grade in relation to HER-2/neu status we found that 25 (50%) of our patients had poorly differentiated carcinoma. When subset analysis was done 50% of them were HER-2/neu positive and 50% HER-2/neu negative. When analysis was done only in HER-2/neu positive population, 50% had poorly differentiated grade and 30% moderately differentiated grade and 20% well differentiated grade. This was similar to analysis done by park et al.

CT abdomen was done in all our patients and we had analysed the presence of nodes by imaging, adjacent organ infiltration, liver secondaries and omental deposits .When comparison was done between HER-2/neu positive and negative patients there was no difference between the two groups. There was no correlation between TNM staging and HER-2/neu positivity. This was similar to the study by Gravalos C, Marquez A, Garcıa-Carbonero R et al.

The type of surgery done was analysed to determine whether radical procedure could be done in HER-2/neu positive patients. There was no difference between HER-2/neu positive and negative patients .Radical surgery was feasible in 50% of HER-2/neu positive patients and only 28% of HER 2 neu negative patients underwent radical surgery. This clearly states that HER-2/neu positvity is not aggressive at presentation. Tanner, M. Hollm ´en, T. T. Junttila et al. also observed similar findings in their study.

The Lauren pathological type of tumor and its association with HER-2/neu was looked in to. All our HER-2/neu patients had only intestinal type of tumor . This was similar to studies by Nakajima, et al, Park, et al. and Tanner, et al .The reason for association of HER-2/neu

with intestinal type of cancer has to be investigated further to identify the cause of this association and prevent if feasible. The ideal algorithm for testing HER-2/neu is

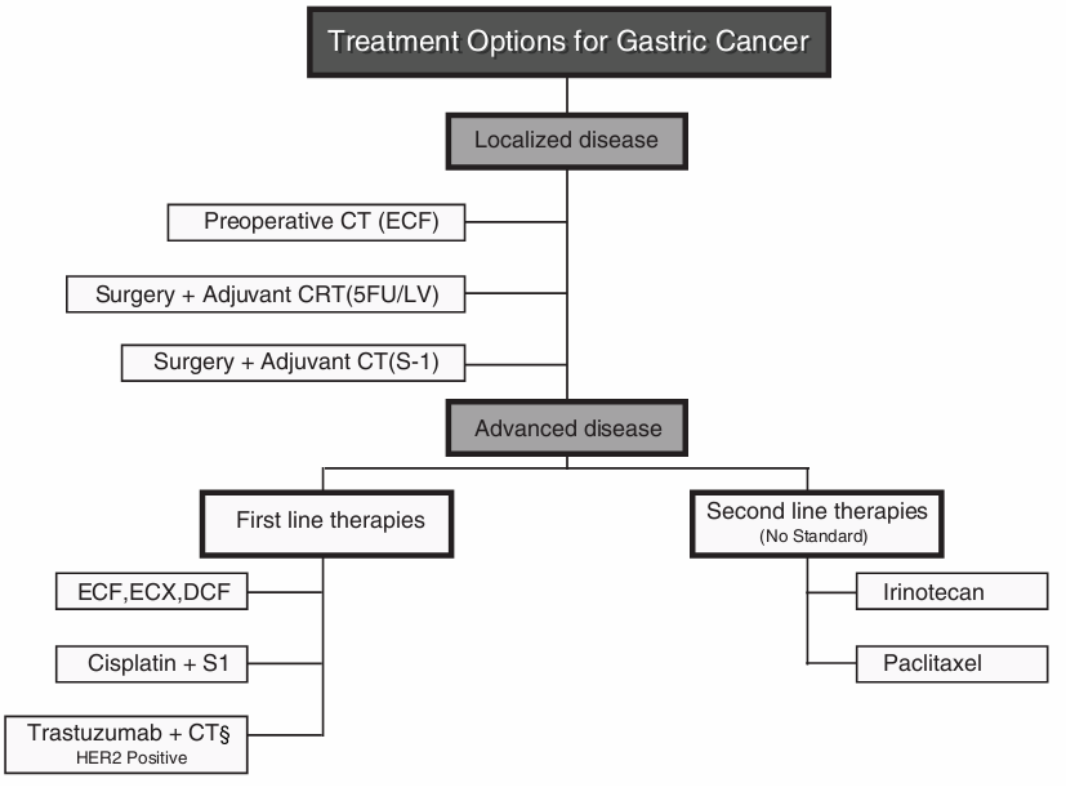


We had 3 patients with equivocal result by IHC .This analysis had the lacuna of not analyzing these equivocal cases by FISH .This was due to the lack of availability of FISH in our institution.

The present study showed that there was no clinicopathological correlation between HER-2/neu positivity and age, sex, presenting complaints, site of lesion, grade of tumor, TNM staging. This indicates that HER-2/neu may be an independent prognostic marker. When overexpressed, it predicts the response to Trastuzumab. Thus, it is the first predictive factor available for gastric cancer and all gastric

carcinoma patients should undergo HER-2/neu testing to obtain its benefit. More centers should be equipped with the availability of IHC facility . Pathologists should to be trained to interpret HER-2/neu in gastric cancer as it is different from interpreting breast cancer.

Trastuzumab in combination with chemotherapy is now considered to be a new standard of care for patients with HER-2/neu positive advanced gastric or esophagogastric junction adenocarcinoma. The current recommended treatment of gastric cancer is



Trials are underway to test the benefit of Trastuzumab in adjuvant and neoadjuvant setting to extend its benefit Further important questions such as duration of treatment, its safety in combination with other regimens like ECF, remains to be determined Trials evaluating Trastuzumab beyond disease progression, and also in pre treated gastric cancer patients to improve their outcome.

Lapatinb, which blocks both HER-1 and HER-2/neu works synergistically with Trastuzumab in. Preclinical studies in gastric cancer cell lines. This is also being looked in to .Also ongoing projects are using Pertuzumab which prevents HER-2/neu dimerization, is being used along with Trastuzumab to increase the benefit. Extended HER-2/neu blockade with enhanced antitumor efficacy makes this combination possible and should be evaluated without delay. Cross-talk between HER-2/neu and other EGFR family members is likely to contribute to trastuzumab resistance.

Highly-selective, small molecule pan-HER inhibitors have the potential to improve antitumor activity and ability to over-come trastuzumab resistance. Strategies to overcome resistance to trastuzumab are being investigated rapidly to improve the survival of gastric cancer patients. As there is clear evidence that Trastuzumab has improved overall survival when given along with cisplatin and 5 fu and since there

are no major side effects with Trastuzumab, it is ideal that all patients with gastric carcinoma are tested for HER-2/neu and be given the benefit of it. The laboratories in the hospital should be well equipped with availability both IHC and FISH to test HER-2/neu gene. Patients may develop pimary and acquired resistance to Trastuzumab due to crosstalk between pathways. Research should also be done with combination of targeted therapies to overcome this resistance to improve the outcome of these patients. Beyond all, only a quarter of gastric cancer patients will have amplification of HER-2/neu gene leaving behind the rest 75% of patients without any benefit. Therefore it is time to concentrate on these patients and find out an effective chemotherapeutic or targeting agents which can really change the outcome of gastric cancer treatment. Also more trials are necessary in adjuvant setup to improve the disease free survival in patients who presented at an early stage though equal importance has to be given to prolong the progression free survival of patients who present in advanced disease. Each country should try to identify if any epidemiological factors are influencing the cause and take steps to get rid of those causative agent which will enable us to decrease the incidence of this disease.

SUMMARY

The study titled “Evaluation of HER-2/neu status in Gastric Carcinoma” was a prospective study of 50 patients with newly diagnosed Gastric Carcinoma, admitted in the Department of Medical Oncology, Rajiv Gandhi Government General Hospital, Chennai. Eligible patients underwent clinical examination, endoscopy, CT Abdomen and Surgery if feasible. All patients underwent HER-2/neu status assessed in the histopathological specimen obtained by endoscopic biopsy or post gastrectomy specimen. The correlation between HER-2/neu status overexpression and clinicopathological features was analysed. There was no significant association between HER-2/neu status and clinicopathological features.

CONCLUSION

The following conclusions were made from this study

- Patients in this study presented at younger age than quoted in the western population.
- The prevalence of HER-2/neu overexpression was 20% in this study population.
- HER-2/neu overexpression was not associated with any major clinical or pathological features.
- Since targeting HER-2/neu has shown an overall survival benefit of 2.7 months in literature, it can be recommended that all patients with newly diagnosed gastric cancer should undergo HER-2/neu testing for subsequent treatment with anti HER-2/neu therapy.

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PROFORMA

S.No.	Complaints	Duration
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		

On Examination:

- Mass Abdomen
- Hepatomegaly
- Ascites
- Supraclavicular node

Investigations:

- Upper Gastro Intestinal Scopy

- Gastro Intestinal Scopic Biopsy

- CT Abdomen

- Surgery

- Per Operative Finding

- Histopathology

Med Onc	Age	Sex	Complaint	Endoscopy		CT Abdomen					Surgery						Mets
				Site	HPE	Nodes	Ascites	Adj Infil	Omen dep	Liver Sec	Done/Not	Size	T stage	Lymphovascular	Perineural	I/D	
207/11	65	M	Pain + Weight Loss	Body	Well	Yes	No	No	No	No	Yes	2x2	T2	Yes	No	Intestinal	No
687/11	55	M	Pain + Weight Loss	Body	Poor	Yes	No	No	No	No	Yes	3x3	T3	Yes	Yes	Intestinal	No
156/11	72	F	Pain	Antrum	Moderate	No	No	No	No	No	Yes	5x4	T3	No	Yes	Intestinal	No
81/11	60	M	Pain + Weight Loss	Antrum	Moderate	No	No	No	No	No	Yes	4x3	T3	Yes	Yes	Intestinal	No
1152/11	50	M	Pain + Weight Loss	Antrum	Poor	Yes	No	No	No	Yes	No					Intestinal	Yes
926/11	32	M	Pain + Weight Loss	Body	Poor	No	Yes	No	No	Yes	No					Intestinal	Yes
358/11	40	F	Pain + Weight Loss	Antrum	Poor	No	No	No	Yes	Yes	Yes					Intestinal	Yes
1150/11	35	M	Pain	Antrum	Poor	No	Yes	Yes	Yes	No	Yes					Intestinal	No
1406/11	67	M	Pain	Antrum	Poor	No	No	Yes	No	No	Yes					Intestinal	No
358/11	40	F	Pain + Weight Loss	Antrum	Poor	No	No	Yes	Yes	Yes	Yes					Intestinal	Yes
94/12	49	M	Pain + Weight	Antrum	Poor	No	No	No	No	No	Yes	3x1.5	T4	Yes	No	Intestinal	No

			Loss													
246/11	45	F	Pain + Weight Loss	Antrum	Well	Yes	No	No	No	No	Yes	8x4	T3	Yes	Yes	Intestinal	Yes
889/11	50	M	Pain + Weight Loss	Antrum	Moderate	Yes	No	Yes	No	No	Yes	9x6	T4	Yes	Yes	Intestinal	Yes
1286/11	52	M	Vomiting + Weight Loss	Antrum	Poor	No	No	No	No	No	Yes	5x5	T3	No	No	Intestinal	No
519/11	57	M	Pain + Weight Loss	Antrum	Poor	Yes	Yes	Yes	Yes	No	Yes	5x3	T3	Yes	No	Intestinal	No
410/11	70	M	Vomiting + Weight Loss	Antrum	Moderate	No	No	No	No	No	Yes	4x6	T3	Yes	Yes	difuse	No
1365/11	65	M	Pain + Weight Loss	Antrum	Moderate	Yes	No	Yes	No	No	Yes	9x10	T4	No	No	Intestinal	No
1254/11	60	F	Pain	Antrum	Poor	No	No	Yes	No	No	Yes	6x9	T3	Yes	Yes	Intestinal	No
261/11	58	M	Vomiting + Weight Loss	Antrum	Well	No	No	No	No	No	Yes	8x6	T3	Yes	No	difuse	No
1301/11	45	F	Vomiting + Weight Loss	Antrum	Well	Yes	Yes	No	Yes	No	No					Intestinal	Yes
2010/11	50	F	Vomiting + Weight Loss	Antrum	Poor	Yes	No	No	No	No	No					difuse	No
1366/11	43	F	Pain + Weight Loss	Antrum	Poor	Yes	No	Yes	No	No	No					Intestinal	No
413/1	70	M	Pain	Antru	Well	No	No	No	No	No	Yes	8x6	T3	No	No	difuse	No

1				m												
219/11	52	M	Pain	Antrum	Moderate	Yes	No	No	No	No	Yes	3.5x3	T3	No	Yes	Intestinal	No
474/11	54	M	Pain	Antrum	Poor	Yes	No	Yes	No	No	Yes	3x3	T4	Yes	No	Intestinal	No
1402/11	31	M	Pain	Antrum	Moderate	Yes	Yes	No	No	Yes	Yes		T3	No	No	Intestinal	Yes
202/11	64	M	Pain + Weight Loss	Body	Moderate	No	No	No	No	Yes	No					Intestinal	Yes
95/11	60	M	Pain + Weight Loss	Body	Moderate	No	No	No	No	No	Yes	8x6	T2	No	No	Intestinal	No
170/11	38	F	Pain	Antrum	Poor	Yes	No	No	No	No	Yes	4x3	T3	Yes	No	difuse	No
730/11	60	F	Pain	Body	Poor	Yes	No	Yes	No	Yes	Yes	5x8	T4	Yes	Yes	difuse	No
1781/11	52	F	Vomiting + Weight Loss	Body	Moderate	No	No	Yes	No	No	Yes	4x6	T4	Yes	Yes	Intestinal	No
1281/11	52	M	Pain + Weight Loss	Body	Poor	No	Yes	No	No	Yes	No					Intestinal	Yes
786/11	50	F	Pain	Body	Poor	Yes	No	Yes	No	No	Yes	5x4	T4	Yes	Yes	difuse	No
619/11	55	M	Pain	Antrum	Poor	No	Yes	Yes	No	No	Yes	8x7	T4	Yes	Yes	difuse	No
439/11	65	F	Vomiting + Weight Loss	Antrum	Poor	No	No	Yes	No	No	Yes	8x6	T4	Yes	Yes	Intestinal	No

499/11	48	M	Pain + Weight Loss	Body	Poor	No	Yes	No	No	Yes	No					Intestinal	Yes
868/11	58	M	Vomiting + Weight Loss	Body	Poor	No	Yes	No	Yes	No	No					difuse	Yes
291/11	53	M	Pain	Body	Moderate	No	Yes	No	No	Yes	No					Intestinal	Yes
592/11	51	F	Vomiting + Weight Loss	Body	Well	No	No	No	No	No	No					Intestinal	Yes
662/11	44	F	Pain	Body	Well	Yes	Yes	Yes	Yes	No	No					difuse	Yes
648/11	42	M	Pain + Weight Loss	Body	Poor	Yes	No	No	No	No	No					difuse	Yes
736/11	35	F	Pain + Weight Loss	Antrum	Well	Yes	No	No	No	Yes	No					difuse	Yes
186/11	60	F	Pain + Weight Loss	Body	Poor	No	No	Yes	No	No	No					difuse	Yes
1374/11	58	M	Vomiting + Weight Loss	Body	Well	Yes	Yes	Yes	No	No	No					difuse	Yes
1126/11	47	M	Vomiting + Weight Loss	Antrum	Moderate	Yes	Yes	No	No	Yes	Yes					Intestinal	Yes
1258/11	62	M	Vomiting + Weight Loss	Antrum	Well	Yes	No	Yes	Yes	No	Yes					difuse	No
1917/11	35	M	Pain	Body	Moderate	Yes	No	No	No	No	Yes					Intestinal	No
185/11	46	M	Pain	Body	Moderate	No	No	No	No	No	Yes					Intestinal	No
754/11	58	M	Pain + Weight Loss	Body	Well	Yes	No	No	No	No	Yes					difuse	No
897/11	42	F	Pain	Body	Moderate	No	No	No	No	Yes	No					difuse	No

ஆராய்ச்சி ஒப்புதல் கடிதம்

ஆராய்ச்சி தலைப்பு

பெயர்	:	தேதி	:
வயது	:	உள் நோயாளி எண்	:
பால்	:	ஆராய்ச்சி சேர்க்கை எண்	:

இந்த ஆராய்ச்சியின் விவரங்களும் அதன் நோக்கங்களும் முழுமையாக எனக்கு விளக்கப்பட்டன.

எனக்கு விளக்கப்பட்ட விஷயங்களைப் புரிந்து கொண்டு நான் எனது சம்மதத்ததை தெரிவிக்கிறேன்.

எனக்கு வயிற்றுப் புற்றுநோயில் சதை பரிசோதனை செய்துகொள்ளவும் சம்மதம்.

இந்த ஆராய்ச்சியில் பிறரின் நிர்பந்தமின்றி என் சொந்த விருப்பத்தின்பேரில்தான் பங்கு பெறுகிறேன் மற்றும் நான் இந்த ஆராய்ச்சியிலிருந்து எந்நேரமும் பின்வாங்கலாம் என்பதையும் அதனால் எந்த பாதிப்பும் ஏற்படாது என்பதையும் புரிந்து கொண்டேன்.

நான் வயிற்றுப் புற்றுநோய் குறித்த இந்த ஆராய்ச்சியின் விவரங்களைக் கொண்ட தகவல் தாளைப் பெற்றுக் கொண்டேன்.

எனக்கு அறுவைச்சிகிச்சை செய்யப்பட்டு நோய்க் குறியியல் துறையில் சதைப் பரிசோதனைக்கு பயன்பட்ட மெழுகுக்கட்டிகளை வைத்து ஆராய்ச்சி மற்றும் சிறப்புப் பரிசோதனை செய்து கொள்ள சம்மதம் தெரிவிக்கிறேன்.

சதை பரிசோதனை செய்வதற்கு முன் வலி தெரியாமல் இருப்பதற்காக ஊசி (லிக்னொகெய்ன் இஞ்செக்ஷன்) போடுவதற்கும் சம்மதிக்கிறேன். மேற்கண்ட ஊசி போடுவதற்கு முன் ஒவ்வாமை (அலெர்ஜி) பரிசோதிக்க மேற்கண்ட ஊசியை தோலில் போட்டுக் கொள்ளவும் சம்மதிக்கிறேன்.

மேற்கண்ட ஊசியை போடும் போதோ அல்லது சதை பரிசோதனை செய்யும் போதோ ஏதேனும் பின் விளைவுகள் (அரிப்பு, தோல் வீக்கம், மயக்கம், தலைச்சுற்றல், வாந்தி முதலியன) ஏற்படலாம் என மருத்துவர் மூலம் தெரிந்துக் கொண்டேன்.

கையொப்பம்

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI -3

Telephone No: 044 25305301
Fax: 044 25363970

CERTIFICATE OF APPROVAL

To
Dr. K.B. Akila
PG in DM Medical Oncology
Madras Medical College, Chennai -3

The Institutional Ethics committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled "Evaluation of Her-2 / Neu status in Gastric Carcinoma" No. 18022011

The following members of Ethics Committee were present in the meeting held on 17.02.2011 conducted at Madras Medical College, Chennai -3.

1.	Prof. S.K. Rajan. MD	-- Chairperson
2.	Pro. A. Sundaram MD Dean i/c, Madras Medical College, Ch -3	-- Member Secretary
3.	Prof. R. Sathianathan MD Director , Institute of Psychiatry, MMC, Ch-3	-- Member
4.	Prof. R. Nandhini MD Director, Institute of Pharmacology ,MMC, Ch-3	-- Member
5	Prof. Pregna B. Dolia MD Director , Institute of Biochemistry, MMC, Ch-3	-- Member
6.	Prof. C. Rajendiran, MD Director , Inst. Of Internal Medicine, MMC, Ch-3	-- Member
7.	Prof. Geetha Subramanian, MD DM Prof & Head , Dept. of cardiology, MMC,Ch-3	-- Member
8.	Thiru. A. Ulaganathan Administrative Officer, MMC, Ch-3	--- Layperson
9.	Thiru. S. Govindsamy. BA BL	-- Lawyer
10.	Tmt. Arnold soulina MA	-- Social Scientist

We approval the proposal to be conducted in its presented form.

Sd/ chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and patients information / informed consent and asks to be provided a copy of the final report.

Member Secretary, Ethics Committee

Evaluation of HER-2/neu status in Gastric Carcinoma

ABSTRACT

INTRODUCTION

Gastric carcinoma is the fourth most commonly diagnosed cancer in the world and is the second leading cause of cancer related death. More than 50% of patients with gastric cancer present in advanced, unresectable stages, making cure impossible. Two-thirds of these patients who undergo radical surgery will experience. Systemic treatment is the only option for the patients presenting in advanced stages. The median survival after diagnosis of metastatic disease is approximately 10-11 monhs with currently available treatments. Many single agents and combination chemotherapeutic agents are active in the treatment of metastatic disease. Objective response rates ranging from 10% to 30% for single-agent and 30% to 60% for combination regimens have been reported. The role of targeted therapy has recently come into play after the establishment of the role of HER-2/neu over-expression in gastric malignancies. Trastuzumab ,an anti HER-2/neu antibody ,in combination with Cisplatin and 5FU in advanced gastric carcinoma patients has shown to improve overall survival by 2.7 months in TOGA trial.

This study was undertaken to find out the prevalence and the clinicopathological correlation in HER-2/neu overexpression in gastric carcinoma patients.

METHODS

IHC evaluation of HER-2/neu was done in paraffin embedded tissue samples of 50 gastric carcinoma histopathology specimen. Of the 50 samples evaluated, 25 were endoscopic specimens and 25 were post gastrectomy specimens. Immunohistochemistry scoring was done with modified Hoffmann score. Results were tabulated and analysed for prevalance of HER-2/neu prevalence and the clinicopathological correlation in HER-2/neu overexpression in gastric carcinoma patients.

RESULTS

Out of 50 patients, 10 (20%) were 3+, 3 patients (6%) had 2+, 10 patients (20%) had 1+ and 27 patients (54%) had 0 score by IHC. HER-2/neu overexpression was not associated with any major clinical or pathological features like age,sex, TNM staging, type of growth and grade of tumor.

CONCLUSION

The prevalence of HER-2/neu overexpression was 20% in this study population.

Since targeting HER-2/neu has shown an overall survival benefit of 2.7 months in literature, it can be recommended that all patients with newly diagnosed gastric cancer should undergo HER-2/neu testing for subsequent treatment with anti HER-2/neu therapy.

KEYWORDS

Carcinoma Stomach, HER-2/neu in CA Stomach, Trastuzumab.