ANALYSIS IN CARCINOMA STOMACH Her-2/neu POSITIVITY BY IMMUNO-HISTOCHEMISTRY

DISSERTATION SUBMITTED FOR

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BRANCH - I (GENERAL MEDICINE)

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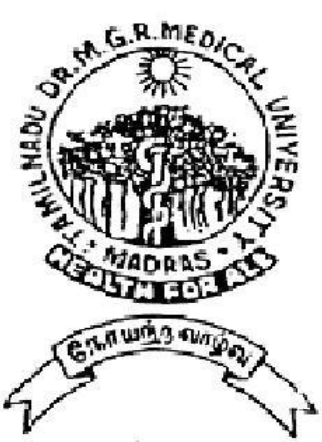

THE TAMILNADU

DR.M.G.R.MEDICAL UNIVERSITY

CHENNAI

BONAFIDE CERTIFICATE

This is to certify that the dissertation entitled **"ANALYSIS IN CARCINOMA STOMACH Her-2/neu POSITIVITY BY IMMUNO-HISTOCHEMISTRY"** submitted by **Dr.V.MARIAPPAN** to the Tamil Nadu Dr. M.G.R.Medical University, Chennai in partial fulfillment of the requirement for the award of M.D Degree Branch I (General Medicine) is a bonafide research work was carried out by him under my direct supervision & guidance.

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DECLARATION

I, **Dr.V.MARIAPPAN** declare that, I carried out this work on, "**ANALYSIS IN CARCINOMA STOMACH Her-2/neu POSITIVITY BY IMMUNO-HISTOCHEMISTRY**" at the Department of Medicine, Govt. Rajaji Hospital during the period of March 2011 to August 2012. I also declare that this bonafide work or a part of this work was not submitted by me or any others for any award, degree, diploma to any other University, Board either in India or abroad.

This is submitted to The Tamilnadu Dr.M.G.R.Medical University, Chennai in partial fulfillment of the rules and regulations for the M.D degree examination in General Medicine.

Place : Madurai **Dr. V. MARIAPPAN**

Date :

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CONTENTS

INTRODUCTION

Carcinoma stomach remains the leading cause of death in both developed and developing countries with mortality ranks second in the world. Early stages of tumour are amenable to surgical correction, but usually the patients presents in advanced stage with signs of inoperability. Inspite of newer modalities of therapies available for treatment, survival rate is poor in advanced stage .

The genesis of molecular biology led to the development of newly designed therapeutic molecules, which interferes with the pathogenesis of cancer cells. The Human epidermal growth receptor 2 gene (Her-2, otherwise called as ERBB 2 and Her-2 nu) is now considered as the cornerstone in solid human cancers, especially gastric cancer.

There is now a strong correlation that exists between Her-2 overexpression and poor outcome in gastric cancer patients, which has been evidenced by many international studies.

Most of the studies regarding Her-2 is from the foreign literature. This study is an attempt to analyse Her-2 positivity in gastric carcinoma patients at MADURAI GOVERNMENT RAJAJI HOSPITAL which primarily caters the rural population in and around MADURAI.

AIMS & OBJECTIVES

1. To assess Her-2/neu content in our gastric cancer patients.
2. To assess the correlation between this receptor tumour content and clinicopathologic characteristics.

REVIEW OF LITERATURE

INCIDENCE

Gastric cancer is the fourth most common type of cancer worldwide, preceded by lung, breast and colorectal cancers. The incidence rate of this disease presents considerable variation according to age, gender, socio-economical conditions and geographical location. Thus, most of the gastric cancer patients are older than 50 years at the time of diagnosis , and the global incidence is two times more common in men than in women. The most substantial variations in the incidence rates of this malignancy are, however, observed in relation to geographical regions.

In general, the incidence of gastric cancer is high in Asian and European populations, while it is low in American and Australian population. Nearly seventy percentage of gastric cancer occurs in developing countries. Gastric cancer exhibits worldwide distribution and in not specific to any geographical pattern. Among the Asian countries China, Japan, Korea harbour the high risk for gastric cancer with other Asian countries comes under low risk .

In India it ranks fifth in men and seventh in women as a leading cause of death. Highest incidence is noted in southern and north-east parts of India with maximum number of cases reported in MIZORAM.

TIME TRENDS IN INCIDENCE:

The incidence rates for gastric cancer have undergone a steady decline during the past decades. This downward trend is equally observed among both sexes and in high and low risk areas, but has been more pronounced in developed countries. Interestingly, the fall in the incidence is particularly associated to distal gastric carcinoma, in contrast to proximal cancer that seems to experience a permanent slight increase. Similarly, epidemiological studies have shown that the general decrease in incidence is mainly attributed to the fall in intestinal subtype of gastric cancer while the diffuse subtype shows a rather small change.

The reasons underlying the generalized decline in the incidence of this malignancy are not well understood, but it has been hypothesized that this may be associated to newer techniques

in the processing and preservation of food, better nutrition and reduced transmission of *H. pylori* in childhood.

Despite the notable fall in the incidence rate, the absolute number of cases of gastric cancer continues to increase globally as a result of the population growth and ageing. In the year 1980 gastric cancer was the most common type of cancer globally, with approximately 669350 new cases diagnosed, representing 10.5% of the cancer burden. Ten years later, in 1990, approximately 798500 new cases occurred.

For the year 2000, the number of new cases of gastric cancer reached 876000. In 2002, the number of new cases was estimated to be 934000, which meant 8.6% of the total number of cancer cases. For 2010, the number of new cases of gastric cancer is expected to be 1.1 million.

MORTALITY AND SURVIVAL:

Following lung cancer, carcinoma stomach ranks second cause of mortality worldwide , accounting for nearly 700000 deaths in 2002. Wide geographical variation in mortality rates exist

throughout the world, particularly high in the developing world. Similar to the incidence, a constant rate of decline in mortality in both sexes, and in low and high risk countries has occurred in the last decade.

The decline in mortality, however, seems to occur faster than with the incidence, and is particularly pronounced in certain populations. Mortality rate are notably high because in most cases, the disease is diagnosed at advanced stages when the treatment is likely to fail. In general, the five-year survival for patients of gastric cancer is below 30% in most countries, despite some variations according to the country/geographical region.

It is noteworthy, however, that the survival rate have reached more than 50% in the last decade among Japanese. This is thought to be associated with the implementation of X-ray (photofluorography) based gastric cancer mass screening programs since early in 1960´s.

Similar experience with X-ray based mass screening interventions in other high risk countries have demonstrated a significant impact of early detection in the mortality of gastric

cancer. Nevertheless, studies in population groups with same ethnic background but dissimilar access to health care suggest that environmental and biological factors may also play an important role in explaining differences in mortality and survival of gastric cancer between high and low risk countries or developing versus developed economies.

HISTOLOGICAL AND ANATOMICAL CLASSIFICATION OF GASTRIC CANCER

Various classifications have been designed for carcinoma stomach on the basis of macroscopic or histological features, which include Borrman, Japanese system, World Health Organization (WHO) system and Lauren. The Lauren classification system is most commonly used and describes the tumors in relation to microscopic configuration and growth pattern.

According to the Lauren system, gastric cancer is divided into intestinal and diffuse histological subtype. These two subtype present marked differences in pathology, epidemiology, etiology and biological behavior. Intestinal subtype gastric cancer is the

most frequent globally and is particularly common in geographical regions with high-risk of the malignancy.

INTESTINAL SUBTYPE:

Intestinal subtype tumors are often localized in the lower part of the stomach (antrum), and are characterized by having well defined glandular formation, similar to the microscopic appearance of colonic mucosa. The development of intestinal subtype gastric cancer follows a stepwise sequence of precursor lesions starting with superficial gastritis, continuing through chronic atrophic gastritis, followed by intestinal metaplasia and dysplasia and progress to overt carcinoma.

For unknown reasons, the multistep process often does not lead to neoplasia, as it stops at one of the stages and undergoes regression. The aetiology of intestinal subtype gastric cancer is mainly associated to environmental factors, the tumour frequently develops late in life (after 50 years of age), and is twice more common in males than females.

DIFFUSE SUBTYPE:

Diffuse subtype gastric cancer commonly develops in the corpus of the stomach which do not have any glands and cellular adhesion, with small clusters of neoplastic cells infiltrating the stomach wall uniformly. No pre-neoplastic lesions have been observed during the development of diffuse cancers.

Diffuse subtype tumours are commonly associated with genetic predisposition, and single-cell mutations in normal gastric glands. The diffuse subtype has a relatively constant or even slightly increase in incidence rates, more often occurs in younger individuals and presents a similar prevalence in males and females, and is associated with a poor prognosis than the intestinal subtype.

ANATOMICAL LOCATION:

The anatomical location of tumour in the stomach is considered as an important parameter for the classification of gastric cancer. On the basis of anatomical location two subtypes of gastric cancer can be distinguished: tumours from the distal region of the stomach and those arising at the most proximal part of this

organ. These two anatomical subtype of tumours present remarkable etiological differences. Non-cardia cancer is generally thought to develop as a result of the interaction between host, environmental and *H. pylori* factors.

In contrast, two etiological mechanisms have been proposed for cardia gastric cancer. One is associated with atrophic gastritis and resembles the development of non-cardia malignancies. The second arises in similar fashion to oesophageal carcinomas, as a result of frequent refluxing of acidic gastric juice into the distal oesophageal mucosa, which leads to the transformation from squamous to columnar metaplastic epithelium to, ultimately, overt cancer. Epidemiological dissimilarities also exist between these two anatomical subtypes of gastric tumours. Non-cardia gastric cancer accounts for the majority of the cases worldwide and is the predominant type in high-risk areas. In contrast, cardia cancer is more homogeneously distributed all over the world and its incidence tends to be increasing.

RISK FACTORS FOR GASTRIC CANCER

Several parameters were correlated as risk factors, which by establishing complex interactions may ultimately lead to development of this malignancy. Among the most recognized gastric cancer risk factors are nutritional and dietary aspects, genetic predisposition and sporadically occurring mutations, and *Helicobacter pylori* infection. More recently, aspects related to the inflammatory response against the bacterial infection have emerged as important determinants for the risk of this malignancy.

Dietary and nutritional aspects

Diet plays a dual role in gastric cancer aetiology, providing a number of elements and vitamins that reduce the formation of carcinogens, but also as the source of well established carcinogenic molecules or precursors of them. Evidence indicates that diet high in fruits and vegetables may protect against gastric cancer while salted foods, consumption of processed foods and inappropriate preservation and storage of aliments could increase the risk of this malignancy.

In general, epidemiological studies show a favourable outcome with consumption of fruits and vegetables and gastric cancer, which seems to be more pronounced in case of citrus fruits and raw allium vegetables. It has also been suggested that fruits may have stronger potential than vegetables to protect against gastric cancer development. These associations differ according to anatomical and histological subtypes of gastric malignancies, sex and lifestyle behaviours (e.g. smoking and alcohol consumption). On the basis of the existing evidence, the International Agency for Research on Cancer (IARC/WHO) has considered that high intake of fruits and high intake of vegetables reduce the risk of gastric cancer. Still, it remains unknown which constituents in fruit and vegetables specifically protect against the development of this malignancy.

Epidemiological studies have evaluated the association between specific antioxidant nutrients known to reduce the formation of carcinogenic molecules and the risk of gastric cancer. In general, antioxidant molecules such as lycopene, vitamin A, vitamin E, vitamin C and micro-nutrients like selenium found to

decrease the risk of carcinoma stomach. But the association between β-carotene, vitamin A and vitamin E and gastric cancer is more controversial. As in the case of fruits and vegetables, the potential significance of antioxidant nutrients as protector factors varies substantially depending on the anatomical and histological subtype of gastric cancer, sex, lifestyle behaviours and interactions between antioxidant molecules.

Diets high in salt and preserved meats have been suggested to play a role in the aetiology of gastric cancer. Salt may act as an irritant of the stomach wall and in connection to *H. pylori* infection may contribute to the damage of the mucosal layer, enhancing thus the susceptibility of epithelial cells to carcinogenic molecules that accumulate in this organ. Meat products like bacon, sausage, salami and ham are often rich in salt, nitrite, nitrosamines, and can also be the source of *N*-nitroso compounds, of established carcinogenic properties. A number of case-control and cohort studies have found that higher consumption of red meat and salted foods resulted in increased risk of carcinoma stomach.

Genetics in gastric cancer

Genetics play a fundamental role for the origin and progression of carcinoma stomach. It is well established that a number of inherited germ-line mutations and genetic syndromes predispose to the development of this malignancy. Likewise, a diverse set of genetic and epigenetic *de novo* alterations are often found in gastric cancer, which probably occur at different stages during the development of the malignancy, and differ according to the histological subtype of the disease. Familial clustering of cases is reported in ten percent of population, in which two or more relatives from the same family are affected.

In general, the risk of first degree relatives developing carcinoma stomach is expected to be 2 to 3 fold higher than in persons with no familiar background of the disease. This, however, should be cautiously analyzed due to the fact that, besides the common genetic background, environmental and cultural factors (e.g. *H. pylori*, diet, lifestyle behaviours) may be similarly shared among the family members and in some cases are difficult to differentiate. Nevertheless, the genetic susceptibility to develop this

malignancy has been clearly established in a fraction of these familial-clustered gastric cancers.

Germ-line mutation:

Mutations involving the E-cadherin gene (*CDH1*) are the most recognized genetic aberrations found in hereditary gastric cancer, accounting for approximately 1-3% of the cases. Epithelial cells express high content of E-cadherin and exerts cellular adhesion and suppression of invasion. *CDH1* associated familial gastric cancer follows an autosomal dominant pattern of inheritance, with more than 70% penetrance, and is caused by several alterations in the *CDH1* gene, mainly truncating mutations.

Most of the gastric cancer cases attributed to *CDH1* aberrations are of diffuse subtype, particularly signet-ring cell adenocarcinomas, and are predominantly observed in young individuals. A considerable number of genetic and epigenetic alterations have been identified both in preneoplastic lesions leading to gastric cancer and neoplasia itself. These spontaneously occurring events can trigger aberrant effects at several molecular levels, including reactivation of telomerase, activation of

oncogenes, inactivation of tumour suppressor genes, over-expression of growth factors and cytokines, altered expression of cell-cycle regulators and DNA-repairing enzymes, and increased microsatellite instability. It is worth noting that genetic and epigenetic events may alter the expression of known oncogenes (*c-met, K-ras*), tumour suppressor genes (*APC, p53*), DNA-repairing enzymes (*hMLH1*) and cell-adhesion molecules (E-cadherin, β-catenin, γ-catenin) that are central for the cellular homeostasis.

Somatic mutation:

Many types of somatic mutation in gastric cancer have been described in at the molecular level. The mechanisms behind this type of mutation are not fully established. The p53 gene is mutated in 60% of gastric cancer. Overexpression of p53 can be identified by immuno-histochemistry techniques but the its association with regard to prognosis was not clear.

A target of amplification on 17q in gastric cancers was also identified using a combination of comparative genomic hybridization and oligonucleotide microarray studies.

Overexpression of DARP32 and a novel isoform t-DARP were both found in many cases of gastric cancer . PCR has detected no somatic mutations of $p16^{INK4}$ among 60 cases with gastric cancer . On the other hand, $p16^{INK4}$ somatic mutations were noted along with loss of heterozygosity of 9p in several oesophageal adenocarcinomas, which are related to gastroesophageal junctional cancers. Other cases of these cancers were observed to have loss of p16 expression. In a study of p16's promoter region in gastric cancers, a significant number (41%) exhibited CpG island methylation. Many cases with hypermethylation of promoter regions displayed the MSI-H phenotype and multiple sites of methylation, including the hMLH1 promoter region.

Evidence of a tumor suppressor locus on chromosome 3p has accumulated from a variety of studies and includes allelic loss at 3p in primary gastric tumours and homozygous deletion of 3p in a gastric cancer cell line . The FHIT gene was isolated from the common fragile site region (FRA3B) at 3p14.2 and found to have abnormal transcripts with deleted exons in five of nine gastric

cancers. Furthermore, loss of FHIT protein expression was demonstrated immunohistochemically in the majority of gastric carcinomas in one study. Also, a somatic missense mutation was identified in exon 6 of the FHIT gene during a coding region analysis of 40 gastric carcinomas. Additional studies are needed to identify the critically altered targets on chromosome 3p and clarify the role that FHIT plays in gastric tumorigenesis.

Deletion of the trefoil peptide TFF1 has been found in 60% of gastric cancer. Mice with homozygous deletion of Tff1 by homologous recombination all developed antral dysplasia, and 30% were reported to have multifocal gastric carcinoma.

Loss of p27, a cell-cycle regulator, correlates with advanced stage in gastric cancer. Amplification and overexpression of the c-met gene, which encodes a tyrosine kinase receptor for the hepatocyte growth factor, have been reported in gastric carcinomas, and the epidermal growth factor and its receptor are expressed in approximately one-fourth of gastric cancers. Alterations of fibroblast found by a polymerase chain reaction based assay in carcinoma stomach correlated with worst prognosis. Amplification

of c-erbB-2 has been demonstrated in a small subset of gastric cancers and overexpression observed correlates with worst prognosis. The expression of angiogenesis factors such as vascular endothelial growth factor has been observed in a subset of gastric cancers, indicating the potential role of angiogenesis inhibitor therapy. Membrane-type matrix metalloproteinase is preferentially expressed in some gastric cancer cells with co-localization and activation of the zymogens proMMP-2. Advanced gastric cancer patients express increased activation of plasminogen. Specific alterations such as these need true prevalence determination and further characterization in gastric cancer before genetic tests can be designed for clinical use.

In addition, they are molecules that have been consistently linked with the development and progression of other types of cancer. These gene dysregulations are likely to occur during the course of the multistep gastric carcinogenesis as a result of replication errors, mutations, amplifications, defective DNA-repair, aberrant methylation, loss of heterozygosity (LOH), or a combination of two alterations.

CLINICAL FEATURES:

Gastric cancers, when superficial and surgically curable, usually do not produce any symptoms. As the tumour becomes more extensive, patients may complain of an insidious epigastric discomfort, early satiety to a severe and constant upper abdominal pain. Loss of appetite, nausea and vomiting is very common but is not the usual presenting complaint.

Weight loss may commonly be observed, and nausea and vomiting are particularly prominent with tumours of the pylorus; dysphagia and early satiety may be the major symptoms caused by diffuse lesions originating in the cardia. There are no early recognisable physical signs. A palpable abdominal mass indicates a long-standing growth and predicts regional extension.

Metastases commonly occurs to the intraabdominal organs , supraclavicular lymph nodes, ovary, periumbilical region, peritoneum and rectum. Malignant ascites may also develop. The liver is the most common site for hematogenous spread of tumor.

The presence of iron-deficiency anaemia and of occult blood in the stool mandates a search for an occult gastrointestinal tract lesion.

Other clinical features associated with gastric adenocarcinomas include migratory thrombophlebitis, micro-angiopathic hemolytic anaemia, diffuse seborrheic keratoses (so-called Leser-Trélat sign), and acanthosis nigricans.

INVESTIGATIONS:

The main modalities of investigating gastric adenocarcinoma and thus guiding therapy are

1. ENDOSCOPY AND ENDOSCOPIC ULTRASOUND:

Flexible endoscopy remains the essential tool for diagnosis of gastric cancer. It allows visualisation of the tumour, provides tissue for pathologic diagnosis, and can serve as a treatment for patients with obstruction and bleeding. Increasingly, flexible endoscopy combined with ultrasound is being used to stage and risk-stratify patients with gastric cancer properly. The predictive accuracy of EUS for T and N stages was found to be

58% and 50% respectively. Its role in the evaluation of metastatic disease is currently limited.

2. COMPUTED TOMOGRAPHY:

CT remains the primary method for detection of intra-abdominal metastatic disease, with an overall detection rate of approximately 85%. The ability to image peritoneal metastases remains only 50%. The accuracy of T and N stages determined by CT is less accurate than EUS.

3. POSITRON EMISSION TOMOGRAPHY:

PET is not currently a primary staging modality for gastric cancer. Only 50% of gastric cancers are PET avid, which limits its application. However, PET positive patients are presumed to have advanced disease and are considered for neoadjuvant therapy. It may be an effective modality for monitoring response to therapy.

4. LAPAROSCOPY:

The high rate of occult metastatic disease makes laparoscopy an attractive staging modality. The overall sensitivity of

laparoscopy for detecting metastatic disease was higher than 95%. Unresectable disease not detected by prior imaging was found in 35% of gastric cancer patients undergoing staging laparoscopy. More than 70% of these patients had occult peritoneal or liver metastasis.

5. TUMOUR MARKERS:

The carcinoembryonic antigen (CEA) level is elevated in approximately one-third of patients with primary gastric cancer. The sensitivity of CEA as a marker of gastric cancer is low, but when the CEA level is elevated, it generally correlates with stage. Combining CEA with other markers, such as the sialylated Lewis antigens CA19-9 or CA50, can increase sensitivity, compared with CEA alone.

A large study of patients with gastric cancer evaluated the prognostic significance of serum levels of CEA , alpha-fetoprotein , human chorionic gonadotropin, CA19-9 , and CA125 , as well as tissue staining for C-erb B-2 and Î²-HCG . In a multivariate

analysis, only a serum Î²-HCG level of 4 IU/L or greater and CA125 level of 350 U/mL or greater had prognostic significance.

Elevated serum Î²-HCG and CA125 levels in gastric cancer before chemotherapy may reflect not just tumour burden but also aggressive biology; however, the utility of these markers in staging must be compared to that of other known preoperative markers of stage, such as on T- and N-stage endoscopic ultrasonography.

TNM STAGING OF CARCINOMA STOMACH:

Stage	TNM	Features
0	$T_{is}N0M0$	Node negative; limited to mucosa
IA	T1N0M0	Node negative; invasion of lamina propria or submucosa
IB	T2N0M0 T1N1M0	Node negative; invasion of muscularis propria
II	T1N2M0 T2N1M0	Node positive; invasion beyond mucosa but within wall
		Or
	T3N0M0	Node negative; extension through wall
IIIA	T2N2M0 T3N1-2M0	Node positive; invasion of muscularis propria or through wall
IIIB	T4N0-1M0	Node negative; adherence to surrounding tissue
IIIC	T4N2-3M0 T3N3M0	>3 nodes positive; invasion of serosa or adjacent structures 7 or more positive nodes; penetrates wall without invading serosa or adjacent structures
IV	T4N2M0	Node positive; adherence to surrounding tissue
		or
	T1-4N0-2M1	Distant metastases
Abbreviation: ACS, American Cancer Society; TNM, tumor, node, metastasis		

HER2 BIOLOGY

The Her-2 protein (p185, Her-2/neu, ErbB-2) has a molecular weight of 185- KDa transmembrane tyrosine kinase receptor and belongs to the family of EGFRs . This family is consists of four types: HER1, HER2, HER3, HER4.These receptors consists of an ligand binding domain situated extracellularly, a small transmembrane domain, and a domain with tyrosine kinase activity present intracellularly. The binding of different substances to the extracellular domain initiates a series of events that resulted in proliferation of cancer cells, cell death, cell to cell adhesion, cellular differentiation and migration.

The gene for Her-2 is present in the chromosome 17q2 1 which is situated adjacent to topoisomerase IIa genes . It bears direct relationship with oncogene V-erbB of the avian erythroblastosis virus.

In carcinomas Her-2 is considered as an oncogene because amplification of this gene induces protein overexpression in the

cellular membrane and as a result it acquires the properties of a malignant cell.

HER-2 AS A PROGNOSTIC FACTOR

Various studies enumerating the prognosis of gastric cancer have been conducted across the world. In Japanese studies, conducted among 200 patients Her-2 positivity was noted in 23% by IHC. 13% positivity was noted among 166 patients in Spanish group. The median survival was poor for patients with Her-2 overexpression by IHC when compared with Her-2 negative patients in both of these studies. This shows Her-2 overexpression as an independent predictor of mortality. It is considered as the second poorest prognostic factor after lymph node status in early stage of the tumour. Her-2 staining intensity was also correlated with the metastasis to lymph node, invasion of serosa and size of tumour.

Allgayer showed a increased rate of membranous or cytoplasmic Her-2 expression by IHC among 203 gastric cancer patients. He also concluded Her-2 expression as a poorest

prognostic factor. This was further confirmed by the studies conducted by Tanner who showed that median survival of patients with Her-2 negativity was one year, while patients with Her-2 positivity had only median survival of six months.

HER-2 AND E-cadherin EXPRESSION

The E-cadherin mediated cell adhesion system acts as an invasive suppressor system, and tumours which expresses E-cadherin frequently had lymph node involvement and metastasis to different sites. This abnormal E-cadherin expression seems to be an early event in tumorigenesis.

The diffuse type of gastric cancer is usually associated with decreased expression of the E-cadherin molecule. Direct evidence of an E-cadherin mutation that triggers tumorigenesis has been associated with detection of germ line mutation of the gene CDH1 in hereditary diffuse gastric cancer. This molecule is down regulated in sporadic diffuse type gastric cancers because of a somatic mutation or hypermethylation of the promotor region in the early stages of tumorigenesis.

HER-2 AND MICROSATELLITE INSTABILITY:

Microsatellite instability (MSI) comprises length mutation in tandem oligonucleotide repeats. It is a hallmark of replication error phenotype observed in some sporadic tumours from different sites. In human, atleast six proteins (hMLH1, hMSH2, hPMS1, hPMS2, hMSH6 and hMLH3) comprise the mismatch repair enzyme system (MMR). Defective DNA MMR usually results from genetic or epigenetic alteration in hMLH1 or hMSH2.

MSI has been reported in early and advanced gastric cancers caused by hypermethylation of the hMLH1 promoter region. Such gene inactivation strongly correlates with microsatellite instability-high phenotype and results in a loss of protein expression identifiable by IHC.

The intestinal type is usually associated with microsatellite instability. Gastric carcinomas with increased frequency of MSI has certain special pathological features such as more antral location of the tumour, increased lymphoid infiltrate, minimal lymph node metastasis and increased median survival rate. Among the

European population groups, Rugge showed that DNA repair mechanism alterations are early molecular events for gastric carcinogenesis and suggested that IHC should be considered as a suitable method for MSI assessment in gastric precancerous lesions. This may imply that impairment in the function of a repair enzyme system may provide a situation for genetic alteration and Her-2 overexpression.

CONCORDANCE BETWEEN HER-2 OVEREXPRESSION AND GENE AMPLIFICATION

There has been controversial reports regarding the concordance of protein expression and gene amplification of Her-2 in carcinoma stomach. Among 40 cases, Lemoine observed 26% of patients had increased protein expression, 13% had gene amplification. Kameda also observed the same result in which he detected overexpression without amplification and came to a conclusion that gene amplification is not only the primary mechanism by which Her-2 protein is overexpressed in carcinoma stomach.

However there are other mechanisms by which Her-2 protein is overexpressed which includes transcriptional activation by other genes or post-transcriptional events. ToGA trial showed the concordance between Her-2 positivity by IHC and FISH was 85% and these results were largely due to the fact that FISH positive cases were among those IHC 1+/2+.

In 2006, Hoffman considered that differences observed between IHC and FISH occurred mainly due to the staining of the basolateral membrane of glandular cells increased percentage of heterogenous tumours in gastric cancer when compared with breast cancer. As a result of these studies they proposed modification in assessing Hercep Test score for gastric cancer.

The Hercep Test TM Kit

The Hercep Test is a semi quantitative method for assessing HER2 protein overexpression which was primarily used for breast cancer and now modified for using in gastric adenocarcinoma.

Following incubation with the primary antibody to human HER-2 protein, this kit is ready to be used. Visualization Reagent

is based on dextran technology. This reagent consists of both secondary goat anti rabbit molecules and horse-radish peroxidise molecules linked to a common dextran polymer backbone, thus eliminating the need for sequential application of link antibody and peroxides conjugate. The enzymatic conversion of the subsequently added chromogen results in formation of a visible reaction product at the antigen site. The specimen may then be counterstained and cover slipped. Control cell in line slides are provided.

Hercep Test TM test kit consists of the following:

Peroxidise – Blocking Reagent
Rabbit Anti human HER2 Protein
Visualization Reagent
Negative control reagent
DAB Buffered substrate
DAB chromogen
Epitope Retrieval solution
Wash Buffer (10%)

Guidelines for Scoring

Her-2 scoring should be performed on the basis of pathologist's previous experience and judgment for interpreting IHC stains. Only biopsy samples from patients with stomach or gastroesophageal junction adenocarcinoma should be used for be scoring. Those cases containing intestinal metaplasia are not considered for scoring.

SURGICAL SPECIMENS

Score to Report	HER-2 Protein Overexpression Assessment	Staining Pattern
0	Negative	Membranous staning involving less than 10% of tumour cell.
1+	Negative	Membranous staining involving more than 10% of tumour cells which is faintly perceptible. Cells are reactive only in part of their membrane.
2+	Equivocal	Lateral or basolateral membranous staining involving more than 10% of tumour cells which is weak to moderately complete.
3+	Positive	Lateral membranous staining involving more than 10% of tumour cells which is strongly complete.

BIOPSY SPECIMENS

Score to Report	HER-2 Protein Overexpression Assessment	Staining Pattern
0	Negative	No staining or membranous staining in any tumour cell
1+	Negative	Tumour cell cluster with a faint/barely perceptible membranous staining irrespective of percentage of cells stained
2+	Equivocal	Tumour cell cluster with a weak to moderate complete, basolateral or lateral membranous staining irrespective of percentage of cells stained
3+	Positive	Tumour cell cluster with a strong complete, basolateral or lateral membranous staining irrespective of percentage of cells stained

HercepTest should be carried out according to the guidelines published in the package insert and within the context of best practices and the pathologist's experience and accurate medical judgment.

ARTIFACTS IN STAINING:

Edge artifacts

Edge artifacts are usually linked to the pre-analytic handling of the tissue. Often the method of surgical extraction is the cause. This phenomenon is more frequently observed for stereotactic needle biopsies.

Increased staining intensity is frequently observed around the periphery of the tissue section, known as “the edge effect’.

The edge effect represents artifacts due to tissue drying prior to fixation. If staining is only observed at the edge of the tissue section, scoring of the tissue specimen should be avoided.

Inadequate fixation of tissue samples rendering the central portion of the tissue sub-optimal fixed relative to the peripheral areas, may mimic edge artifact. In these circumstances, the immunoreactivity in the sub-optimal central portion may be mistakenly interpreted as false negative as compared to the correct immunoreactivity observed at the section periphery which has optimal fixation.

Crush artifacts

Crush artifacts are related to edge artifacts. The artifact may be encountered more often in needle biopsies. It is presumed that the tissue injury occurs during the extraction of the tissue from the needle rather than from the actual biopsy process. Regardless, the compression of the tissue along the edges of the needle core can produce a linear staining that should be interpreted as an artifact.

Tissue areas with crushed cells typically demonstrate condensed nuclei and should be avoided in scoring. Deposition of the chromogen is characteristic in areas where the cells are crushed, while well preserved cells are devoid of immunoreactivity.

Retraction artifacts

Retraction artifacts are small spaces in the tissue where antibody and chromogen can pool forming circumferential depositions. Retraction of epithelial cells from stroma may create small spaces where the reagent pool around the epithelial cells forms a circumferential deposition of the brown end product. This

artifact requires thorough examination of the intercellular areas (i.e. cell to cell interface not the cell to stroma interface).

TREATMENT-OVERALL:

Surgical removal of tumour is the mainstay of treatment for both early and advanced stages. Adenocarcinoma is relatively radio resistant. Major role of radiotherapy is palliation of pain.

The administration of combinations of cytotoxic drugs to patients with advanced gastric carcinoma has been associated with partial responses in 50% of cases; responders appear to benefit from treatment. Such drug combinations have generally included cisplatin combined with epirubicin or docetaxel and infusional 5-FU, or with irinotecan.

Despite this encouraging response rate, complete remissions are uncommon whereas, the partial responses are transient, and the overall influence of multidrug therapy on survival has been unclear. The use of adjuvant chemotherapy alone following the complete resection of a gastric cancer has only minimally improved survival.

However, combination chemotherapy administered before and after surgery as well as postoperative chemotherapy combined with radiation therapy reduces the recurrence rate and prolongs survival.

Rationale for adjuvant chemotherapy:

Because the risk of recurrence with surgery alone is high, the use of adjuvant systemic therapy and, in the postoperative setting, additional regional treatment with radiation have been extensively explored. Two different strategies have been tested: postoperative (adjuvant) chemotherapy or chemoradiation therapy or preoperative (also known as neoadjuvant or primary) chemotherapy. More recently, some clinical trials have begun exploring preoperative chemoradiation.

The rationale for neoadjuvant therapy is that systemic treatment with its attendant risks is best given when a patient is most fit to tolerate treatment (i.e., before surgery), that tumour regression with neoadjuvant therapy may improve the likelihood of an R0 resection, and that the early introduction of systemic therapy allows simultaneous treatment of regional and distant disease. In

contrast, the rationale for postoperative therapy is that higher-risk patients will have already been identified by more accurate pathologic staging and lower-risk patients will be spared the risks for toxicity associated with preoperative treatment based on less accurate pre-treatment staging. In addition, because surgery is the most effective therapeutic modality, with initial surgical resection one would not be taking the risk of giving potentially ineffective therapy while delaying effective treatment.

Intraperitoneal chemotherapy

The rationale for the use of intraperitoneal chemotherapy after resection of primary gastric cancer is the high risk of peritoneal metastasis as an initial component of treatment failure. Autopsy series and second-look laparotomy series have reported that up to 50% of gastric cancer patients who undergo potentially curative resections have clinically evident peritoneal carcinomatosis as a site of failure.

Immuno-chemotherapy

Japanese and Korean investigators have performed a number of trials studying the use of immunochemotherapy as adjuvant treatment after curative resection of gastric cancer. Many of these trials involve using a protein-bound polysaccharide (PSK) alone or combined with chemotherapy after gastrectomy. PSK is a polysaccharide extracted from Coriolis vesicular, whose mechanism of action is not fully understood. The control arm in most of these studies, however, also received chemotherapy.

Nakazato et al. reported the results of a study involving patients who were randomly assigned to receive mitomycin plus FU (given by mouth) or the same chemotherapy plus PSK. The experimental arm received treatment with PSK for 36 months after surgery. As part of the eligibility process, patients had to have a positive purified protein derivative of tuberculin (PPD) test. Both groups received ten cycles of chemotherapy. With a minimum follow-up of 5 years, a significant survival advantage was seen for the PSK group. 70% of the PSK group versus 59% of the standard treatment group were alive and disease free at 5 years.

In other trials, Korean investigators have studied the use of chemotherapy plus immunostimulants after potentially curative resection. In one trial, chemotherapy with mitomycin, FU, and cytosine arabinoside plus OK432 (a Streptococcus pyogenes preparation) was given to 74 patients, whereas a control group of 64 patients underwent surgery alone. 265 Of the group receiving postoperative treatment, 44.6% were alive at 5 years, compared to 23.4% of those randomized to surgery only. In a follow-up three-arm trial, patients were randomized to receive immunotherapy with OK432 plus chemotherapy with mitomycin and FU. A second group received chemotherapy alone, whereas the third arm was a control arm of observation after surgery. At 5 years, 45.3% of the immunochemotherapy group were alive, compared to 29.8% of the chemotherapy group and 24.4% of the surgery group. Kim et al. performed a similar trial using FAM chemotherapy with or without OK432. Fifty patients received chemotherapy alone, and 49 patients received chemotherapy plus OK432. These authors reported a significant improvement in survival for chemotherapy plus immunotherapy versus chemotherapy alone .

In summary, data from Japanese and Korean investigators suggest that immunotherapy may improve outcome for patients undergoing potentially curative resection. The number of patients in any given trial is small, and it is unclear how these trials should be translated to Western patient populations.

COMBINATION CHEMOTHERAPEUTIC DRUGS

FAM and FAMTX

The FAM combination was widely used for metastatic gastric cancer in the 1980s but is rarely used in current clinical practice. Phase III trials failed to demonstrate a significant improvement in survival with FAM, and the response rates in those trails were substantially lower than the rates reported in the initial studies of FAM.

Substitution of FU by methotrexate and leucovorin led to the development of the FAMTX regimen. Phase II studies in patients with metastatic gastric cancer indicated high response rates to FAMTX, and phase III studies comparing FAMTX with etoposide, doxorubicin, and cisplatin (EAP) showed FAMTX to be better

tolerated. These led to a European Organization for Research and Treatment of Cancer study comparing FAMTX and the older FAM regimen. The response rate with FAMTX was superior to that with FAM. The 2-year overall survival rate for patients receiving FAMTX was 9%, compared with 0 for those receiving FAM; this was not better than the rate expected with the best supportive care. Subsequently, FAMTX was found to be inferior to cisplatin-containing combinations, and it is not widely used currently.

Cisplatin-Based Chemotherapy

Although many phase II studies of cisplatin-containing regimens have been performed, only a few RCTs have been reported. Several recent trials have compared cisplatin-based combinations to non-cisplatin-based combinations, allowing a fuller evaluation of these regimens.

The in vitro synergy between cisplatin and FU, the activity of cisplatin as a single agent in gastric cancer, and the potential advantage of cisplatin over older drugs such as mitomycin C led to the development of cisplatin-FU regimens. Response rates to many of these regimens are in the 30% to 50% range. For example, the

cumulative overall response rate for FU, doxorubicin, and cisplatin (FAP) is approximately 35%. As is the case for other combinations, only 3% to 5% of patients achieve complete clinical remissions. Cunningham et al. substituted epirubicin for doxorubicin in the ECF combination (epirubicin, cisplatin, and FU). In phase II trials of ECF, the overall response rate ranged from 37% to 71%.

These preliminary results led Waters et al. to perform a comparison of ECF to FAMTX. In a randomized MRC study, 126 patients received ECF and 130 patients received FAMTX. The overall response rate for ECF was significantly higher than that for FAMTX . Median survival was also longer for ECF (8.7 vs. 6.1 months). In an update by Waters et al., the 2-year overall survival rate was 14% for patients receiving ECF versus 5% for those receiving FAMTX.

Ross et al. performed a two-arm randomized trial comparing ECF (289 patients; reference arm) to mitomycin, cisplatin, and protracted infusional FU (MCF; 285 patients). The trial allowed entrance of patients who had esophageal or gastric cancers, and the overwhelming majority of patients had adenocarcinoma. Patients

with locally advanced disease were also allowed to take part; only a small percentage of these patients subsequently underwent operation.

No significant difference was seen in response (42% for ECF vs. 44% for MCF), median survival (9.4 months vs. 8.7 months), or 1- and 2-year overall survival rates. The analysis included a quality-of-life assessment in which ECF was generally superior to MCF. The authors concluded that ECF should continue to be one of the reference treatments for advanced esophagogastric cancer.

Cisplatin-Etoposide Variants

Because etoposide and cisplatin may be synergistic, these drugs have been combined in the treatment of many tumors. Several phase II studies in patients with metastatic gastric cancer demonstrated that the combination is well tolerated. Preusser et al. then added doxorubicin to create the EAP regimen. Phase II trials of EAP reported high response rates. In subsequent phase III studies, although response rates of approximately 50% were reported, toxicity was high, with treatment-related mortality ranging between 10% and 14%. In a randomized comparison of

EAP to FAMTX, Kelsen et al. showed similar response rates for the two regimens but significantly less toxicity with FAMTX.

Wilke et al. developed etoposide, leucovorin, and 5-FU (ELF). Because of the toxicity of EAP, particularly in older patients, phase II study data indicated a substantial response rate, approximately 50%, and a median duration of response of 9 to 10 months. ELF was subsequently studied in phase III trials .

Cisplatin-Fluorouracil

The combination of 5FU and Cisplatin are extensively studied in gastric cancer. Phase II studies very good response with minimal toxicity. This regimen has been considered by some U.S. investigators to be a reference regimen and typically consists of 75 to 100 mg/m^2 cisplatin and 750 to 1000 mg/m^2 FU given as a 4 or 5 day infusion.

Two phase III randomized studies compared cisplatin-FU to other regimens. Vanhoefer et al. compared ELF, cisplatin-FU, and FAMTX in 399 patients with advanced gastric cancer. No significant differences were seen in response rates among the patients with measurable disease (9% for ELF, 20% for cisplatin-

FU, and 12% for FAMTX). Also, no differences were found in median survival, which ranged from 6.7 to 7.2 months.

Ohtsu et al. compared cisplatin-FU to FU alone and to uracil and tegafur (UFT) plus mitomycin C in 280 Japanese patients with unresectable advanced gastric cancer. The UFT-mitomycin arm was inferior and was closed after an interim analysis. The overall response rate was higher for cisplatin-FU (34%) than for FU alone (11%) or for UFT-mitomycin (9%), as was the progression-free-survival rate. However, there was no difference in overall survival.

Docetaxel-Containing Therapy

Phase II trial results for taxane-containing, irinotecan-containing, and oxaliplatin-containing regimens are being reported, but there are no reports of completed phase III trials involving these regimens. Interim results of one randomized study comparing a docetaxel, cisplatin, and FU regimen to a cisplatin and FU regimen have been reported in abstract form. In this study, Ajani et al. compared 75 mg/m^2 docetaxel with 75 mg/m^2 cisplatin on day one plus 750 mg/m^2/d FU by continuous intravenous infusion over 5 days every 3 weeks with 100 mg/m^2 cisplatin followed by a 5-day

infusion of 1000 mg/m^2 FU every 4 weeks. The result of this study showed good response with increase in median survival rate. At the time of the interim analysis, 111 patients receiving the three-drug regimen and 112 receiving the two-drug regimen had been analyzed. A significantly higher time to tumor progression was reported for the docetaxel, cisplatin, and FU arm. The response and survival rates also appear to be higher in this arm. But the end result of this was not reported.

Another area of investigation is the use of irinotecan-containing regimens. Preliminary results of a randomized phase II trial comparing cisplatin and irinotecan to irinotecan, FU, and leucovorin have been reported. Both of these combinations were found in earlier studies to have substantial response rates. In this study, the two arms (approximately 70 patients in each) were well balanced for major prognostic variables. Toxicity was slightly greater in the cisplatin-irinotecan arm, and the overall response rate was higher and median survival longer for patients receiving irinotecan, FU, and leucovorin. Therefore, the three-drug arm was chosen for a definitive phase III trial comparing it to cisplatin-FU.

This study has completed the accrual, and no data are available at this time.

In addition to new classes of cytotoxic agents, there is also interest in the use of oral fluorinated pyrimidines. These are FU prodrugs that offer the ease of oral administration and mimic the benefits of long-term infusional therapy. Different mechanisms of action offer another advantage. Three fluorinated pyrimidines have been studied in gastric cancer as single agents: UFT, S1, and capecitabine. In most studies, the overall response rate for all three drugs is approximately 20% to 30%, similar to the rate reported in the past for FU. Whether the oral fluoropyrimidines are equivalent to infusional FU in gastric cancer is not yet clear.

TARGETED THERAPY

Anti Her-2 therapy has been found to increase the survival of patients who express Her-2 receptor in carcinoma stomach. Two groups of drugs that act against EGFR and HER-2 are increasingly used to prolong the survival in gastric cancer.

1. Monoclonal antibodies, 2. Tyrosine kinase inhibitors

Monoclonal antibodies: It acts by three mechanism

1. Blocks the intracellular cascade by inhibiting ligand – receptor binding.
2. Decreasing the expression of receptors on cell surface by downregulation of receptors and endocytosis.
3. Activation of immune system and complement by inducing Antibody dependent cell cytotoxicity (ADCC).

Drugs commoly used are Cetuximab, Panitumumab, Matuzumab, Nimotuzumab, Trastuzumab. All these drugs are administered by intravenous infusion.

Tyrosine kinase inhibitors:

It acts by preventing the binding of ATP with tyrosine kinase domain and inhibits the autophosphorylation of EGFR and HER-2.

Drugs used in trial are Erlotinib, Geftinib, Lapatinib.

Cetuximab

Cetuximab has been tried as monotherapy in both oesophageal and gastric cancer. But the response rate was found to only 20 to 30 percent. Cetuximab on combination with other chemotherapeutic regimes showed response rate of 50 to 60 percent with median survival of 10 months. Three trials were conducted with combination of cetuximab. FOLFOX, ECF, Irinotecan and cisplatin were combined with cetuximab. Response rate of 50 percent was achieved only with ECF and FOLFOX combination with median survival of 6 months.

In advanced gastric cancer, it has been tried as a chemosensitivity restoring agent, but the response was poor. Cetuximab has also been tried in patients with metastatic colorectal cancer. The main toxicity profile was skin rash and diarrhoea.

Panitumumab

Panitumumab is a humanized IgG2 monoclonal antibody used in combination with Oxaliplatin and Capecitabine. The response

rate was found to be inferior when compared with cetuximab. Diarrhoea is the predominant side effect.

Matuzumab

Matuzumab is a humanized IgG1monoclonal antibody directed against EGFR which acts by ADCC. Results of various trials showed Matuzumab had showed lower response rate and poor survival both as monotherapy and combination therapy. As a result it is not recommended for treatment of advanced gastric cancer.

Nimotuzumab

Nimotuzumab is a humanized IgG1 monoclonal antibody used both as monotherapy and combination with Irinotecan. The median survival with combination therapy was lower on compared with Irinotecan alone.

Trastuzumab

Trastuzumab is a humanized IgG1 monoclonal antibody. It is used as combination therapy with either cisplatin or cisplatin and

docetaxel. Tumours which expressed Her-2 positivity either by using IHC or FISH were enrolled for this combination therapy.

Preliminary trials were satisfactory with good subjective response and minimal side effects which paved way for ToGA trial. 3665 patients with GEJC and gastric cancer who tested Her-2 positivity were randomly assigned into two groups. The control group received only chemotherapy with cisplatin, 5 FU, capecitabine and the study group received chemotherapy with above drugs and trastuzumab.

The response rate and median survival was significantly high in patients receiving trastuzumab with chemotherapy but side effect profile diarrhoea was found to be higher in trastuzumab group. As a result of these studies trastuzumab was approved by FDA for treating Her-2 positive advanced gastric cancer.

Lapatinib

Results of ToGA trial led to the development of another anti Her-2 agent Lapatinib. It induces G1 cell cycle arrest through AKT and MAPK pathways. The overall survival was poor when

Lapatinib was used as a monotherapy. The response rate was 25 percent when it was combined with Capecitabine. The role of Lapatinib in Her-2 negative cases is also being studied. TYTAN trial compares the efficacy of Paclitaxel with and without Lapatinib. LOGiC trial compares Capecitabine and Oxaliplatin with and without Lapatinib in Her-2 positive cases. Results of both these studies are awaited.

The HercepTest identifies Her-2 overexpression in patients with both carcinoma stomach and carcinoma breast. As a result of this, it was approved by FDA for treatment with Trastuzumab.

Her-2 overexpression has been designed into four levels:0,1,2,3. Trastuzumab do not show any response in patients with level 0 and 1. Level 2 expression has some benefit. Patients who express level 3 shows significant responseby increase in median survival and overall response rate.

Trastuzumab when combined with Doxorubicin was associated with increased of heart failure. Fatal infusion reactions and pulmonary fibrosis were also described with trastuzumab. The

infusion reaction occurred within 24 hours of infusion. Trastuzumab infusion should be stopped for patients with dyspnoea or clinically significant hypotension. Patients should be monitored until signs and symptoms completely resolve. Trastuzumab is contraindicated in those patients manifesting as anaphylaxis, diarrhoea, angioneurotic oedema, interstitial lung disease and acute respiratory distress syndrome.

The standard initial dose of Herceptin is 8 mg/kg as a 90-min intravenous infusion which is subsequently followed by doses of 6mg/kg as an intravenous infusion over next 30 to 90 mins and repeated every 3 weeks until disease progression.

Predicting Response to treatment

The development of techniques that will allow physicians to prospectively choose chemotherapeutic agents that are most likely to work in an individual patient is a high priority. This is particularly important because currently available cytotoxic chemotherapy for gastric cancer has only modest to moderate effectiveness, with objective regressions in 25% to 40% of all

patients treated, and toxicities can be substantial. In vitro assays of live tumor cells have not proved to have adequate sensitivity; however, several studies have suggested that molecular analysis of tumor tissue might provide a more accurate predictor of outcome. The hypothesis is that levels of expression of molecular targets or of molecules associated with the mechanism of action of an individual agent are associated with response or resistance.

Preliminary studies of several new techniques for molecular analysis have been performed in gastric cancer. These studies were generally retrospective evaluations of prospectively accrued data and tissue. For example, the majority of correlative studies have involved collecting tissue before therapy, treating a group of patients with the same treatment (e.g., cisplatin-FU chemotherapy), and then correlating the molecular analysis findings with clinical outcome. In some cases, tissue has been collected at the time of definitive surgery after initial chemotherapy and these studies are more difficult to evaluate because the correlative analysis is performed after treatment.

The molecular analysis approaches used to date include evaluation of single genes or small numbers of genes by immunohistochemistry or by the use of reverse transcriptase polymerase chain reaction. Gene expression profiling involving hundreds or thousands of genes has also been studied. These techniques are being explored extensively for cancers in general, but data on their use in gastric cancer are limited.

Most of the data currently available involve small groups of patients treated with preoperative chemotherapy in whom molecular analysis of pre-treatment biopsy specimens and post treatment surgically resected tissue is performed. In older trials from the University of Southern California, Metzer et al. and Lenz et al. reported on an analysis of a subgroup of patients who received neoadjuvant cisplatin-FU chemotherapy, followed by resection and intraperitoneal floxuridine.

Response and survival were correlated with molecular markers assessed by reverse transcriptase polymerase chain reaction for several genes of interest, primarily thymidilate synthase (TS) and ERCC1 (excision repair cross complementing

gene), a putative marker of cisplatin sensitivity. Patients with low levels of relative expression of these two genes had a significantly longer median survival and higher long-term survival rate than did patients with high levels of expression.

Drug resistance

Resistance to chemotherapy has been reported by many investigators to be associated with mutations of the p53 oncogene. Several studies of this association have been performed in gastric cancer. Cascinu et al. performed immunohistochemical analysis of pre-treatment endoscopic biopsies from 30 patients with locally advanced but not metastatic gastric cancer.

Assessing response to treatment in such patients can be difficult, but 10 of 12 responding patients had p53-negative tumors, whereas patients overall had high levels of p53 expression. A definitive study in which adequate numbers of patients with advanced gastric cancer receive the same chemotherapy irrespective of their molecular marker profile and are followed prospectively has not yet been performed.

Histopathologic Assessment of Response

The use of preoperative systemic therapy has led to an interest in evaluating histologic changes as a surrogate marker of efficacy. Histologic assessment of response typically includes identification of residual cancer cells and determination of the extent of fibrosis. In one study, a tumor regression scale of grades 1 to 5 was used, with 1 denoting a complete pathologic regression and 5 meaning no evidence of chemotherapy effect.

Regression was defined by the replacement of cancer with fibrous tissue and scattered inflammatory cells. Percent histologic response was determined, ranging from no evidence of treatment effect (0%) to a complete response with no viable tumor identified (100%). Complete or near complete responses were associated with improved long-term survival rates.

The use of immunohistochemistry in assessing treatment response has also been evaluated. In one study, p53, Ki-67, and epidermal growth factor receptor expression were the most reliable tissue markers of response. Initial evaluations of the induction of apoptosis by chemotherapy in gastric cancer have used the TUNEL

assay. Satomi et al. correlated traditional assessment of histologic response and TUNEL assay findings. However, the use of histology as a surrogate for therapeutic effect is still an experimental approach.

Positron Emission Tomography and Treatment Response

Another potential role for FDG-PET is the evaluation of treatment response. A growing body of evidence suggests that FDG-PET can be used to identify response to therapy early in treatment. This approach has been studied in several tumours, including non-Hodgkin's lymphoma, breast cancer, and tumours of the gastroesophageal junction. In these studies, PET was used to assess response to chemotherapy or to chemo radiation, and results were correlated with histology, traditional imaging modalities, and survival.

The positive and negative predictive values for FDG-PET for histologic response were 77% and 86%, respectively. The 2-year overall survival rates of PET responders versus PET nonresponders were 89% and 26%. Many studies concluded that a

significant decrease in FDG-PET SUV identified at day 14 is associated with histologic response and with survival.

FISH ASSAY:

Apart from IHC, FISH assay is also used for standardization of Trastuzumab therapy. It allows direct visualisation of the gene as it contains direct labelled DNA probe that binds to Her-2 gene. FISH assay approved by FDA quantifies Her-2 amplification. The HER2/neu is also over-expressed in other tumours like ovarian, bladder, pancreatic, salivary gland, endometrial and non-small-cell lung cancer.

METHODS AND MATERIALS

Thirty gastric adenocarcinoma patients paraffin block was obtained from Department of Pathology, Madurai Medical College. It consists of both surgical and biopsy block. All patients had undergone supra and infra diaphragmatic imaging studies with chest X-ray, Ultrasound abdomen, CT abdomen and chest. TNM staging had bee n performed on all patients according to American Joint Committee on Cancer. Ten patients undergone surgery followed by chemotherapy and remaining twenty patients were receiving palliative chemotherapy.

Representative blocks were chosen for immunostaining. IHC was performed with Hercep test kit. Tumour with more than ten percent of cancer cells showing membranous staining for Her-2 were classified as positive.

RESULTS AND OBSERVATIONS

TABLE – 1: AGE DISTRIBUTION

AGE(in yrs)	Her-2/ neu status		Total
	Positive	Negative	
20-40	2	3	5
41-60	4	14	18
61-80	1	6	7
Total	7	23	30

Out of 30 patients, Her 2 positivity reported was 7. Among seven patients, maximum positivity was seen in age group between 41 to 60, this is because maximum number of cases were reported in these age groups. The P value was 0.695 which was not statistically significant on comparing with other age groups.

TABLE 2: SEX DISTRIBUTION

SEX	Her-2/ neu status		Total
	Positive	Negative	
Male	5	17	22
Female	2	6	8
Total	7	23	30

Of the 30 patients, male to female ratio was 2.75.this is because cancer stomach is more common in males on compared to females Among 22 males, 5 showed positivity and among 8 females, 2 showed positivity, the ratio was 2.5. The P value was 1.000 which was not statistically significant. Also on comparing males and females individually with positive and negative results to their total, P value obtained was 0.063 (males) and 0.464 (females) which was not significant.

TABLE 3: TUMOUR GRADING

GRADING (differentiation)	Her-2/neu status		Total
	Positive	Negative	
Poor	2	8	10
Moderate	4	7	11
Well	1	8	9
Total	7	23	30

Out of 30 patients, all three types of grade were almost equally present. Among 7 positive patients, maximum cases belong to moderate differentiation, on comparing with other two, the P value was 0.494 which was not statistically significant.

TABLE 4: TUMOUR (T) - STAGING

T stage	Her-2/neu status		Total
	Positive	Negative	
T1,2,3	1	8	9
T4	6	15	21
Total	7	23	30

Out of 30 patients, 21 patients belonged to T4. This shows that maximum number of patients were diagnosed in advanced stage. Her2 positivity was also maximum in T4. On comparing with T1,2,3 the P value was 0.393 which was not statistically significant. Also on comparing the positive and negative results of T4 the P value was 0.177 which was not statistically significant.

TABLE 5: NODE (N) - STAGING

N stage	Her-2/neu status		Total
	Positive	Negative	
N0	3	7	10
N1	4	9	13
N2	0	6	6
N3	0	1	1
Total	7	23	30

Among 30 patients, 23 patients belong to N0 and N1. All the 7 patients tested positive comes among the above 23. None of patients from N2 and N3 tested positive. The P value was 2.724 which was not statistically significant.

TABLE 6: METASTASIS (M) - STAGING

M stage	Her-2/neu status		Total
	Positive	Negative	
M0	3	16	19
M1	4	7	11
Total	7	23	30

Out of 30 patients, 11 patients had distant metastasis, Among 11 patients, 4 showed Her2 positivity. But among 19 patients who had no metastasis, 3 showed positivity. This shows that positivity was equally distributed among patients irrespective of metastasis. The statistical P value was 0.372 which was not significant.

TABLE 7: TNM STAGING

STAGING	Her-2/neu status		Total
	Positive	Negative	
Stage 1	1	1	2
Stage 2	0	9	9
Stage 3	2	6	8
Stage 4	4	7	11
Total	7	23	30

Although the number of patients in stage 2,3,4 were almost equally distributed , maximum positivity was present in stage 4. Two patients were positive among 8 belonging to stage 3. The statistical P value was 0.163 which was not significant.

TABLE 8 : HISTOLOGY

HISTOLOGY	Her-2/neu status		Total
	Positive	Negative	
Intestinal	7	22	29
Diffuse	0	1	1
Total	7	23	30

Among 30 patients, 29 patients belong to intestinal sub-type. All 7 positive patients were from above 29. This shows that Her2 positivity was maximum from intestinal sub-type of gastric cancer, but the statistically P value was not significant. This is because the number of patients belonging to diffuse type was less.

TABLE 9 – HER-2 POSITIVITY

Study	No.of cases	Positive Percentage
Yano et al	200	23%
Gravalos et al	166	13%
Lordick et al	1527	22%
ToGA trial	3665	22%
Our study (GRH, Madurai)	30	23%

On comparing with various studies conducted all over the world, our study (GRH, MADURAI) showed SIGNIFICANT positivity. Among 30 patients studied, 7 patients tested positive for Her 2 by IHC. The percentage positivity was 23.3.

TABLE 10: PROGNOSIS

Prognosis	No.of cases	Death
Positive	7	7
Negative	23	5

Among 7 patients who tested positive died within one year of follow-up inspite of effective chemotherapy and surgical measures. This indicates that Her2 positive patients has poor prognosis inspite of effective treatment. Among 23 patients who were negative, 5 patients died during follow-up for one year, it indicates that staging is also important in prognostic significance. The statistical P value was 0.047 which was SIGNIFICANT.

DISCUSSION

Tumours of the upper gastrointestinal tract have been reported to show a wide range of overexpression of HER-2 protein. In our study we investigated the expression of this receptor protein in 30 patients in GRH MADURAI retrospectively. Our observed prevalence of HER2 protein overexpression by IHC was 23% which fits the range previously reported (8.2%- 27.5%).

However, at the upper range there are some limitations inherent in IHC technique, such as those caused by variability in fixation, and different standardization techniques and scoring of the staining. The results of ToGA trial analysed by FISH technique showed HER-2 was found to be amplified in about 18% of cases. Our percentage can be modified as well if we employ FISH method alternative to IHC on the same samples. This is designed as the future direction of this project.

The results of ToGA trial showed that the intestinal type of carcinoma stomach shows higher prevalence of HER2 amplification in contrast to diffuse type. But our results failed to

confirm that HER2 Protein overexpression is strongly associated with intestinal subtype than diffuse subtype. However, so far none of the studies evaluated the clinical importance of this feature.

HER2 gene amplification has been associated with the degree of differentiation of adenocarcinomas, in that well differentiated adenocarcinomas have shown a very high incidence of HER2 amplification. In our study, moderately differentiated tumours were more likely to overexpress HER-2 protein. However, not all studies agree, and a positive relation with poor tumour differentiation has been reported as well.

Further in our study, HER2 positivity does not correlate with the AGE, SEX and TNM staging which was already proven in various studies. Relating to prognosis, none of the seven HER2 POSITIVE patients survived beyond one year of follow-up of study, which showed HER2 overexpression as a poor prognostic factor. On the other hand five patients with HER--2 NEGATIVITY also died during follow-up, which showed surgical stage is by itself another proven prognostic factor status . The absence of correlation between HER-2 expression status and staging might be

due to the fact that HER-2 positive tumours have a more aggressive pathologic behaviour, which is reflected in more common micrometastatic disease at presentation and more distant failures later after treatment.

Additionally, it should be noted that there are inconsistencies in the literature regarding the clinical significance of HER2 overexpression which may be due to problems of selecting the best methodology, standardizing techniques, and determination of appropriate cut off points.

CONCLUSION

Gastric cancer is one of the leading causes of cancer mortality in INDIA. This is due to the fact that most of the patients were diagnosed at end stage where only palliation is possible. In patients where surgical resection is not possible, systemic chemotherapy is the main treatment option.

Although many chemotherapeutic regimes have been extensively studied both as monotherapy and combination therapy active in metastatic diseases, there is no internationally accepted standard of care and survival remains poor. In order to increase the life span newer therapeutic strategies are needed. There is increasing evidence that HER2 overexpression in patients with gastric cancer is correlated with poor outcomes and more aggressive disease.

Various trials showed TRASTUZUMAB inhibits the growth of human gastric cancer in patients with HER2 overexpression both in vitro and vivo. These clinical trials paved way for starting ToGA trial which was studied in gastric cancer patients conducted

in various countries all over the world. The results of these studies will contribute to a better knowledge of the efficacy and treatment of TRASTUZUMB- based therapy in HER2 POSITIVE gastric cancer in the future.

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PROFORMA

NAME :

AGE/SEX :

OCCUPATION :

ADDRESS :

GENERAL EXAMINATION :

Anaemia, Jaundice

Lymph nodes

Pedal oedema

SYSTEM EXAMINATION :

CVS :

RS : E/O Pleural effusion

ABDOMEN: E/O Hepatic secondaries, ascitis,

epigastric mass

CNS :

OTHER SYSTEMS : E/O Cutaneous metastasis, acanthosis nigricans.

INVESTIGATIONS :

X-RAY CHEST

ULTRASOUND ABDOMEN AND PELVIS

CT ABDOMEN AND THORAX

ENDOSCOPY

PARAFFIN BIOPSY BLOCK

STAGING: TNM

ABBREVIATIONS

Her 2	-	Human epidermal growth factor receptor 2
ERBB 2	-	Erythroblastic leukemia viral oncogene homolog 2
H.pylori	-	Helicobacter pylori
IARC	-	International Agency for Research on cancer
ACS	-	American cancer society
MSI	-	Microsatellite instability
ToGA	-	Trastuzumab for gastric cancer
FISH	-	Fluorescent in-situ Hybridisation
TKI’s	-	Tyrosine kinase inhibitors
MAbs	-	Monoclonal antibodies
CRC	-	Colorectal cancer
ASCO	-	American society of clinical oncology
EMA	-	European medicines agency
ADCC	-	Antibody dependent cell cytotoxicity

MASTER CHART

S.No.	NAME	AGE	SEX	TUMOUR TYPE	INVASION	NODAL STATUS	META STASIS	STAGING	T	N	M	HER 2 NEU	GRADING
1	Sathan	50	M	INTESTINAL	MUSCLE	NO	NO	STAGE 1B	T2	N0	M0	3+	MOD DIFF
2	KALYANI	40	F	INTESTINAL	SEROSA	COELIAC, PARA AORTIC	LIVER	STAGE 4	T4a	N1	M1	3+	WELL DIFF
3	ABBAS	53	M	INTESTINAL	SEROSA	PERIGASTRIC	NO	STAGE 3A	T4a	N1	M0	3+	MOD DIFF
4	NALLIAH	63	M	INTESTINAL	SEROSA	NO	LIVER	STAGE 4	T4a	N0	M1	3+	MOD DIFF
5	SELVI	35	F	INTESTINAL	SEROSA	10 PERIGASTRIC	NO	STAGE 3A	T4a	N1	M0	3+	POORLY DIFF
6	AYEPILLAI	50	M	INTESTINAL	SEROSA	HEPATODUODENAL	LIVER	STAGE 4	T4b	N1	M1	3+	MOD DIFF
7	SOMASUNDARAM	51	M	INTESTINAL	SEROSA	NO	PLEURAL EFFUSION	STAGE 4	T4b	N0	M1	3+	POORLY DIFF
8	KANDASAMY	76	M	INTESTINAL	SEROSA	COELIAC	NO	STAGE 3A	T4a	N1	M0	2+	MOD DIFF
9	MUTHURAJ	74	M	INTESTINAL	SEROSA	COELIAC	NO	STAGE 3A	T4a	N1	M0	2+	MOD DIFF
10	BALAMMAL	50	F	INTESTINAL	MUSCLE	3/6 NODES	NO	STAGE 2B	T2	N2	MO	2+	WELL DIFF
11	ALAGAR	45	M	INTESTINAL	SEROSA	NO	PERITONEAL	STAGE 4	T4a	NO	M1	2+	MOD DIFF

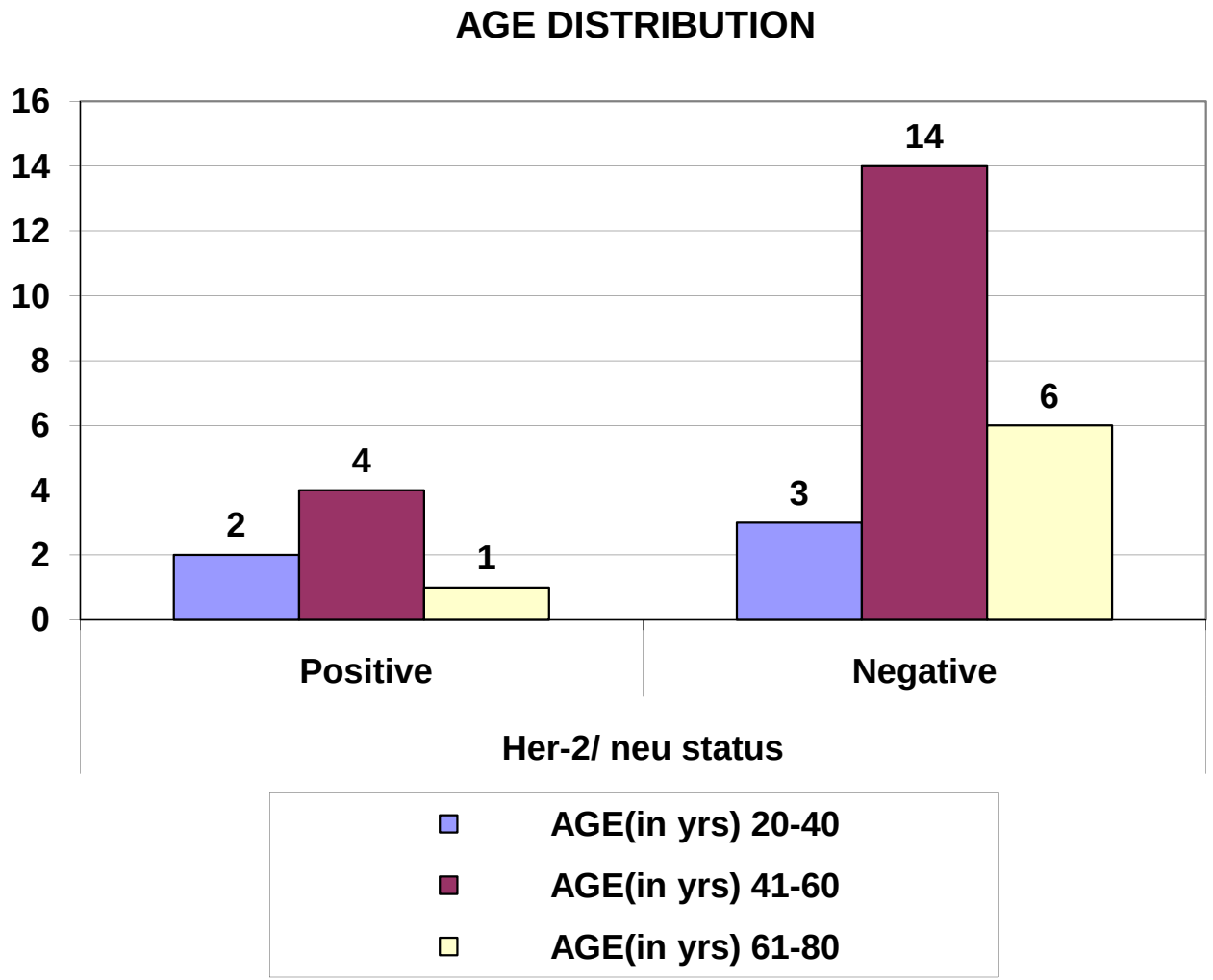
12	MUNIANDI	63	M	INTESTINAL	SEROSA	3/13 NODES	LIVER	STAGE 4	T4a	N2	M1	2+	MOD DIFF
13	KARUPIYA	50	M	INTESTINAL	SEROSA	3/7 NODES	NO	STAGE 3B	T4a	N2	M0	2+	POORLY DIFF
14	PERUMAL	50	M	INTESTINAL	SEROSA	NO	NO	STAGE 2B	T4a	NO	M0	2+	WELL DIFF
15	MACHAKALAI	52	M	INTESTINAL	SEROSA	COELIAC, PERIGASTRIC	NO	STAGE 3A	T4a	N1	M0	2+	WELL DIFF
16	ANTHONY	61	M	INTESTINAL	SEROSA	2/4 NODES	NO	STAGE 2A	T2	N1	M0	2+	POORLY DIFF
17	MUTHIAH	41	M	INTESTINAL	MUSCLE	COELIAC, PERI PANCREATIC	NO	STAGE 2A	T2	N1	M0	2+	POORLY DIFF
18	MUTHUMANI	67	M	INTESTINAL	SEROSA	PORTAL	LIVER	STAGE 4	T4a	N1	M1	2+	WELL DIFF
19	PANCHAVARNAM	45	F	INTESTINAL	MUSCLE	COELIAC, PERIGASTRIC	LIVER	STAGE 4	T2	N2	MI	1+	POORLY DIFF
20	VALLIAMMAL	66	F	INTESTINAL	SEROSA	NO	NO	STAGE 2B	T4a	N0	M0	1+	POORLY DIFF
21	POONGODI	48	F	INTESTINAL	MUSCLE	NO	NO	STAGE 1B	T2	N0	M0	1+	MOD DIFF
22	MURUGESAN	38	M	INTESTINAL	SEROSA	OMENTAL	NO	STAGE 3A	T4a	N1	M0	1+	MOD DIFF
23	PONNIAH	60	M	INTESTINAL	SEROSA	3/7 NODES	LIVER	STAGE 4	T4a	N2	M1	1+	WELL DIFF
24	THARMAR	27	M	DIFFUSE	SEROSA	NO	NO	STAGE 2B	T4a	N0	M0	1+	POORLY DIFF
25	SARKARAI ABDUL	30	M	INTESTINAL	MUSCLE	6/7 NODES	NO	STAGE 2B	T2	N2	M0	1+	WELL DIFF
26	MARIAPPAN	45	M	INTESTINAL	SEROSA	COELIAC	ASCITIS	STAGE 4	T4a	N1	M1	0	POORLY DIFF

27	JANAKI	42	F	INTESTINAL	SEROSA	7/8 NODES	NO	STAGE 3C	T4a	N3	M0	0	WELL DIFF
28	GURUVAMMAL	42	F	INTESTINAL	SEROSA	NO	NO	STAGE 2B	T4a	N0	M0	0	WELL DIFF
29	RAMASAMY	60	M	INTESTINAL	MUSCLE	PERIGASTRIC	ASCITIS	STAGE 4	T2	N1	M1	0	MOD DIFF
30	KARUPIAH	60	M	INTESTINAL	SEROSA	NO	NO	STAGE 2B	T2	N0	M0	0	POORLY DIFF

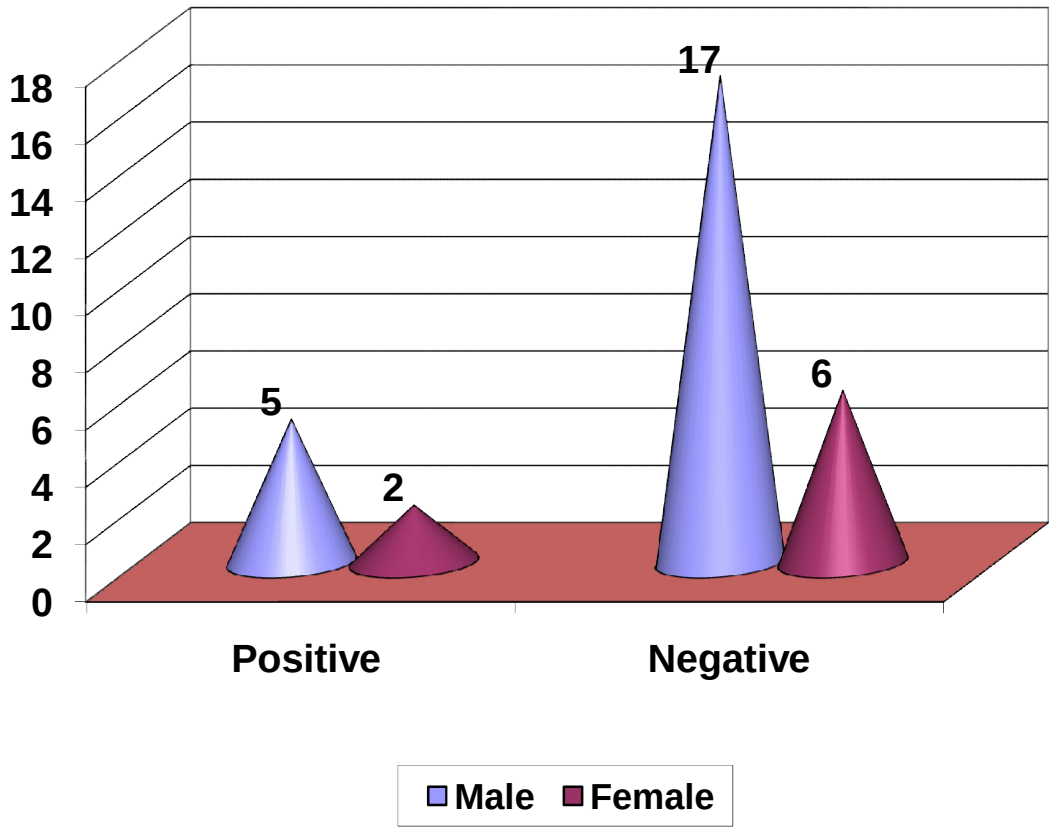
MASTER CHART

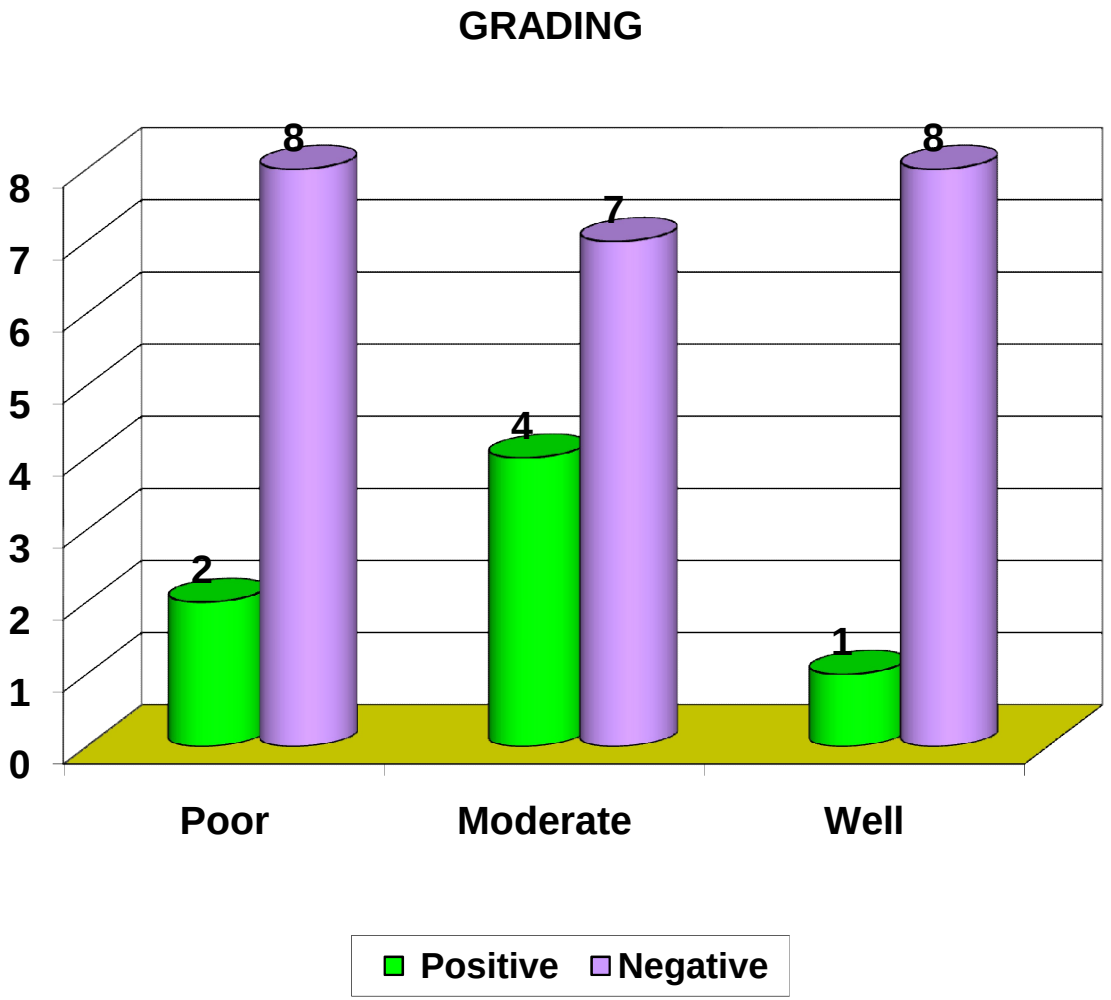
S.No.	NAME	AGE	SEX	TUMOUR TYPE	INVASION	NODAL STATUS	META STASIS	STAGING	T	N	M	HER 2 NEU	GRADING
1	Sathan	50	M	INTESTINAL	MUSCLE	NO	NO	STAGE 1B	T2	N0	M0	3+	MOD DIFF
2	KALYANI	40	F	INTESTINAL	SEROSA	COELIAC, PARA AORTIC	LIVER	STAGE 4	T4a	N1	M1	3+	WELL DIFF
3	ABBAS	53	M	INTESTINAL	SEROSA	PERIGASTRIC	NO	STAGE 3A	T4a	N1	M0	3+	MOD DIFF
4	NALLIAH	63	M	INTESTINAL	SEROSA	NO	LIVER	STAGE 4	T4a	N0	M1	3+	MOD DIFF
5	SELVI	35	F	INTESTINAL	SEROSA	10 PERIGASTRIC	NO	STAGE 3A	T4a	N1	M0	3+	POORLY DIFF
6	AYEPILLAI	50	M	INTESTINAL	SEROSA	HEPATODUODENAL	LIVER	STAGE 4	T4b	N1	M1	3+	MOD DIFF
7	SOMASUNDARAM	51	M	INTESTINAL	SEROSA	NO	PLEURAL EFFUSION	STAGE 4	T4b	N0	M1	3+	POORLY DIFF
8	KANDASAMY	76	M	INTESTINAL	SEROSA	COELIAC	NO	STAGE 3A	T4a	N1	M0	2+	MOD DIFF
9	MUTHURAJ	74	M	INTESTINAL	SEROSA	COELIAC	NO	STAGE 3A	T4a	N1	M0	2+	MOD DIFF
10	BALAMMAL	50	F	INTESTINAL	MUSCLE	3/6 NODES	NO	STAGE 2B	T2	N2	MO	2+	WELL DIFF
11	ALAGAR	45	M	INTESTINAL	SEROSA	NO	PERITONEAL	STAGE 4	T4a	NO	M1	2+	MOD DIFF
12	MUNIANDI	63	M	INTESTINAL	SEROSA	3/13 NODES	LIVER	STAGE 4	T4a	N2	M1	2+	MOD DIFF
13	KARUPIYA	50	M	INTESTINAL	SEROSA	3/7 NODES	NO	STAGE 3B	T4a	N2	M0	2+	POORLY DIFF
14	PERUMAL	50	M	INTESTINAL	SEROSA	NO	NO	STAGE 2B	T4a	NO	M0	2+	WELL DIFF
15	MACHAKALAI	52	M	INTESTINAL	SEROSA	COELIAC, PERIGASTRIC	NO	STAGE 3A	T4a	N1	M0	2+	WELL DIFF
16	ANTHONY	61	M	INTESTINAL	SEROSA	2/4 NODES	NO	STAGE 2A	T2	N1	M0	2+	POORLY DIFF
17	MUTHIAH	41	M	INTESTINAL	MUSCLE	COELIAC, PERI PANCREATIC	NO	STAGE 2A	T2	N1	M0	2+	POORLY DIFF
18	MUTHUMANI	67	M	INTESTINAL	SEROSA	PORTAL	LIVER	STAGE 4	T4a	N1	M1	2+	WELL DIFF
19	PANCHAVARNAM	45	F	INTESTINAL	MUSCLE	COELIAC, PERIGASTRIC	LIVER	STAGE 4	T2	N2	MI	1+	POORLY DIFF
20	VALLIAMMAL	66	F	INTESTINAL	SEROSA	NO	NO	STAGE 2B	T4a	N0	M0	1+	POORLY DIFF

21	POONGODI	48	F	INTESTINAL	MUSCLE	NO	NO	STAGE 1B	T2	N0	M0	1+	MOD DIFF
22	MURUGESAN	38	M	INTESTINAL	SEROSA	OMENTAL	NO	STAGE 3A	T4a	N1	M0	1+	MOD DIFF
23	PONNIAH	60	M	INTESTINAL	SEROSA	3/7 NODES	LIVER	STAGE 4	T4a	N2	M1	1+	WELL DIFF
24	THARMAR	27	M	DIFFUSE	SEROSA	NO	NO	STAGE 2B	T4a	N0	M0	1+	POORLY DIFF
25	SARKARAI ABDUL	30	M	INTESTINAL	MUSCLE	6/7 NODES	NO	STAGE 2B	T2	N2	M0	1+	WELL DIFF
26	MARIAPPAN	45	M	INTESTINAL	SEROSA	COELIAC	ASCITIS	STAGE 4	T4a	N1	M1	0	POORLY DIFF
27	JANAKI	42	F	INTESTINAL	SEROSA	7/8 NODES	NO	STAGE 3C	T4a	N3	M0	0	WELL DIFF
28	GURUVAMMAL	42	F	INTESTINAL	SEROSA	NO	NO	STAGE 2B	T4a	N0	M0	0	WELL DIFF
29	RAMASAMY	60	M	INTESTINAL	MUSCLE	PERIGASTRIC	ASCITIS	STAGE 4	T2	N1	M1	0	MOD DIFF
30	KARUPIAH	60	M	INTESTINAL	SEROSA	NO	NO	STAGE 2B	T2	N0	M0	0	POORLY DIFF

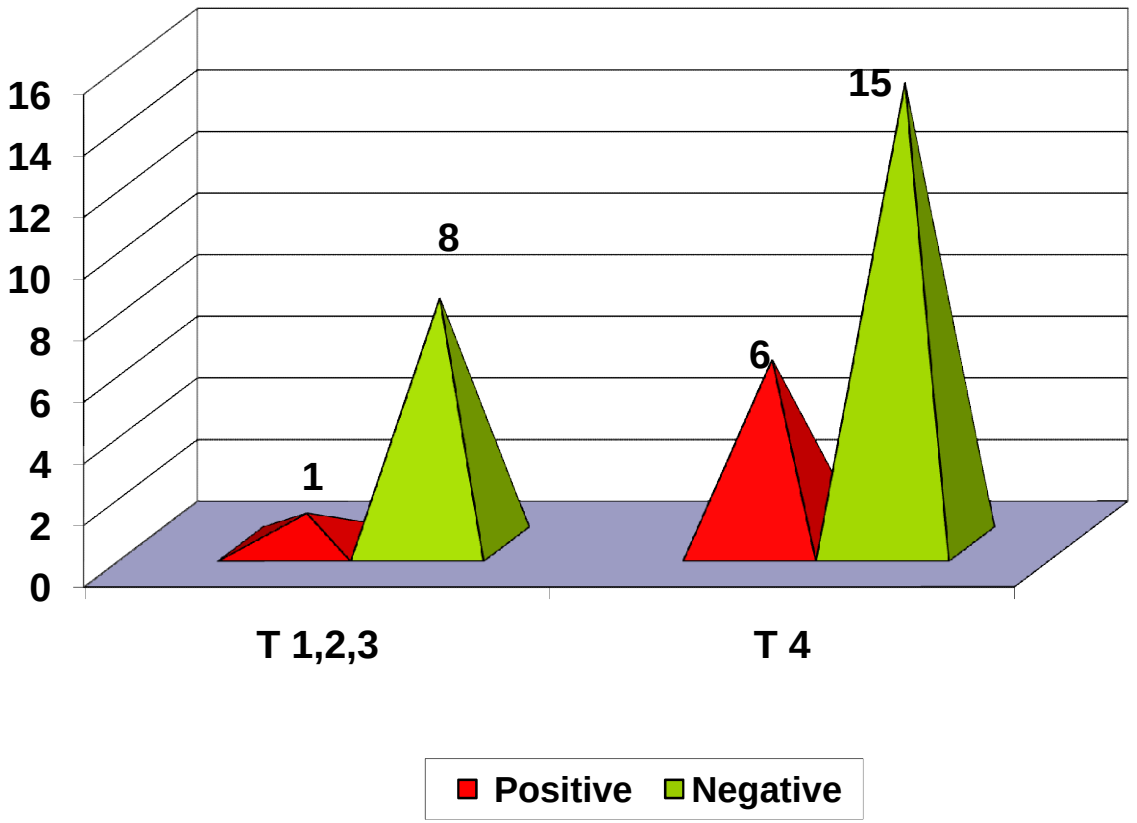

AGE DISTRIBUTION
16
14
12
10
8
6
4
2
0
2
4
1
3
14
6
Positive
Negative
Her-2/ neu status
AGE(in yrs) 20-40
AGE(in yrs) 41-60
AGE(in yrs) 61-80

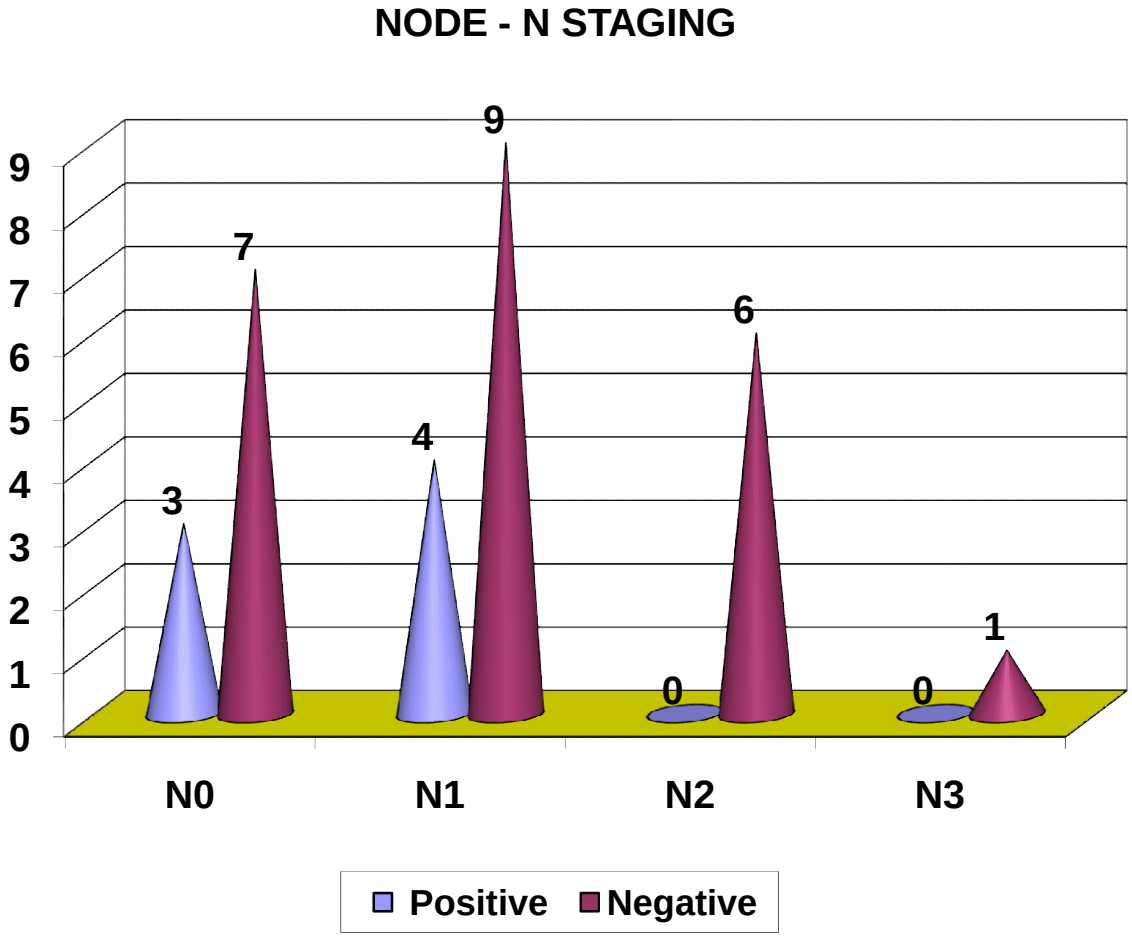
SEX DISTRIBUTION

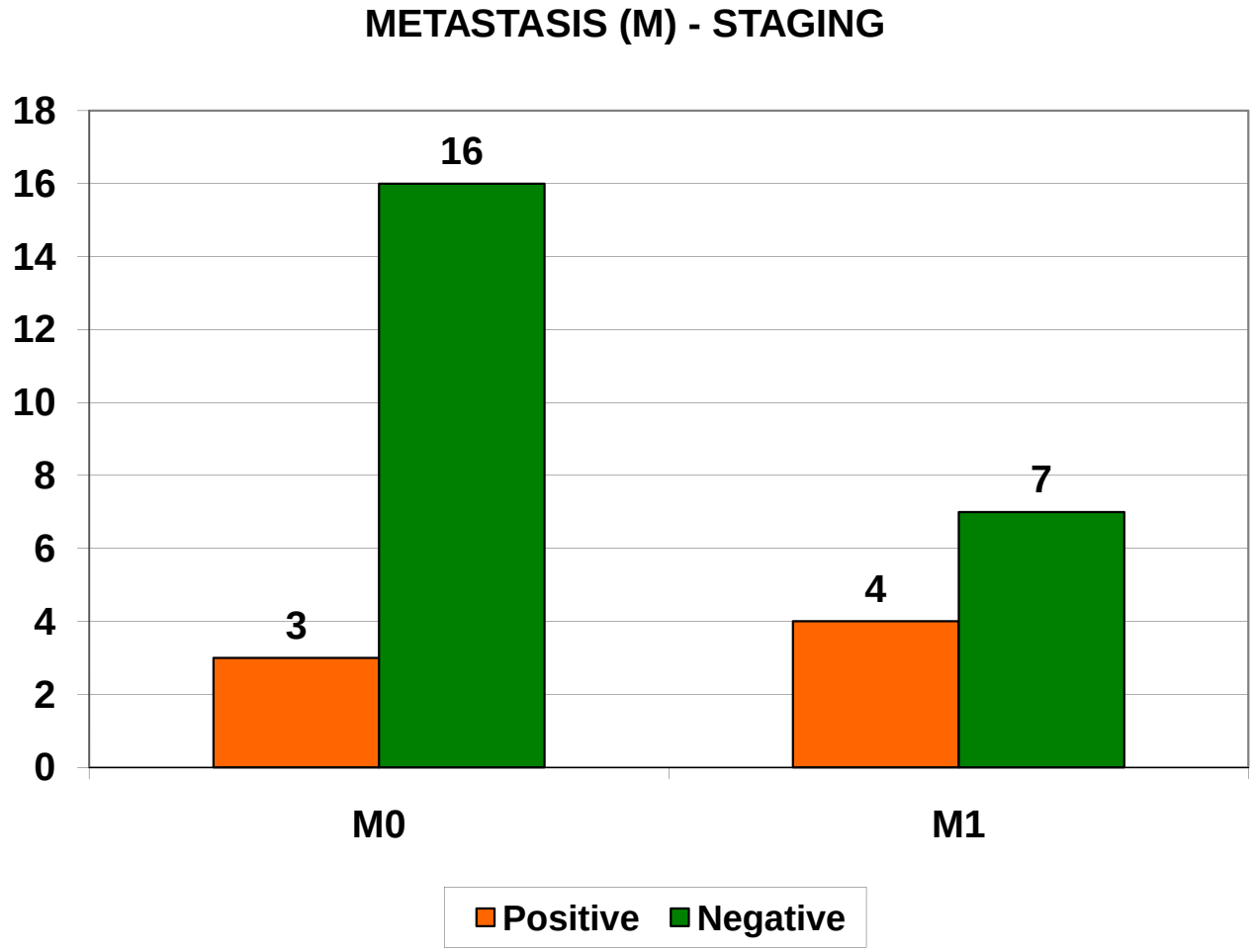



GRADING
8
7
6
5
4
3
2
1
0
2
8
4
7
1
8
Poor
Moderate
Well
Positive
Negative

TUMOUR T STAGING




NODE - N STAGING
9
8
7
6
5
4
3
2
1
0
3
7
4
9
0
6
0
1
N0
N1
N2
N3
Positive
Negative


METASTASIS (M) - STAGING
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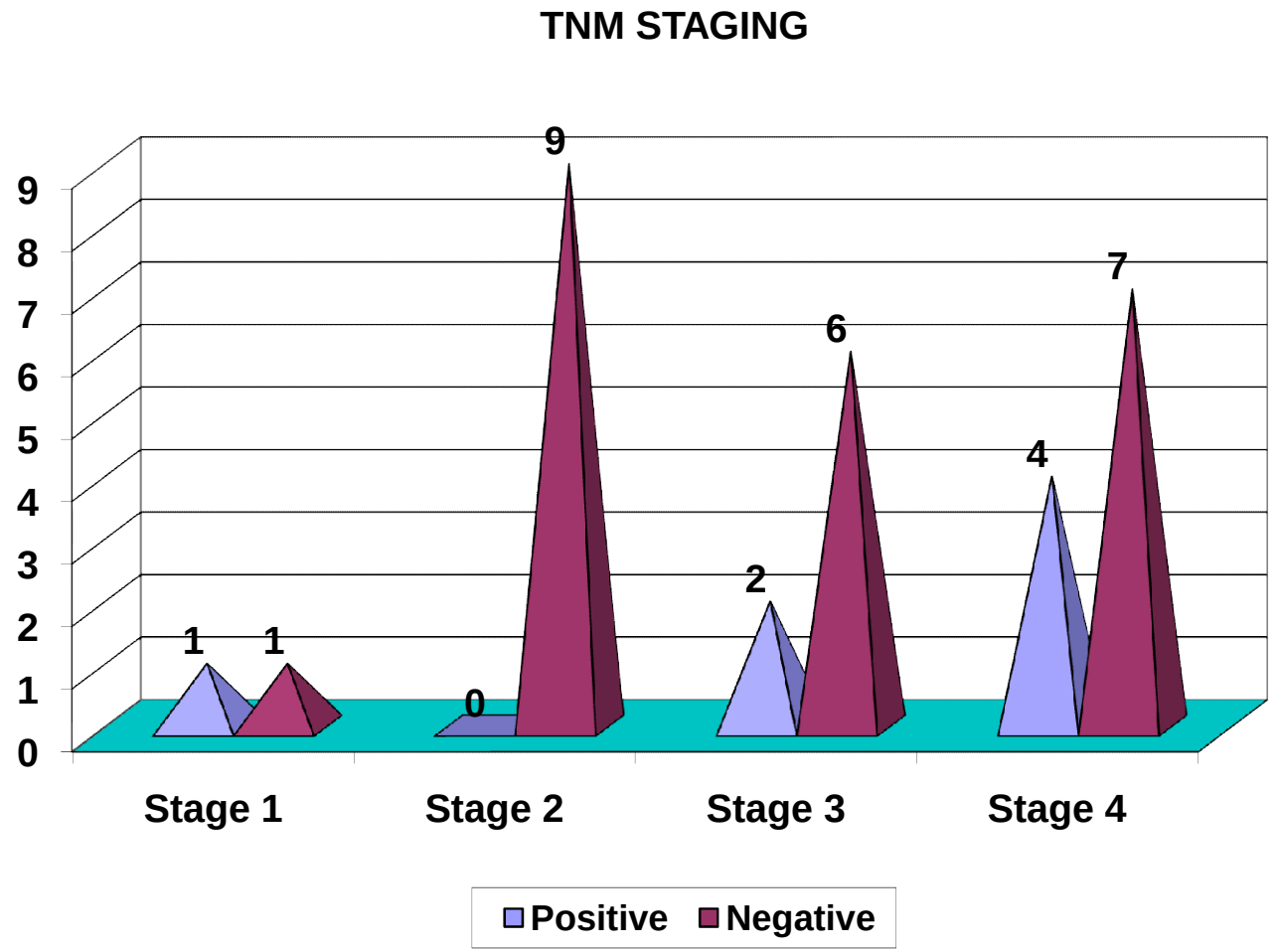
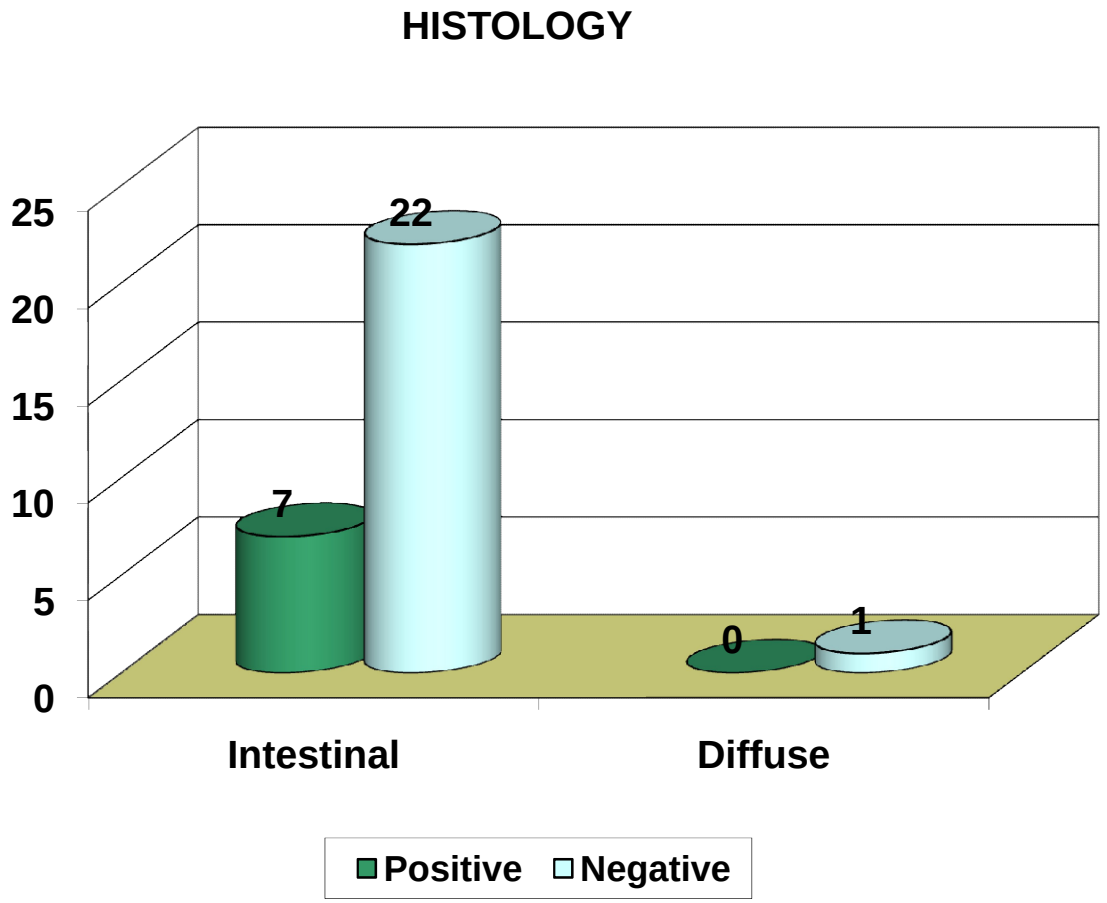
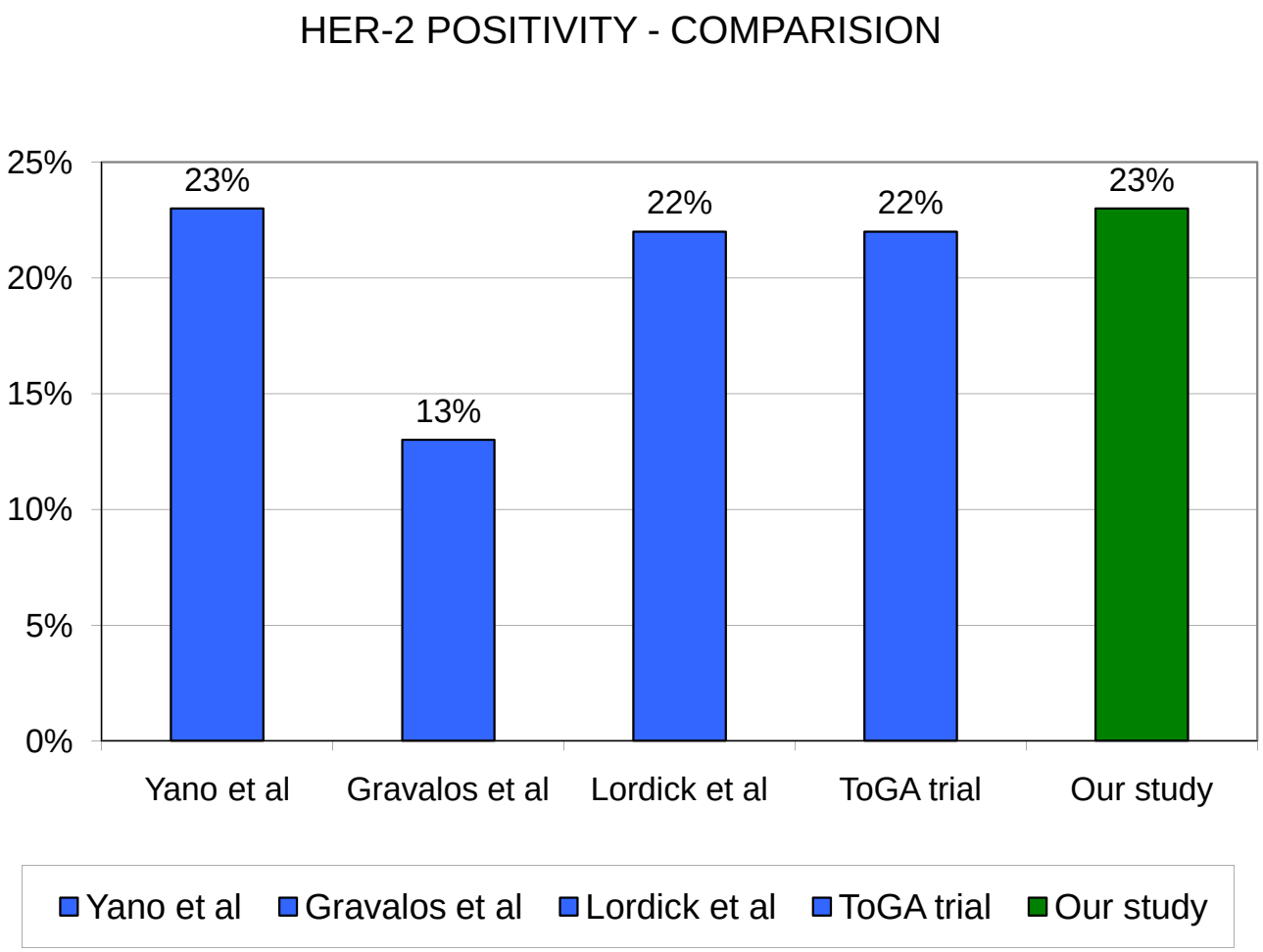
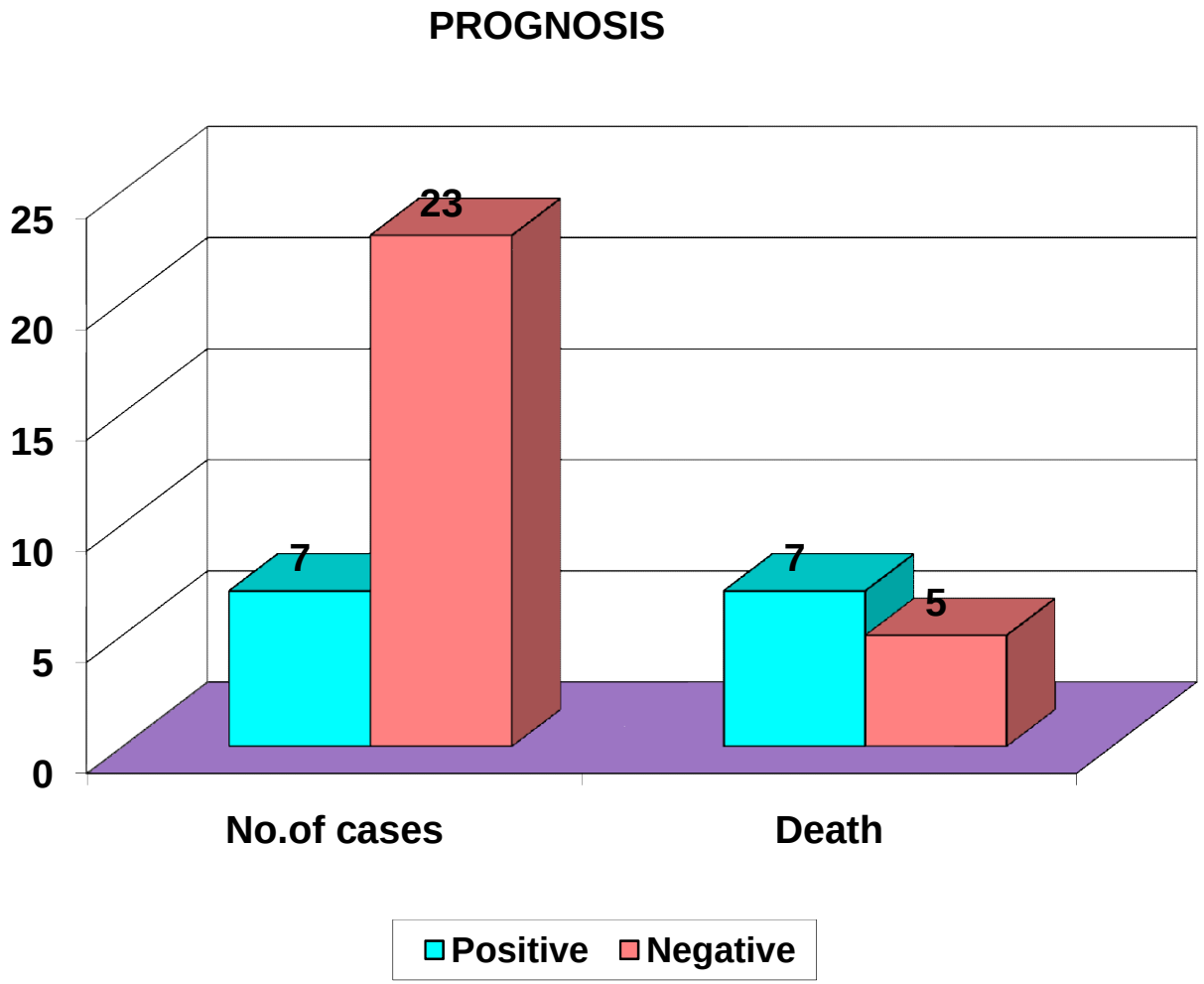
14
12
10
8
6
4
2
0
16
3
7
4
M0
M1
Positive
Negative


TNM STAGING
9
8
7
6
5
4
3
2
1
0
1
1
0
9
2
6
4
7
Stage 1
Stage 2
Stage 3
Stage 4
Positive
Negative


HISTOLOGY
25
20
15
10
5
0
22
7
0
1
Intestinal
Diffuse
Positive
Negative


HER-2 POSITIVITY - COMPARISION
25%
20%
15%
10%
5%
0%
23%
13%
22%
22%
23%
Yano et al
Gravalos et al
Lordick et al
ToGA trial
Our study
Yano et al
Gravalos et al
Lordick et al
ToGA trial
Our study


PROGNOSIS
25
20
15
10
5
0
23
7
7
5
No.of cases
Death
Positive
Negative

AGE(in yrs)	Her-2/ neu status	
	Positive	Negative
20-40	2	3
41-60	4	14
61-80	1	6

	Her-2/ neu status	
	Positive	Negative
Male	5	17
Female	2	6

GRADING (differentiation)		
	Positive	Negative
Poor	2	8
Moderate	4	7
Well	1	8

	Her-2/neu status	
	Positive	Negative
T 1,2,3	1	8
T 4	6	15

	Her-2/neu status	
	Positive	Negative
N0	3	7
N1	4	9
N2	0	6
N3	0	1

M stage	Her-2/neu status	
	Positive	Negativ
M0	3	16
M1	4	7

STAGING	Her-2/neu status	
	Positive	Negative
Stage 1	1	1
Stage 2	0	9
Stage 3	2	6
Stage 4	4	7

HISTOLOGY		Her-2/neu status	
		Positive	
Intestinal	7	22	
Diffuse	0	1	

Study	No.of cases	Positive Percentage	Study	Positive Percentage
Yano et al	200	23%	Yano et al	23%
Gravalos et al	166	13%	Gravalos et al	13%
Lordick et al	1527	22%	Lordick et al	22%
ToGA trial	3665	22%	ToGA trial	22%
Our study (GRH, Madurai)	30	23%	Our study	23%

Prognosis	No.of cases	Death
Positive	7	7
Negative	23	5

/e		Positive	Negative
	M0	3	16
	M1	4	7

Negative		Positive	Negative
	Intestinal	7	22
	Diffuse	0	1

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ANALYSIS IN CARCINOMA STOMACH Her-2/neu POSITIVITY BY IMMUNO-HISTOCHEMISTRY DISSERTATION SUBMITTED FOR DOCTOR OF MEDICINE BRANCH - I (GENERAL MEDICINE) APRIL 2013 THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY CHENNAI BONAFIDE CERTIFICATE This is to certify that the dissertation entitled “ANALYSIS IN CARCINOMA STOMACH Her-2/neu POSITIVITY BY IMMUNO- HISTOCHEMISTRY” submitted by Dr.V.MARIAPPAN to the Tamil Nadu Dr. M.G.R.Medical University, Chennai in partial fulfillment of the requirement for the award of M.D Degree Branch I (General Medicine) is a bonafide research work was carried out by him under my direct supervision & guidance. Prof.Dr.J. Sangumani M.D., Unit Chief, Department of Medicine,...

TURNITIN CERTIFICATE