A Dissertation on

EPIDERMAL GROWTH FACTOR RECEPTOR STATUS IN GASTRIC MALIGNANCIES

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CHENNAI - 600 010.

MARCH 2007

CERTIFICATE

This is to certify that **Dr. S. SELVA SEETHA RAMAN,** a bonafide MS General Surgery Post Graduate Student, from Government Royapettah Hospital, Kilpauk Medical College, Chennai – 600 010 has submitted the dissertation on **EPIDERMAL GROWTH FACTOR RECEPTOR (EGRF) STATUS IN GASTRIC MALIGNANCIES** in partial fulfillment of the requirements for M.S. General Surgery (Branch – I) Degree Examination of **THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY, GUINDY, CHENNAI,** to be held in MARCH 2007.

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PREFACE

In this era of technical advancement both in medical and surgical fields, there are still cancers for which cure cannot be established and only supportive care or symptomatic management is possible. Advanced cancers of stomach are apt instances for this. Though surgery and chemotherapy are complimentary to each other in treatment, the acquaintance of knowledge about these malignancies in the cellular level paves way for new molecular targets.

A plethora of recently acquired information about specific molecular abnormalities that "drive" the malignant phenotype, together with profound advances in biotechnology, has resulted in the beginning of an era of abundant novel therapeutic options to treat patients with a variety of malignant diseases.

Hence future research directions should focus on tailoring therapy to specific patient populations, such as those with genetic mutations on receptors, for optimal therapeutic effect.

Hence this study focuses on the possible role of EGFR in Indian patients with gastric malignancy.

INTRODUCTION

Cells are constantly exposed to a variety of external stimuli, ranging from soluble endocrine and paracrine factors to signaling molecules on neighboring cells. Thus, it is extremely important that the cell correctly interprets these extracellular signals to create an appropriate developmental or proliferative response.

Beatson's original observation on breast cancer regression after ovariectomy published in 1896 provided the first insight into the hormone dependent nature of the tumours. In the 1950s Ludwig Gross first showed the clear evidence of tumor specific immunogenicity with sarcoma arising after exposure to methylcholanthrene. In 1989 Salmon et al discovered the expression of erb-b2 in breast and ovarian cancer. Since then, the discovery of the various receptors and development of corresponding antagonists has improved the survival rate of many lethal cancers.

The identification of parameters that reflect biological behavior of individual cancer tissues correlating with tumor aggressiveness is a key determinant of prognosis and a fundamental issue for the improvement of cancer therapy. Despite recent progress in defining the molecular mechanisms of cancer development and tumor progression, only a few individual biomarkers providing prognostic information and a therapeutic potential have been identified. Among them, the EGFR pathways attracted the most attention of cancer investigators.[54].

The epidermal growth factor receptor (EGFR) was the first identified growth factor receptor and is implicated in the widest number of human cancers.

In this study we have attempted to identify the mutant EGFR in gastric cancer patients using Immunohistochemistry methods.

PURPOSE OF STUDY

Worldwide, Gastric Cancer is the fourth most common cancer and the second leading cause of cancer death (1, 2). In India, Gastric cancer is the most common cancer and the most common cause of cancer death. Worldwide esophageal cancer ranks fifth in the mortality rate among tumour sites (3). In fact, gastric and esophageal cancers together accounted for nearly 1.3 million new cases and 980,000 deaths worldwide in 2000-more than lung, breast, or colorectal cancer [4]. For gastric cancer, the 5-year overall survival rate remains poor, even in comparison with the dismal survival rates from the 1970s [5].

The underlying reasons for this disappointingly low survival rate are multifold:

(a) Ineffective screening tools and guidelines;

(b) Cancer detection at an advanced stage, with over 50% of patients with unresectable disease or distant metastasis at presentation

(c) High risk for recurrent disease after gastrectomy or definitive chemotherapy [6]

(d) Unreliable noninvasive tools to measure complete response to Chemotherapy [7,8]; and

(e) Limited survival achieved with palliative chemotherapy alone for patients with metastatic or unresectable disease.

Over the past decade, the field of drug development has been transformed with the identification of and ability to direct treatment at specific molecular targets. The EGFR monoclonal antibodies has already been shown to have promising results in metastatic breast cancer, head and neck cancer and in lung cancer [31-33].The results are encouraging from the colorectal and head and neck cancer trials for antibodies against EGFR. Active clinical research in esophageal cancer patients towards antibody inhibition of EGFR is in their phase I trials.

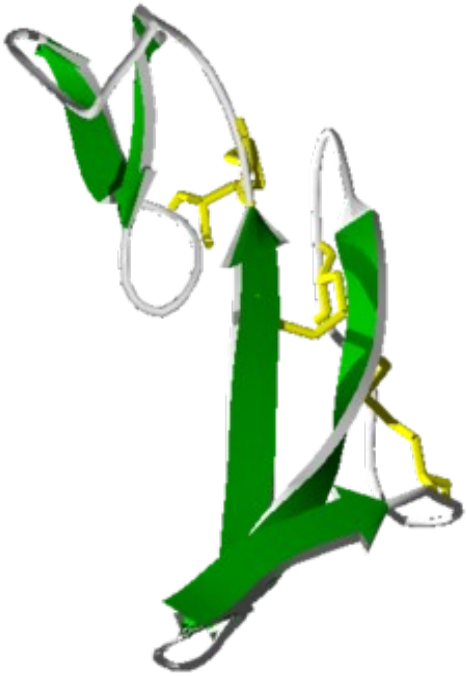
This study focuses on the identification of EGFR status in gastric cancers in Indian population.

EPIDERMAL GROWTH FACTOR

Epidermal growth factor or EGF is a growth factor that plays an important role in the regulation of cell growth, proliferation and differentiation. Human EGF is a 6045 Da protein with 53 amino acid residues and three intramolecular disulfide bonds[22].

EGF acts by binding with high affinity to epidermal growth factor receptor (EGFR) on the cell surface and stimulating the intrinsic protein-tyrosine kinase activity of the receptor. The tyrosine kinase activity in turn initiates a signal transduction cascade which results in a variety of biochemical changes within the cell - a rise in intracellular calcium levels, increased glycolysis and protein synthesis, and expression of certain genes including the gene for EGFR , that ultimately lead to DNA synthesis and cell proliferation. The active EGFR is responsible for activating other proteins through phosphorylation, with two principal and divergent pathways: the PI3 kinase pathway and the MAP kinase pathway. Several further downstream proteins are then activated along these two pathways. Additionally, other signaling pathways can modulate proteins downstream of EGFR, such as cyclooxygenase-2 (COX-2) and RAS proteins.

EPIDERMAL GROWTH FACTOR

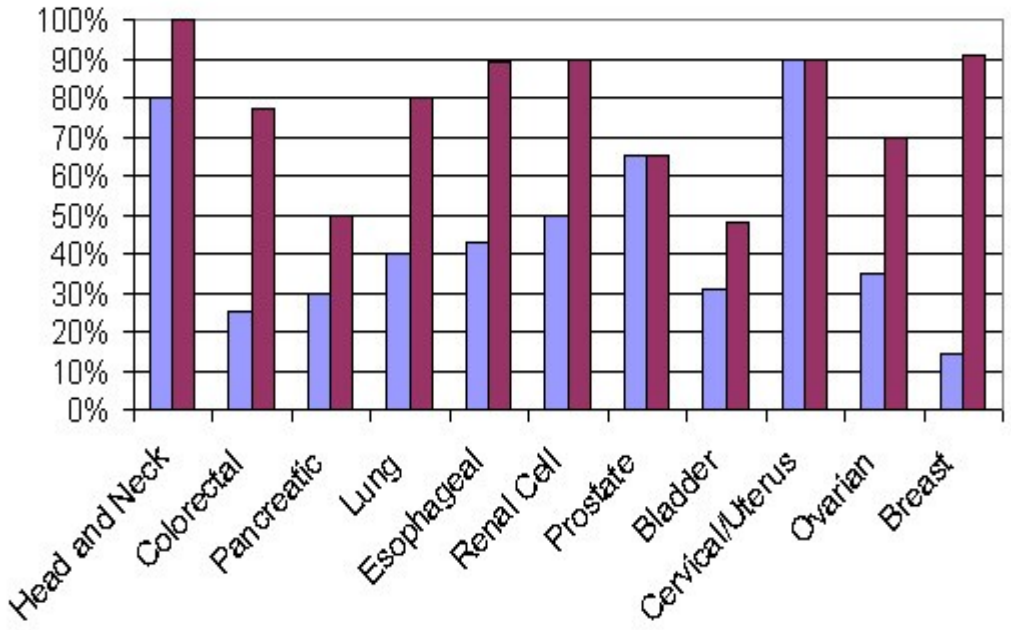


Three dimensional structure of EGF.

EPIDERMAL GROWTH FACTOR RECEPTOR

EGFR (ERBB1) is a member of the ERBB receptor tyrosine kinase family that includes ERBB2 (HER-2), ERBB3, and ERBB4 [9]. It consists of an extracellular ligand-binding domain, a transmembrane region that anchors the receptor to the plasma membrane, and a cytoplasmic region containing a tyrosine kinase domain. The known natural ligands of EGFR include epithelial growth factor (EGF) and transforming growth factor alpha (TGF), which both activate the receptor by binding to the extracellular domain and inducing the formation of receptor homodimers or heterodimers, which is followed rapidly by activation of the receptors' intrinsic tyrosine kinase. In this signal network, ERBB2 is the major partner of EGFR because activated heterodimer complexes containing ERBB2 are more stable at the cell surface than complexes containing other EGFR family members [10,11].

Ligand stimulation of EGFR initiates one of the most important cellular growth-regulatory pathways. As a trans-membrane glycoprotein, the extracellular domain of the EGFR is a ligand-binding site for TGF-alpha and EGF ligand cellular mechanisms that regulate cell growth [12].



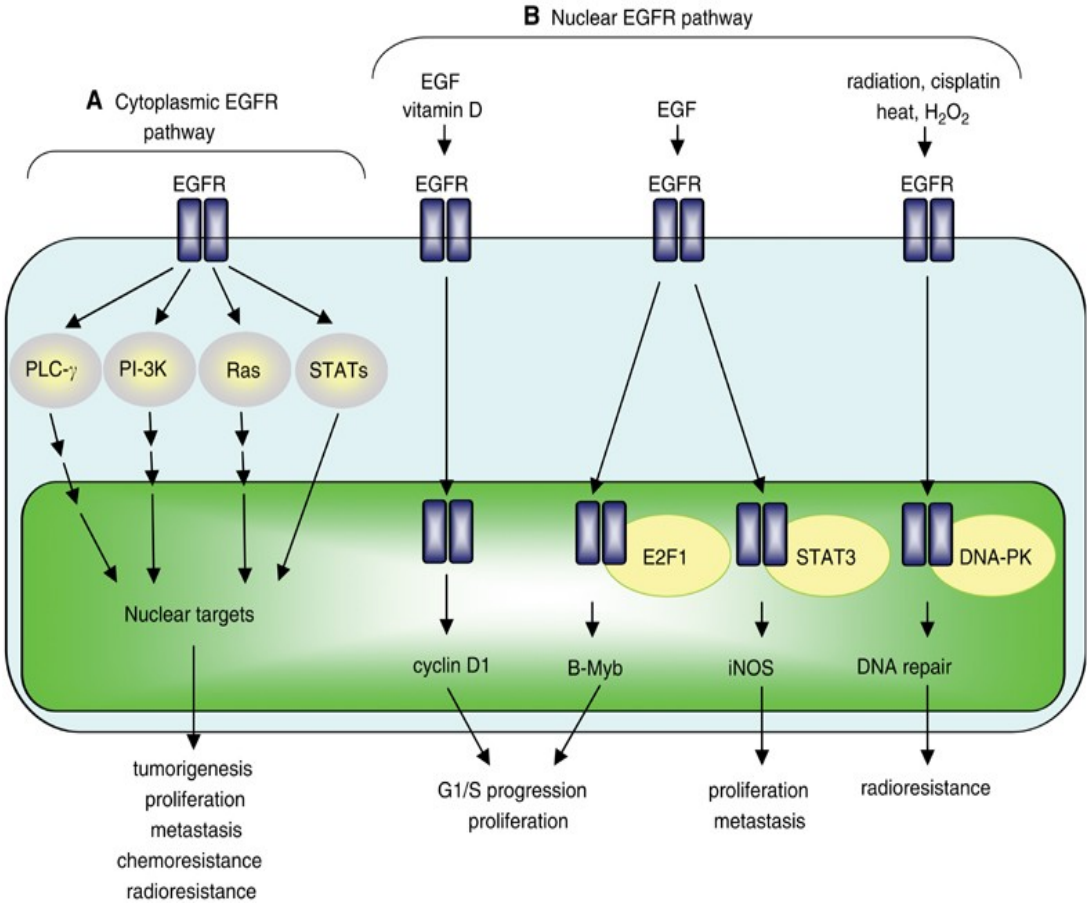
Various studies done on the EGFR status in different organs has showed encouraging results. This bar diagram depicts the outcome of various studies. Not many studies are available in gastric cancer.

Graph showing the higher and lower percentages of EGFR in different tumor types.

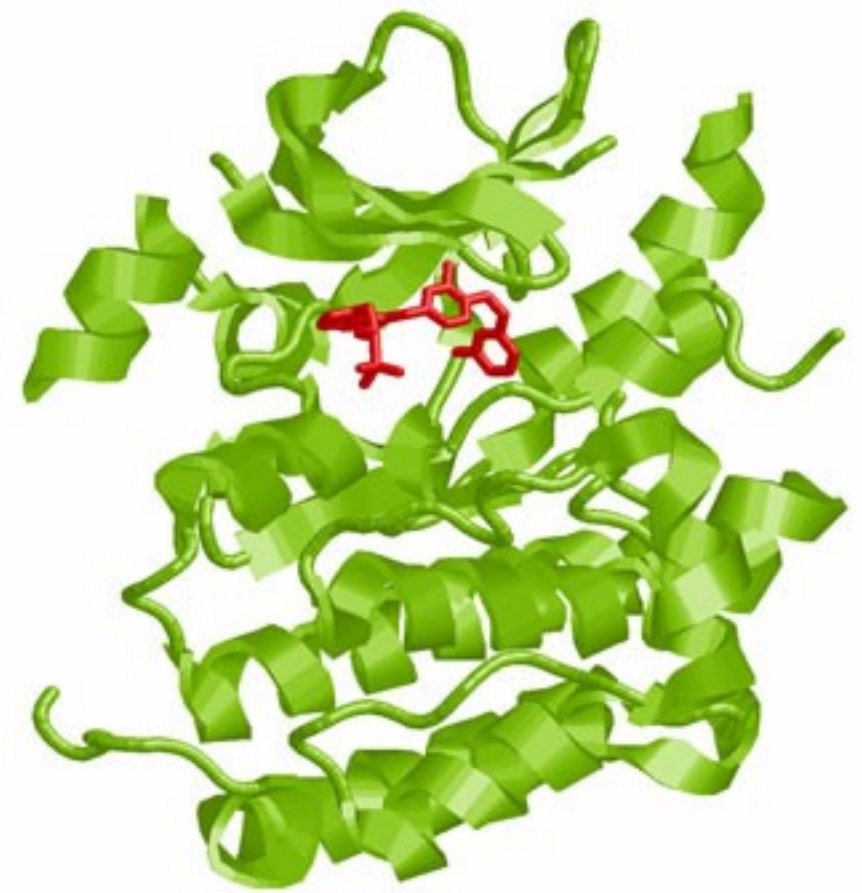
The EGFR is constitutively expressed in many normal epithelial tissues, including the skin and hair follicle. Overexpression of EGFR, as wild type or with mutations,

occurs in many types of human tumors, including esophageal (92%), head and neck (90%), colorectal (72%), prostate (65%), bladder (65%), ovarian (60%), cervical (60%), pancreatic (89%), renal cell (50%), and lung (50%) cancers [13-15]. Expression of EGFR correlates with poor prognosis and advanced disease. The EGFR signal transduction network plays an important role in various tumorigenic processes, including cell-cycle progression, angiogenesis, and metastasis, as well as protection from apoptosis [9,16].

EGFR SIGNALLING CASCADE

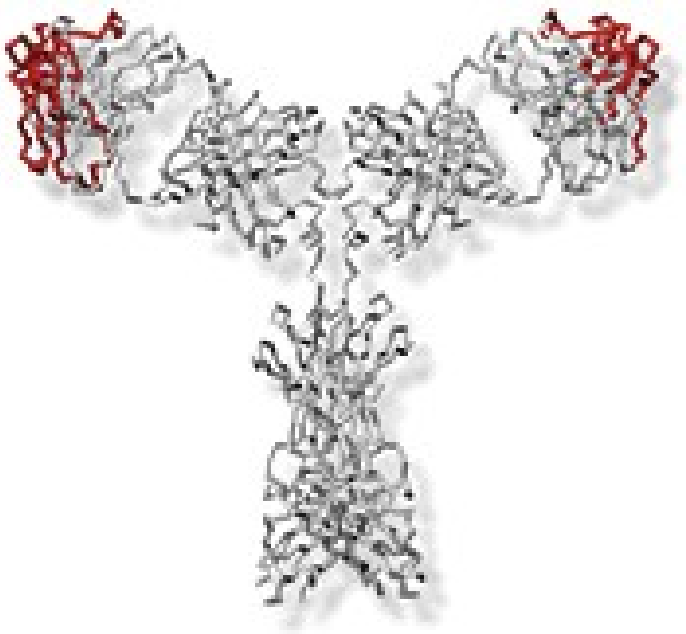


EGFR-MOLECULAR STRUCTURE



EGFR ANTIBODY (CETUXIMAB)

MOLECULAR STRUCTURE:

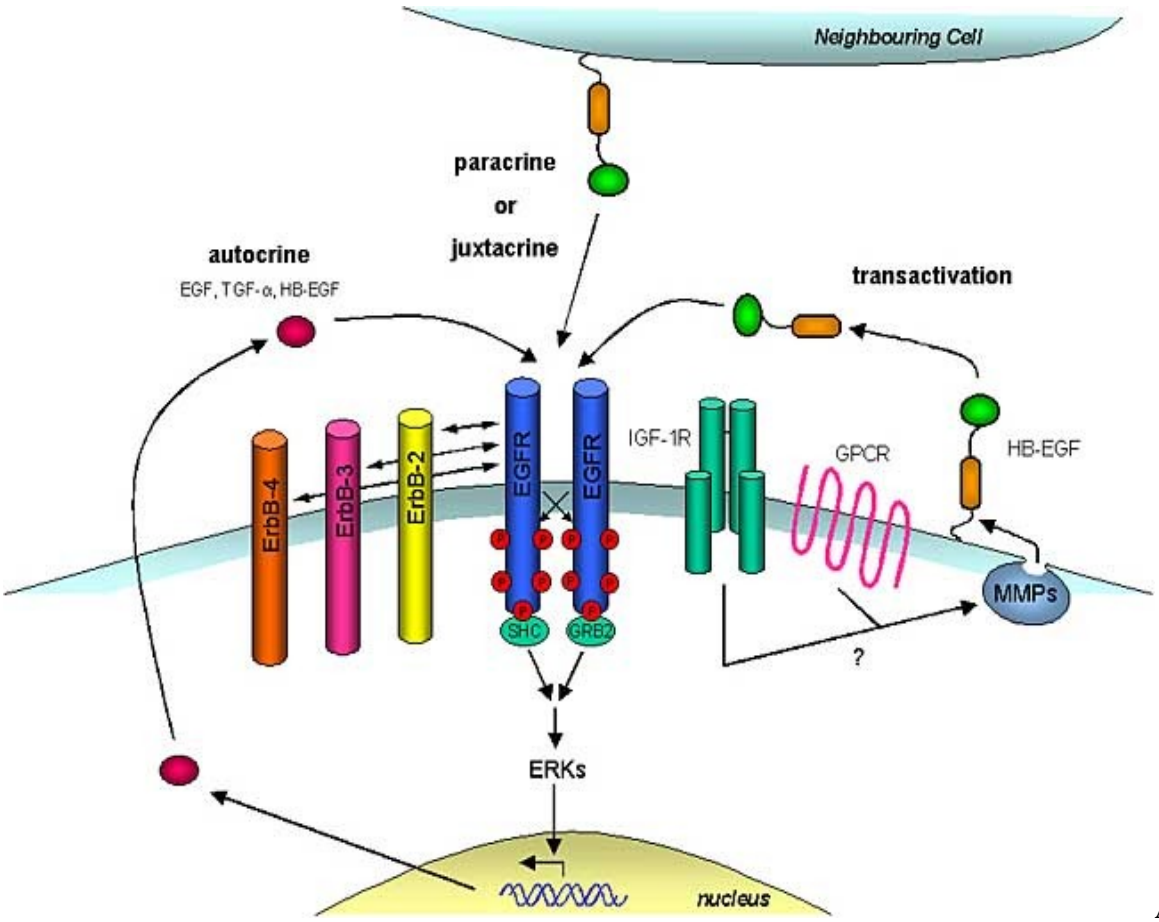


FACTORS AFFECTING EGFR SIGNALS

Signals relayed through EGFR receptors can be affected by multiple factors, including receptor expression levels and constitutive aberrations to the receptor. Several other factors converge to determine the specific quality and magnitude of the signal in the ErbB system. These factors include the nature of the system's ligand, the nature of the dimer upon activation by its ligand, dimer degradation rate, and crosstalk with other pathways [17].

The EGFR is activated via multiple mechanisms. The EGFR receptor is activated by ligands that are derived from within the same cell (autocrine), from neighboring cells (paracrine/juxtacrine) or as a result of the activation of heterologous receptors (transactivation). Receptor ligation results in dimerization with other EGFR monomers (homodimerization) or with other ErbB receptors (heterodimerization), receptor kinase activation and phosphorylation on multiple tyrosyl residues in the C-terminal region. These sites recruit phosphotyrosine-binding proteins such as GRB2 and SHC which trigger signaling pathways such as the ERK pathway. Only the ERK activation pathway is shown for clarity.

FACTORS AFFECTING EGFR SIGNALS



Abbreviations: IGF1-R, insulin-like growth factor-1 receptor; GPCR, G protein-coupled receptor; MMP, matrix metalloproteinase; HB-EGF, heparin-binding epidermal growth facto

Egfr - Targeted Therapeutics in Development

Several therapies have been developed that specifically target the ErbB receptor family. A variety of strategies have been considered for inhibiting ErbB receptor activity.

- In one strategy, an antibody binds to the receptor, blocking ligand binding and

the further cascade pathway.

- Accelerating receptor internalization and degradation is another potential target for EGFR. The receptor degradation process effectively "turns off" the ErbB response; consequently, it is a valuable target for rational therapy. One such therapeutic target is the signaling protein Cbl, which is important in receptor processing. The Cbl protein attracts ubiquitin-loaded molecules that tag the receptor with ubiquitin for recognition and sorting, eventually leading to proteosomal digestion [55].

- Another strategy involves the utilization of a low-molecular-weight inhibitor of RTK activity, which blocks receptor activation.

- In a fourth strategy, a bispecific antibody binding to a receptor and an immune cell facilitates immunologic attack.

- In a fifth approach, an antireceptor antibody conjugated to molecules of a cellular toxin or cytotoxic drug promotes receptor internalization and delivery of the drug or toxin to the interior of the cell.

- Finally, antisense oligonucleotides complementary to the nucleotide sequence of the ligand or receptor block protein translation.

The magnitude and quality of the downstream response to targeted therapies may be determined by a variety of factors that need to be identified in tumors. Thus, another important consideration in the development of targeted therapeutic agents is tumor markers; that is, the subcellular determinants of the ErbB inhibitory response, such as

phosphorylated (p)-ErbB-1, p-Erk, and p-Akt. Following treatment with a particular compound, immunohistochemical staining of receptors, phosphorylated aspects of receptors, and ST elements has permitted seimiquantitation of these determinants [46].

The above discussion focuses on the current level of knowledge acquired in EGFR research. Considering the promising outlook we have tried to identify the EGFR in our population with gastric cancer.

EGFR PATHWAY AND TARGET SITES

Currently, we have two distinct methods to target EGFR: mAbs to the EGFR and small-molecule TKIs.

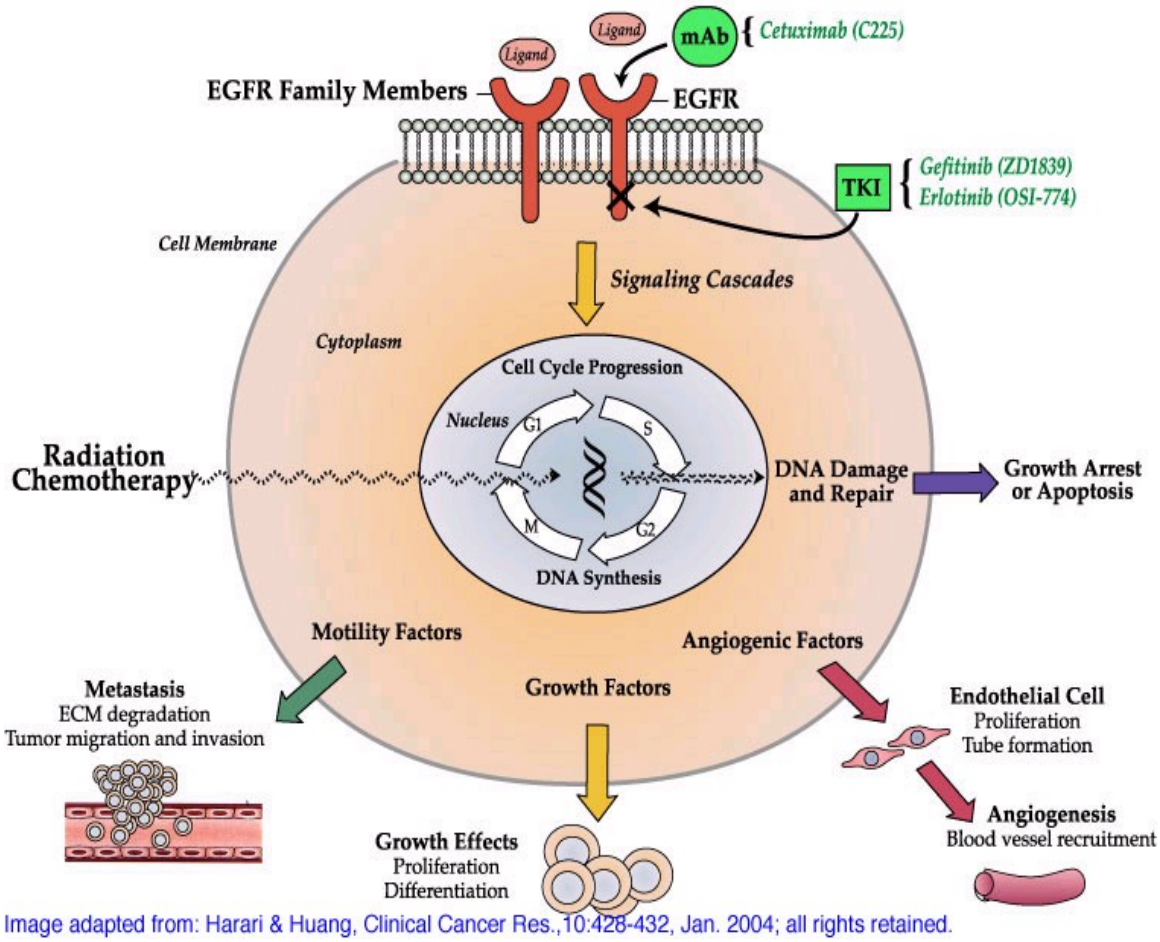


Image adapted from: Harari & Huang, Clinical Cancer Res.,10:428-432, Jan. 2004; all rights retained.

HYPOTHESIS

Agents that target the signal transduction pathways are highly specific for various components of these complex biologic processes and hold great promise for better patient outcomes.

Signaling through ErbB-1 and other family members triggers a powerful network of downstream cellular pathways, ending in responses that range from cell division to cell death, and from motility to adhesion, and include invasiveness and angiogenesis.

By interfering with cell signaling pathways involved in cell proliferation such as inhibition of EGFR-associated tyrosine kinase represents a novel approach to the treatment of solid tumors.

This study addresses the presence of EGFR in cancers of stomach. Since EGFR plays a major role in tumor development, angiogenesis and metastasis, targeting these receptors may help in the management of these cancers.

REVIEW OF LITERATURE

Many targeted therapies against EGFR for esophageal and stomach cancer are in

various phase I/II clinical trials which include monoclonal antibodies (mAbs) and signal transduction/tyrosine kinase inhibitors (TKIs) for EGFR.

In 1989,Yonemura.y et al conducted a study at Department of Surgery II, School of Medicine, Kanazawa University, Japan in 242 cancer stomach patients which revealed the presence of EGF receptor in 76(31.4%) patients and the receptor was more common in diffuse infiltrating types, had significantly higher values of Bromodeoxyuridine labeling indices and associated with a poor prognosis.[18].

In 1993, Demard .F et al found that EGFR levels were higher in tumor samples as compared with healthy control zones. There is no significant difference in EGFR expression according to the various anatomic sites explored or tumoral differentiation status. There is a significant difference of distribution for EGFR levels between stages I and II tumors and stages III and IV tumors.[19].

In 1995, Tokunaga et al published that EGF has been shown Immunohistochemical to be present in 26% of gastric cancers (n = 395). Fifteen percent of gastric cancers (n = 352) were also shown to be positive for both EGF and the EGF receptor immunohistochemically, and the simultaneous occurrence of EGF and the EGF receptor suggests that these tumors grow in an autocrine fashion. Tumors exhibiting EGF and the EGF receptor simultaneously show a greater degree of local invasion and

lymph node metastasis.[47]

In 1995, D'Agnano.I analysed the expression of EGF-R which revealed that 88% of aneuploid tumors were positive for receptor expression. On the contrary, diploid tumors showed the presence of EGF-R only in 56% of cases ($p = 0.01$). EGF-R expression was not related to histological grade. The close relationship between EGF-R positive expression aneuploidy, node involvement, and tumor invasion suggests that these parameters may be indicators of high malignancy. Finally, the results also show that aneuploidy and EGF-R-positive expression are indicative of a worse prognosis in gastric cancer patients.[52].

In 1996, Kitagawa et al showed that the cumulative survival rate for patients with EGFR gene amplification in their primary tumors was significantly lower than that for patients without amplification ($p < .001$). A significant correlation was also observed between extensive lymph node involvement at the time of surgery and EGFR gene amplification
($p < .05$) [35].

In 1999,Senekowitsch-Schmidtke et al in identified the sufficient uptake of the EGFR antibodies to the receptors and it can be used for immunotherapy and, after labeling with an appropriate radionuclide, called radioimmunotherapy[40].

Garcia j, Vizoso et al in 2001 found EGFR levels were significantly higher

($p<0.0005$) in neoplastic tissue than in paired adjacent mucosa samples (median) (n= 84; 8.7 vs. 3.9 fmol/mg protein). Intratumoral EGFR levels were significantly correlated with tumor stage ($p<0.05$), and were higher in patients with stage III tumors (median) (7.6, 6.4, 12.3 and 7.5 fmol/mg protein for stages I, II, III and IV, respectively). In addition, the tumor/mucosa ratios of the EGFR content were significantly higher ($p<0.05$) in patients with stage III tumors (1, 1.8, 3.9, and 0.92, respectively) [24].

In 2002 ,Wang ZH et al published their study showing that the level of expression of EGFR was higher in the patients with metastasis and residual gastric cancer(61.11%, 66%, 66.67%) than in the patients with other cancers, normal gastric mucous membrane, atypical hyperplasia(47.83%, 0%, 35%) ($P < 0.01$) [20].

In 2004 ,Gamboa-Dominguez A et al evaluated the membrane staining of EGFR in the neoplastic cells and graded using a semiquantitative score (0-3+). Of the 89 carcinomas examined, staining of neoplastic cells was weak in 17 (19.1%, score 1+), moderate in 16 (18.0%, score 2+), and strong in nine cases (10.1%, score 3+). EGFR reactivity score correlated with distant metastases ($P=0.002$) and clinical stage ($P=0.033$). EGFR score 0/1+ was significantly associated with an increase in patient survival when compared to score 2+/3+ ($P=0.0006$). In a multivariate analysis, EGFR positive cells in muscularis or subserosa ($P=0.004$), distant metastases ($P=0.016$) and residual disease ($P=0.039$) were significantly correlated with decreased survival. EGFR reactivity in neoplastic cells is an independent prognostic factor in gastric

adenocarcinoma.[53]

Evaluation of PCNA and EGFR status of pretreatment biopsies may identify a group of patients likely to derive the greatest benefit from chemoradiotherapy before surgery in terms of histologic response and long term survival[21].

In 2003, Ghaderi et al retrospectively investigated in 146 southern Iranian gastric cancer patients. Indirect immunostaining was used to evaluate the expression of these two receptors in formalin-fixed paraffin-embedded tissue samples. C-ErbB-1 expression was observed in 47 (32.2%) and c-erbB-2 expression was observed in 24 (16.4%) of tumors. Significant positive correlations were observed between c-erbB-1 expression and tumor size, local invasion, lymph node involvement and tumor stage. [48]

In 2004, Wilkinson et al in a retrospective review of 38 patients with resected gastroesophageal adenocarcinoma, demonstrated that poorly differentiated adenocarcinomas of the esophagus demonstrated higher EGFR expression than low-grade tumors based on IHC analysis (57% versus 13%, $p = .02$). The median overall survival times were 35 months for EGFR-negative patients and 16 months for EGFR-positive patients [39]. Overexpression of EGFR via IHC analysis occurs in 30%-90% cancer cases and correlates with poor prognosis [25-30].

In 2004 , Bonner JA et al in his Pre-clinical in vitro and in vivo model systems demonstrated radio sensitization with EGFR signaling inhibition addition of cetuximab

to radiation resulted in a significantly longer median survival time (54 months versus 28 months, p = .02) and significantly greater 3-year survival rate (57% versus 44%)(36).

In 2004, Radovich.D *et al* reported the phase II trial evaluating single agent erlotinib in patients with metastatic esophageal cancer regardless of EGFR expression who had received up to one prior chemotherapy regimen for metastatic disease has shown positive response in squamous cell carcinoma. [23].

In 2004, Van Groeningen et al did a multicenter, phase II trial in Japan (n=75) and evaluated the efficacy, tolerability, and pharmacokinetics of gefitinib in pretreated patients with advanced gastric cancer, showed despite fairly limited antitumor activity, it appears that the gefitinib did reach its target, suggesting that tumor control may require more than inhibiting EGFR alone.[37-39]

STUDY DETAILS

Type of Study

Prospective descriptive experimental study.

Study Duration

Jan 1, 2005 to December 31, 2005.

Collaborating Institutions

1. Department of Surgery, Government Royapettah Hospital, Kilpauk Medical College, Chennai - 600 010, India.

2. Department of Surgical Gastroenterology, Government Royapettah Hospital, Kilpauk Medical College, Chennai - 600 010, India.

3. Division of Immuno Histochemistry, R&D Histopath Lab, Mylapore, Chennai - 600 004, India.

EXPERIMENTAL DESIGN

Tissue Specimens

Formalin fixed, paraffin embedded, tissue from endoscopic biopsy of gastric malignancy patients were used for this study. Histological sections were studied by the collaborating pathologist at Immuno histochemistry division of the R&D Histopath Lab, Mylapore, Chennai - 4.

Technique of Detection

Immunohistochemistry is a multi step process that requires specialized processing of the tissue, the selection of appropriate reagents and interpretation of the stained tissue sections. In general, Immunohistochemistry staining techniques allow for the visualization of antigens, by sequential application of a specific antibody to the antigen, a secondary antibody to the primary antibody which serves as a link between the primary antibody and streptavidin enzyme conjugate, an enzyme conjugate and a chromogenic substrate. The enzymatic activation of the chromogen results in a visible product at the antigen site.

We have used this method in identifying EGF receptor in gastric malignancies, in our study.

REAGENTS


REF/Cat. No. 85-9643
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For In Vitro Diagnostic Use
REF/Cat. No. 85-9643
HRP-STREPTAVIDIN
C
Ready-to-use
Store at 2-8°C
For In Vitro Diagnostic Use
D1
BUFFER
(20x) Concentrate
Store at 2-8°C
D2
SOLUTION
(20x) Concentrate
Store at 2-8°C
D3
(20x) Conc
Store at 2-8°C
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MATERIALS AND METHODS

The primary antibody mouse anti-EGRF receptor clone-EGFR-31G7 was obtained from Zymed Laboratories Inc, South Sanfrancisco, CA 94080, USA. The necessary reagent, buffers and humidifying chambers were utilized from the Immunohistochemistry division R&D Histopath Lab; Mylapore, Chennai-4. The primary monoclonal antibody is generated in ascitic fluid, protein A purified. The vial is filled to 0.5 ml with reagent containing PBS, 1% BSA, and 0.1% sodium azide.

Tissue section of 5 microns cut with the help of Leica microtome. They were applied to Poly-L-Lysine precoated slides. The following staining protocol was followed. Dewaxing done in xylene bath and sections were brought to water through graded alcohol. They were subjected to microwave antigen retrieval in citrate buffer of pH6 for 30 minutes. To block non specific reactivity and staining from endogenous peroxidase, sections were incubated with hydrogen peroxide for 5 minutes.

After rinsing the slides were incubated at room temperature with EGFR receptor primary antibody for 1½ hrs. The slides were washed and biotinylated link was applied and incubated for 30 minutes. The sections were incubated in biotinylated streptavidin HRP for 30 minutes

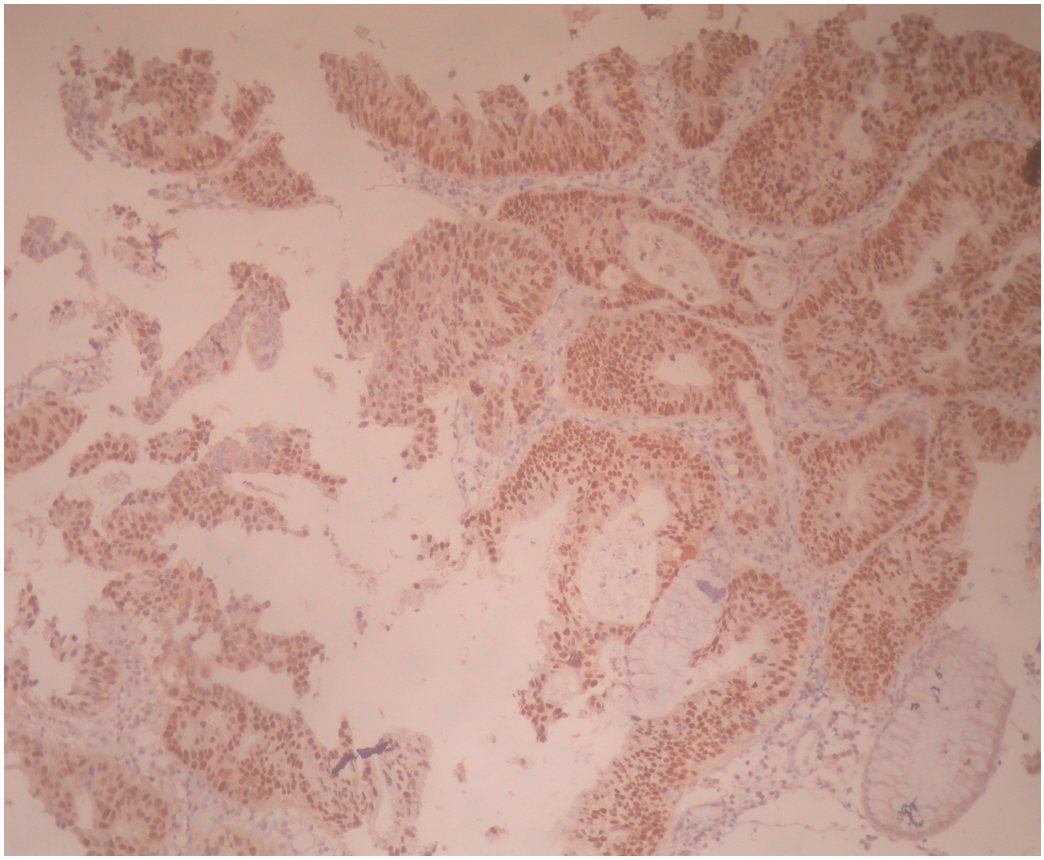
In between these stages, the slides were rinsed in 10mM phosphate buffered saline. DAB, a substrate chromogen was applied and the slides were incubated for 5 minutes. The

slides were thoroughly rinsed and counterstained with Mayer's Hematoxylin for 30 seconds and then covered with glycerol jelly and cover slip applied.

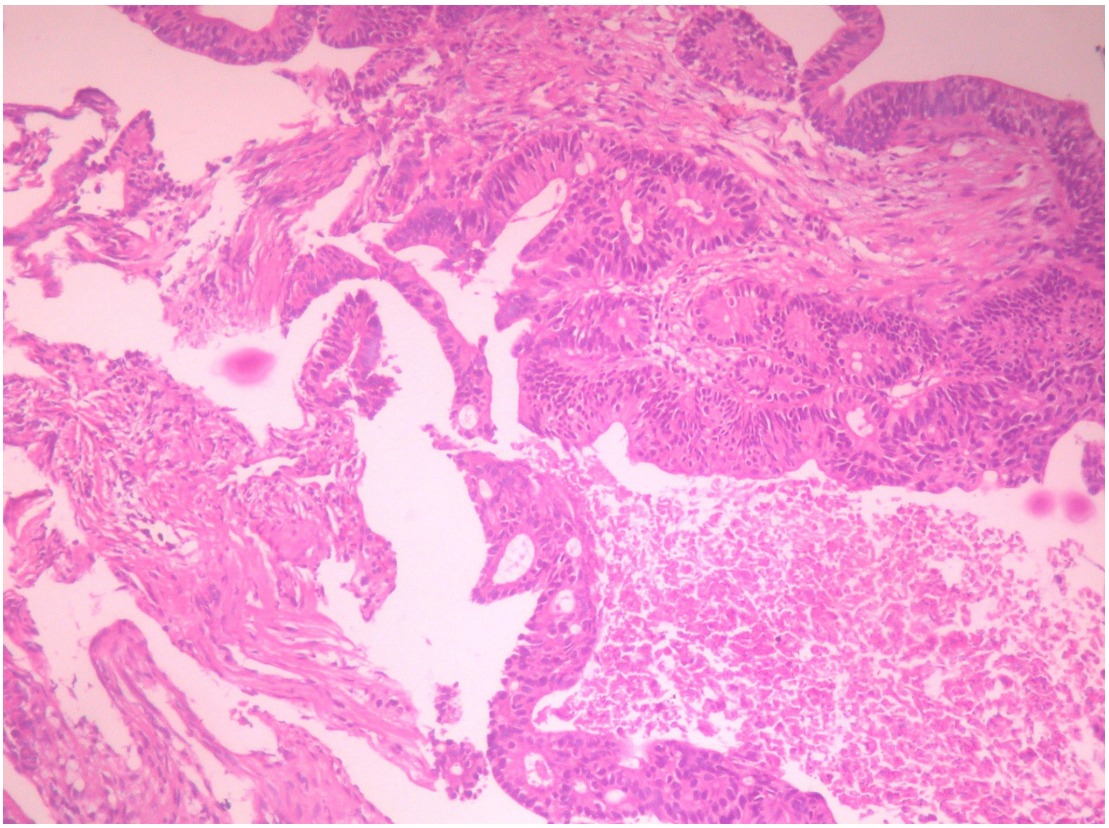
Throughout the procedure 98 to 100% humidity was maintained in a humid chamber. After the above procedure, the slides were ready for screening.

Immunohistochemistry stained positive cells, look brown and negative cells look blue. Staining was graded based on the UICC guidelines.

EGFR POSITIVE



EGFR NEGATIVE



STATEMENT OF LIMITATIONS

This study is based on a limited number of patients. A detailed study of more subjects, in relation to histopathology of cancer and lymph node status and follow up for a longer duration will augment the validity of the data.

Downstream of the EGFR pathway are several proteins that may be activated without EGFR thus allowing blockade to be overcome. Therefore, while blocking the activity of the EGFR protein appears to be a promising anticancer strategy, a simplistic strategy of blocking only EGFR is likely to only impact a minority of patients [41].

Epidermal growth factor receptor is overexpressed in a variety of tumor types with variable frequency [42]. Of note, different studies yield a wide range of overexpression frequencies in part because of the lack of consistent techniques and uniformity for measuring EGFR levels.

The nature of the ligand determines the nature of the dimer formed upon binding; this very complex process involves the possibility of multiple ligands and subsequent dimer profiles that each confer a particular mitogenic response to the cell [43]. Therefore, the magnitude and type of mitogenic response downstream is a contextual summation of the individual effects of multiple ligands and receptor dimerization profiles. Consequently, targeting one specific ErbB subfamily may be insufficient to impart a significant therapeutic benefit [44, 45].

Trials done on manipulating the EGFR has shown varied results. Inhibition of

these drugs and altered responses are due to various factors which influence the carcinogenic pathway. Hence modulating the EGFR along with other rate limiting steps may be essential.

Molecular diagnostics have been improving everyday. The technique of detection based on proteomic molecules, PCR and western blot methods may yield better results as compared the Immunohistochemical staining.

An attempt to quantify the EGFR in normal gastric mucosa will help us to understand the validity of this data.

ETHICAL ISSUES INVOLVED

The study was done in tissue obtained from upper gastrointestinal endoscopy. No ethical conflicts are involved in this study.

EXCLUSION CRITERION

- Non adenocarcinoma histology of gastric malignancy were excluded from the study.

- Patients who were not been able to stratify the differentiation status were not included in this study.

- Patients who have had pre biopsy chemotherapy were excluded.

PATIENT CHARACTERISTICS

Total No. of patients

N = 28

Characteristics		**No. Of patients**
1. Gender		
Male	=	20
Female	=	8
2. Age		
Male	=	41 - 83yrs
Female	=	37 - 76yrs
3. Site		
Stomach	=	28
4. Histopathology		
Adenocarcinoma	=	28

5. Differentiation

a. Well Differentiated = 13

b. Moderately Differentiated = 7

c. Poorly differentiated = 8

VARIABLES		N	%
Age group	31-40	1	5.3%
	41-50	6	19.7%
	51-60	9	32.1%
	61-70	4	14.3%
	71-80	7	25.0%
	81-90	1	3.6%
Sex	Male	20	71.4%
	Female	8	28.6%
Site of biopsy	Stomach	28	100%
Differentiation	Poor	8	28.6%
	Moderate	7	25.0%
	Well	13	46.4%

STATISTICAL ANALYSIS

Categorical variables sex, histopathology type, site, mitosis and EGFR were given in frequencies with percentage. Continuous variable (age) given with mean, standard deviation, minimum and maximum age.

'Epi info 6' Software is used in this analysis.

Categorical variables associations with positivity were analysed using chi square test. Continuous variable associated with positivity were analysed using students ‘t’ test. Correlation between age and positivity were analysed using Pearson correlation coefficient and graphical representation of the same is given in scattered diagram. The Incidence of positivity was given in proportion with 95% confidence interval. The risk factors associated with positivity were analysed using multivariate logistical regression.

OBSERVATIONS

Variables		EGFR status			
		Negative		Positive	
		n	%	n	%
Age Group	31-40	1	5.3%	0	0
	41-50	3	15.8%	3	33.3%
	51-60	6	31.6%	3	33.3%
	61-70	3	15.8%	1	11.1%
	71-80	5	26.3%	2	22.2%
	81-90	1	5.3%	0	0
Sex	Male	13	68.4%	7	77.8%
	Female	6	31.6%	2	22.2%
Differentiation	Poor	4	21.1%	4	44.4%
	Moderate	5	26.3%	2	22.2%
	Well	10	52.6%	3	33.3%

EGFR Positivity = 9/28 = 32% (16%- 52%)

The EGFR status in the gastric malignancy studies is 32% that can vary from 16%-52% when a confidence interval of 95% is applied.

AGE BASED OBSERVATION

Age	Percentage of staining					
	0	**1- 30%**	**31- 60%**	**>60%**	**Total Patients**	**Total Positivity**
31-40	1	0	0	0	1	0
41-50	3	2	0	1	6	2
51-60	6	1	1	1	9	3
61-70	3	0	1	0	4	1
71-80	5	1	1	0	7	2
81-90	1	0	0	0	1	0
Total	19	4	3	2	28	9

Positive were more common in the 41-50 and 51-60 age group. Definitive statistical significance could not be arrived.

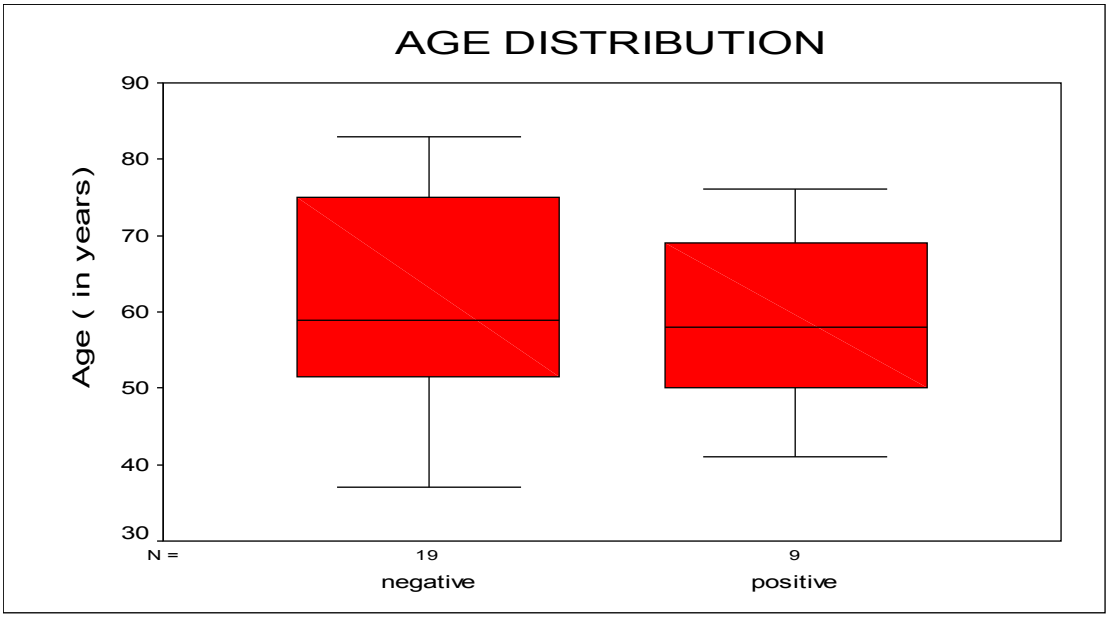
AGE DISTRIBUTION

EGFR Status	N	Mean	Standard Deviation	Student t-test
Positive	9	58.22	12.143	t=0.56
Negative	19	61.21	13.460	P=0.58

CORRELATIONS

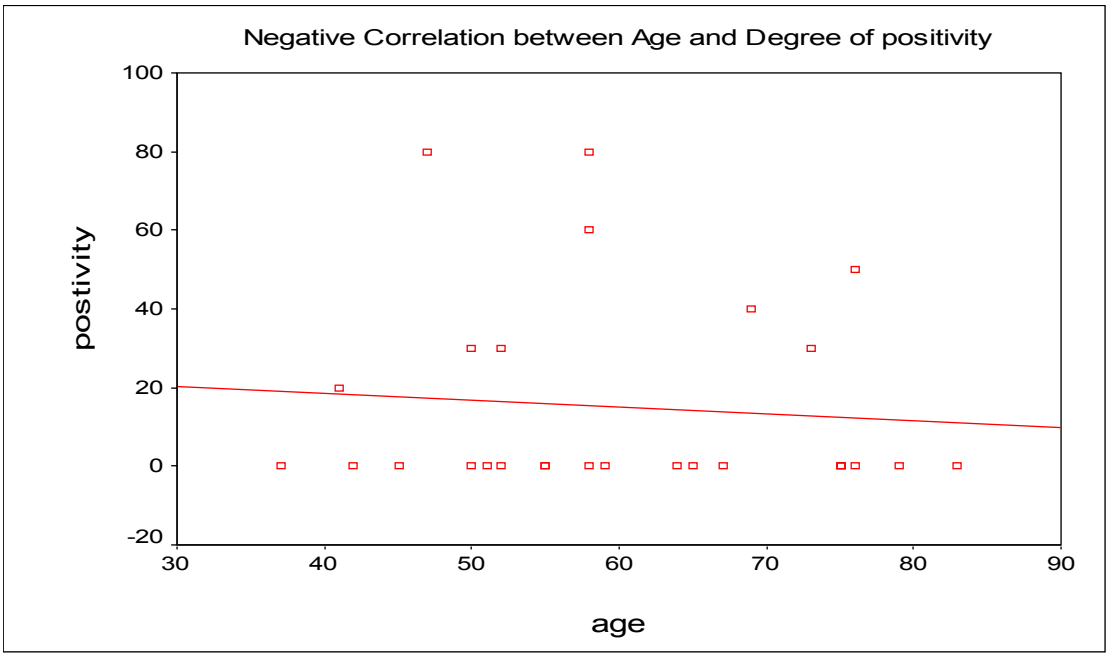
		Grade
age	Pearson Correlation	-.110
	Sig. (2-tailed)	.07
	N	28

AGE BASED OBSERVATION



There is poor negative correlation. It means positivity of EGFR is negatively correlated with age. The mean age of EGFR positivity is about 3 years less than the receptor negative patients.

AGE BASED OBSERVATION

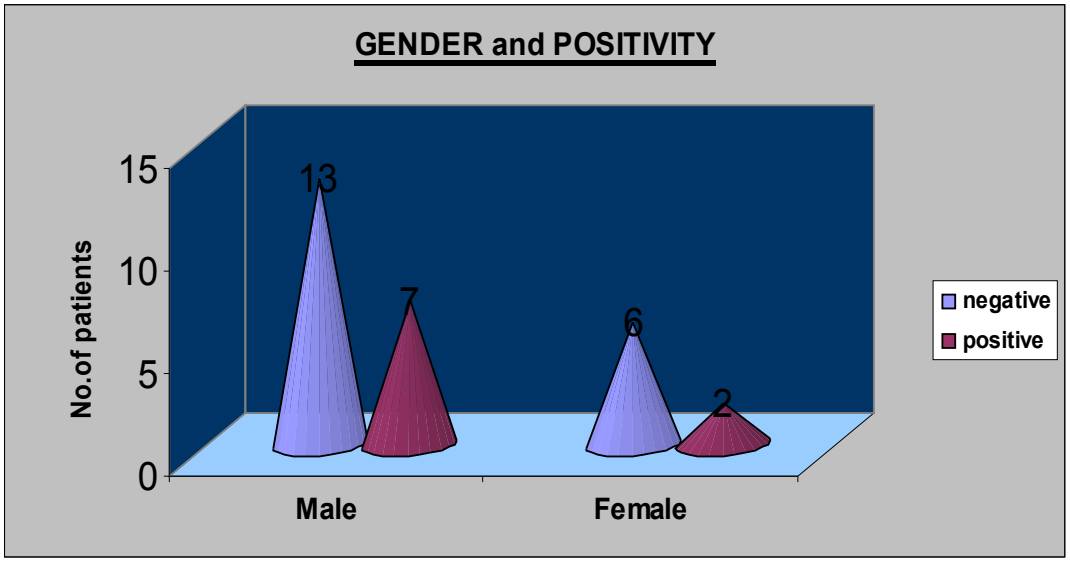


This graph represents the analysis of the age based EGFR positivity with the degree of staining. In this study the intensity of the receptor is negatively correlated to the percentage of staining i.e. staining decreases with increasing age of the patient.

SEX BASED EGFR STATUS

Sex	Percentage of staining					
	0	1- 30%	31- 60%	>60%	Total Patients	Total Positivity
Male	13	4	1	2	20	7
Female	6	0	2	0	8	2
Total	19	4	3	2	28	9

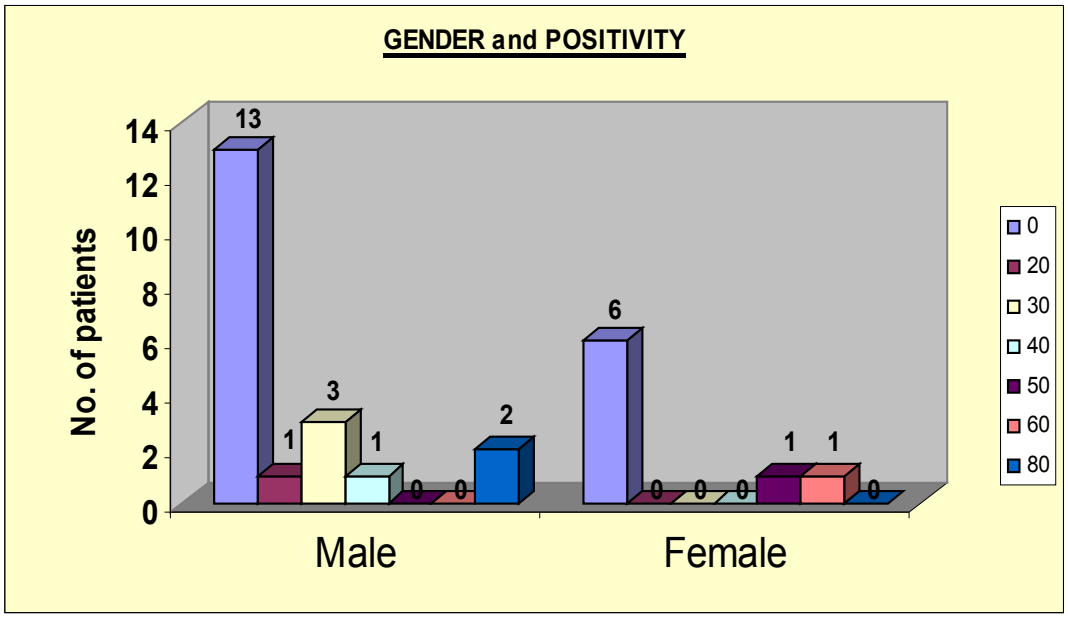
$\chi 2=0.26$ P =0.61 Not significant



In this study there is no difference between EGFR positivity and the sex of the patient.

SEX BASED EGFR STATUS

Sex	Percentage of staining						
	0	20	30	40	50	60	80
Male	13	1	3	1	0	0	2
Female	6	0	0	0	1	1	0

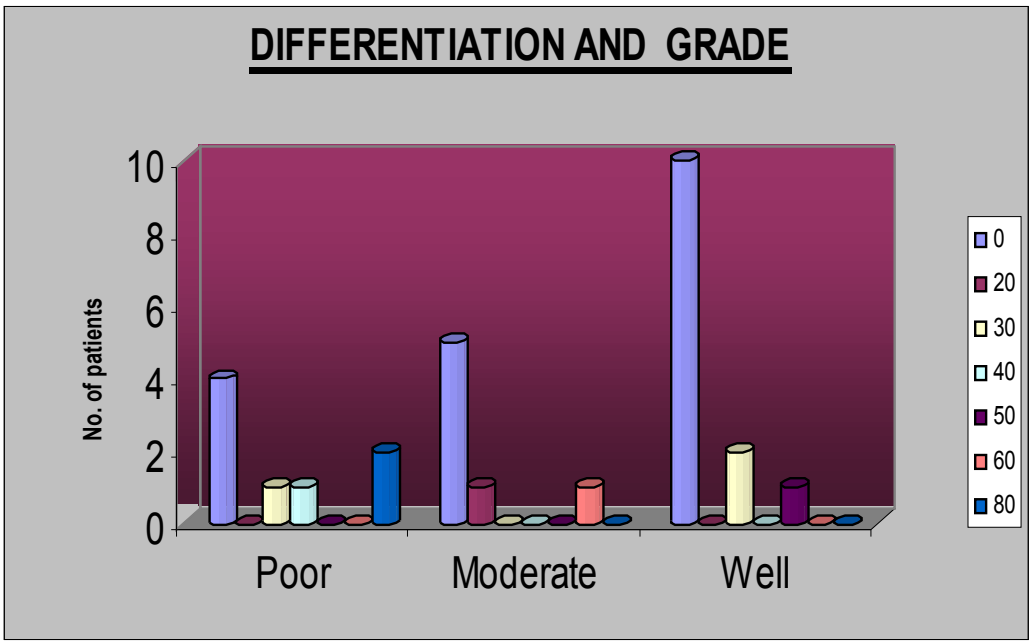


The degree of positivity does not correlate with the sex of the patient.

DIFFERERNTIATION BASED EGFR STATUS

Differentiation type	Percentage of staining					
	0	1- 30%	31- 60%	>60%	Total Patients	Total Positivity
Well differentiated	10	2	1	0	13	3
Moderately differentiated	5	1	1	0	7	2
Poorly differentiated	4	1	1	2	8	4
Total	19	4	3	2	28	9

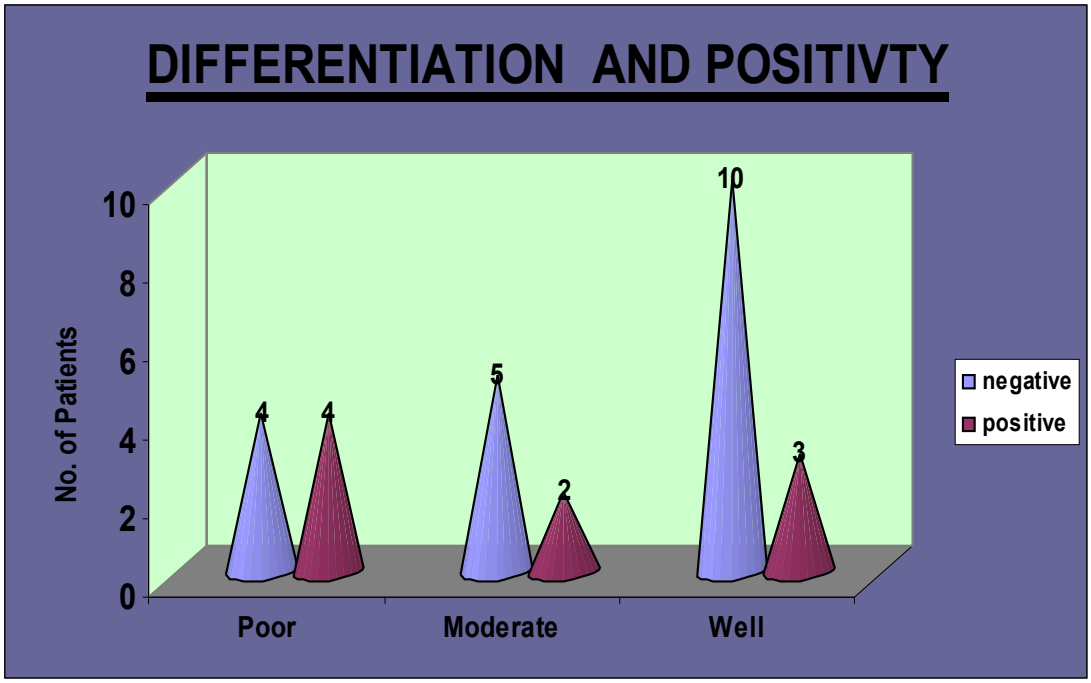
Staining based analysis versus differentiation of tumors did not yield results of statistical significance as the analysed patients volumes is less.



DIFFERENTIATION AND RECEPTOR STATUS

Differentiation type	EGFR status		Total
	Negative	Positive	
Poorly differentiated	4	4	8
Moderately differentiated	5	2	7
Well differentiated	10	3	13
Total	19	9	28

$\chi 2=1.07$ P =0.42 Not significant



Positives were more common among poorly differentiated tumors. Staining for mutant EGFR were least positive among well differentiated tumors. This correlates well with similar studies around the world.

MULTIVARIATE LOGISTIC REGRESSION FOR IDENTIFICATION OF RISK FACTORS FOR POSITIVITY

VARIABLES		SIGNIFICANCE	ODDS RATIO
1	AGE	.097	.987
2	SEX	.888	.868
3	SITE	.879	.000
4	HPE TYPE	.104	.633

COMPARISON WITH INTERNATIONAL STUDIES

STUDY GROUP	NO. OF PATIENTS (n)	EGFR POSITIVITY %	P VALUE
Tokunaga a et al [47]	395	26%	-
Wang zh et al [20]	199	66%	<0.01
Ghaderi et al[48]	146	32.2%	-
He SW et al [51]	104	30.8%	<0.05
Gamboa-Dominquez A et al [53]	89	40%	<0.033
Suzuki et al [49]	66	51.5%	<0.01
Kikkawa et al[50]	65	26.2%	<0.05
D'Agnano I[52]	63	56-88%	<0.01
Wilkinson et al [34]	38	%57	-

The EGFR positivity in our study is 32% which range from 16%-52% with a confidence interval of 95%. This is comparable to the other studies done at various countries.

SUMMARY AND CONCLUSION

Within only a few years, anticancer therapeutic development against advanced cancers has moved from almost a standstill, with a paucity of new agents showing potential for major effect, to the rapid development of agents targeted against the inherent basis of cancer. This transition is based largely on the exponential rate of information acquisition regarding the cancer cell, particularly in terms of aberrant growth signal transduction and the microenvironment of the cell.

In our study, the presence of EGFR in gastric malignancy was 32% ranging from 16%-52% with a confidence interval of 95%. This may form the basis for targeting EGFR in carcinoma of stomach.

The mean age of the patients with EGFR positivity is 3 years less than those showing negative for EGFR receptor. Similarly the degree for positivity is also negatively correlated with age.

The sex of the patient, the histopathology of the tumor, the degree of differentiation is not related to the EGFR status. However the sample studied is small and the significance of these factors are to be studied in a larger population.

The correlation between EGFR positivity and the differentiation of tumors is that the poorly differentiated tumors express the EGFR more commonly than the moderate and well differentiated tumours. Poorly differentiated tumours staining positive for

EGFR is almost 50%.Also the intensity of staining is high in poorly differentiated tumours.

Although the GI malignancies are a heterogeneous group of malignancies, several common features make them excellent candidates for the investigation of EGFR inhibitors such as aggressive tumors with poor prognoses, common overexpression of EGFR, and limited treatment option availability.

Initial studies in a variety of GI malignancies have already shown initial promise and one agent, cetuximab, is already approved for use in refractory colorectal cancer. While further analyses of single-agent EGFR inhibitors and combinations with cytotoxics need to continue, studies that have randomised cohorts will yield definitive results. The EGFR protein is just one target in a network of protein-cell signals. In order to best target this network, we will need to maximize our understanding of the interactions of all the proteins that affect this network.

Hence, adequately designed clinical trials are necessary to ensure that the usefulness of Signal transduction inhibitors is correctly evaluated and that potentially useful agents are not rejected solely on the basis of poor performance in an inadequately designed trial with an inappropriate clinical or biologic end point. The full potential of these new agents may only be realized with the implementation of radically different therapeutic development, evaluation, and treatment paradigms.

There are literature evidence stating that these receptors may be useful for as screening tool, as a sensitizer, for assessing the invasiveness, metastatic potential and the prognosis of the tumor. However we will be able to draw a firm conclusion from large volume studies on Indian population, with long term patients follow up.

Thus identification of the presence of EGFR status in gastric cancers may help to target the tumor cells in addition to the present multimodality management.

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STUDY FORMAT

Name :

Age :

Sex :

a. Male

b. Female

Patient Endoscopy ID No.

Location of the tumor

Stomach

Degree of Differentiation

a. Well differentiated

b. Moderately differentiated

c. Poorly differentiated

Immunohistochemistry

a. Positive

b. Negative

Degree of Staining (in percentage)