"ANALYSIS OF A THREE GENE SIGNATURE IN GASTRIC CANCER PATIENTS"

Dissertation submitted to

THE TAMILNADU Dr. M. G. R. MEDICAL UNIVERSITY

in partial fulfillment of the requirements for the

award of the degree of

M. Ch. BRANCH – VI

SURGICAL GASTROENTEROLOGY AND PROCTOLOGY

THE TAMILNADU Dr. M. G. R. MEDICAL UNIVERSITY

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CERTIFICATE

This is to certify that the dissertation titled **"ANALYSIS OF A THREE GENE SIGNATURE IN GASTRIC CANCER PATIENTS"** submitted by **DR. SATHEESH KUMAR M** appearing for **M.Ch. Surgical Gastroenterology & Proctology** degree examination in August 2013, is a bonafide record, of work done by him under my guidance and supervision in partial fulfillment of requirement of the Tamil Nadu Dr. M. G. R. Medical University, Chennai. I forward this to the Tamil Nadu Dr. M. G. R. Medical University, Chennai.

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DECLARATION

I solemnly declare that this dissertation titled **"ANALYSIS OF A THREE GENE SIGNATURE IN GASTRIC CANCER PATIENTS"** was prepared by me in the department of Surgical Gastroenterology and Proctology, Centre of Excellence for Upper Gastrointestinal Surgery, Madras Medical College & Rajiv Gandhi Government General Hospital, Chennai under the guidance and supervision of **Prof.S.M.Chandramohan,** M.Ch, FACS, Professor & Head of the department of Surgical Gastroenterology and Proctology, Centre of Excellence for Upper Gastrointestinal Surgery, Madras Medical College & Rajiv Gandhi Government General Hospital, Chennai. This dissertation is submitted to the Tamil Nadu Dr. MGR Medical University, Chennai in partial fulfillment of the university requirements for the award of the degree of M.Ch Surgical Gastroenterology & Proctology.

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INTRODUCTION

Incidence of Gastric Cancer

Stomach cancer is the 2nd most common cause of cancer related mortality in both men and women aged 30–69 years in India next only to oral cancer in men and cervical cancer in female. [1]

Worldwide, stomach cancer is the 2nd leading cause of cancer death and 4th most common cancer type. Gastric cancer is more common in East Asia and South America than other parts of the world. Gastric cancer is increasing in developing countries and the rates have been decreasing in the developed countries. Among developed countries, Korea and Japan have the highest rates of incidence. But, the mortality rate has dropped by 50% in the above countries as a result of the screening programme. Distal stomach is the most common site in developing countries and in Japan.

Incidence and Mortality Trends

Worldwide, stomach cancer was the most common cause of cancer-related death. But, the rates have started to decline. The mortality rate has halved in Europe and Russia. This fall is less pronounced in developing than in developed countries. [2]

This fall in stomach cancer rates are because of the following:

1. More affluent diet, with increased consumption of fresh fruits

2. Better food preservation, including refrigeration

3. Effective treatment of Helicobacter pylori infection

4. Decrease in smoking

Trends in Gastric cancer by site and histology

The gastric cancer incidence is declining mainly due to the decrease in the incidence of intestinal type cancers but, the incidence of diffuse type cancers has been generally stable throughout the world.

Also, the cancers arising from the gastric body and antrum are decreasing and the proximal gastric cancers are increasing.

Risk factors for gastric cancer

Advancing age

Advancing age is a risk factor for cancer stomach. The number of cases increases with age. Most deaths occur in the 50-70 age group. The increasing frequency of stomach cancer is due to the accumulation somatic mutations with age.

Sex

Stomach cancer is 2 times more common in men than women. 70% of gastric cancer patients are men and only 30% are women.

Obesity

Obesity results in a 2 times more risk of malignancy of cardia of stomach in comparison to non-obese people. [9]

Diet

Excessive salt, excessive consumption of fish and other seafood is an etiology for gastric cancer. Reduced consumption of vegetables and fresh fruits also plays an important role in etiology. Improvements in food processing and storage and refrigeration have significantly decreased the incidence of gastric cancers worldwide. High red meat consumption is also implicated as an etiological factor for gastric cancer.

The mechanism of carcinogenesis is the conversion of nitrates in the food to carcinogenic N-nitroso compounds in the stomach. This chemical change leads to decreased mismatch repair (MMR) genes activity and increased tolerance to DNA damage thereby resulting in the errors of DNA strands.

Antioxidants in fruits like the ascorbic acid, catechins and tacopherols remove the mutagenic N-nitroso compounds and oxygen free radicals, thereby having anti-cancer effect. Microelements like zinc, selenium and magnesium also have a protective effect. [3]

Helicobacter pylori Infection

Infection of the mucosa of the gastric wall with Helicobacter pylori (H. pylori) leads to acid peptic disease and in the long run, malignancy. H. pylori is designated as a class I carcinogen by the World Health Organization. There is a 7 times increased risk of stomach cancer in H. pylori infected individuals.

The exact mechanism of carcinogenesis is not completely understood. H. pylori with VacA (valulocating cytotoxin A) and cagA (cytotoxin associated gene A) genes induce a more severe infection. The cytotoxin produced by these genes increases the virulence. Cag A positivity increases the risk of cancer by intensifying the immunological response, and by stimulating the release of IL-8, a chemokine which damages the mucosa. Also, a large amount of urease is produced by H. pylori, which break the urea into carbon dioxide and ammonia. The latter, neutralizes the hydrochloric acid thereby producing a increased pH environment enabling the bacterial growth in the gastric wall.

H. pylori induced inflammation results in increase of free radicals and cellular DNA damage thereby increasing the risk of cancer. H. pylori also stimulates the production of an array of inflammatory interleukins and Chemokines which further stimulate the immunological processes and chronic inflammation, paving the way for cancer development.

The molecular alterations described in intestinal metaplasia include overexpression of COX-2 and cyclin D2, low p27 expression, p53 mutations, transcription factors alteration including the CDX1 and CDX2, and microsatellite instability.

H. pylori cause cancer by the following mechanisms:

1. Metabolic products affecting the stomach mucosa directly

or

2. Indirectly by increasing the DNA damage risk, thereby making the mucosa susceptible to malignant change by carcinogens and by-products of infection.

Furthermore, eradication of H. pylori reduces the cancer risk in patients below the age of 40 years. Also, H. pylori status is a significant prognostic factor and negative H. pylori status is an indicator of poor prognosis in patients with gastric cancer.

Epstein-Barr virus (EBV) infection

Epstein-Barr virus infection is present in nearly 15% of stomach cancer subjects. EBV associated gastric carcinoma is non-endemic and has distinct characteristics like more diffuse type tumors, increased incidence of body and fundal tumors and predominance in males. The characteristic molecular abnormality is the promoter area methylation of the malignant genes. But, EBV associated stomach cancer has a better outlook compared to the negative tumors.

Mechanisms of EBVaGC include:

• DNA methylation

• Viral small RNAs

• Epigenetic alterations and

• Altered microRNAs expression of the host cells

Alcohol and Smoking

The duration and the frequency of smoking parallels the stomach cancer risk. Consuming alcohol greater than five occasions in two weeks and smoking more than 20 cigarettes per day increases the risk of stomach

cancer 5 fold. Also, there is an increased risk for passive smokers compared to those who don't smoker. [10]

Carcinogens contained in cigarette smoke include tar, polycyclic hydrocarbons and N-nitroso compounds. Carcinogens form covalent bonds with DNA, altering their function and pave the way for stomach cancer.

Alcohol ingestion also, significantly increases the risk of developing gastric cancer. Consumption of 4 or more drinks a day significantly increases the risk of developing gastric cancer.

Alcohol stimulates gastric motility and gastric juice production. Ethanol causes mucous membrane damage by reducing the blood flow, decreased mucus production, increase in pro-inflammatory cytokines, and causing oxidative stress. Also, the vodka and other alcoholic beverages contain nitrosamines that accentuate the risk of stomach cancer.

Socioeconomic status

Persons belonging to the lower socioeconomic status have an increased risk of developing the stomach cancer. Professions which have an exposure to nitrates and/or herbicides during work, also have an increased risk of developing the gastric cancer.

Migration

Reduction in the incidence of cancer is observed when migrating from a high risk area to a low risk area. For example, Japanese born in the United States have a low occurrence of gastric cancer similar to US population in comparison to recent immigrants.

Hereditary Risk Factors

Gastric cancer is associated with many rare inherited disorders that include:

1. Hereditary Diffuse Gastric Cancer

2. Familial Adenomatous Polyposis

3. Hereditary Nonpolyposis Colorectal Cancer

4. Li-Fraumeni syndrome

Hereditary Diffuse Gastric Cancer

Sporadic stomach cancers account for the majority of stomach cancer cases. But, in about 12% of gastric cancer cases, familial clustering is seen. About 2% of the familial clustering cases have the hereditary diffuse gastric cancer (HDGC).

Germline mutation in the CDH1 gene causes the autosomal dominant HDGC. The CDH1 gene located in chromosome 16 and encodes a 120-kDa protein called E- cadherin. E- cadherin is present on cells of all the epithelium and plays a crucial role in the intercellular adhesions. E- cadherin establishes and maintains the polarity of the epithelium by the intercellular adhesion formation and thereby it suppresses the invasiveness. Decreased expression of E- cadherin is associated with invasiveness and metastatic spread.

The International Gastric Cancer Linkage Consortium (IGCLC) has laid down the criterion for diagnosis of HDGC. Families which don't fulfill the IGCLC criteria but have an index case are grouped into one of the following types:

1. Familial Diffuse Gastric Cancer (FDGC)[4]

2. Familial Gastric Cancer (FGC)

3. Familial Intestinal Gastric Cancer (FIGC)

Genetics of HDGC

Guilford et al. first described the genetic linkage of HDGC in 1998. The various common CDH1 mutations include missense mutations and insertions or deletions. But, irrespective of the type of mutation, most

result in a truncated protein without any function. CDH1 deregulation is an initial event in HDGC.

CDH1 mutation subjects are found concentrated in Canada and New Zealand. But, in Asia, where a high incidence of sporadic gastric cancer is seen, the incidence of CDH1 mutations is very low. The reason for this is not known.

HDGC is diagnosed at a mean age of 40 years. The lifetime risk of developing stomach cancer is about 65% in men and 80% in women. The penetration of CDH1 germline mutation in HDGC is very high. The presence of a CDH1 mutation is also associated with increased risk of lobular breast cancer among women. This high penetrance of CDH1 mutation highlights the importance of identifying the carriers and the early diagnosis of HDGC which may translate to better prognosis and longer survival rates in these patients. [6]

Recommendations for CDH1 screening:

The IGCLC published the recommendations for CDH1 testing in 1999. Shortly after, many other revised guidelines were added which included lobular breast cancer, colon cancer or signet ring cell colon cancer as a criteria for genetic testing. Isolated individuals with the

diagnosis of diffuse gastric cancer prior to the age of 45, as well as individuals with diffuse gastric cancer and lobular breast cancer without any family history, were also included as criteria for CDH1 mutation testing.

Prophylactic gastrectomy:

The New Zealand HDGC Group provided recommendations for both screening and prophylactic gastrectomy. It recommended that patients under 14 years need not be tested for CDH1 mutations, while those over 16 years should be tested. Prophylactic gastrectomy was not recommended for patients aged less than 16 years and delaying prophylactic gastrectomy beyond 30 years of age was proposed to carry a significant risk for cancer.[13]

Outcome after prophylactic gastrectomy

In the young and healthy population, prophylactic gastrectomy was associated with up to 2% mortality and up to 20% major acute morbidity and 100% long-term morbidity. Hence, IGCLC recommended that gastrectomy for HDGC should be done in centers performing at least 25 gastrectomies per year with a mortality of less than 5%.

Timing of prophylactic gastrectomy:

The New Zealand HDGC group has recommended doing the procedure before 30 years of age. The Stanford group recommended prophylactic gastrectomy 5 years earlier than the age at which the youngest family member developed clinically apparent diffuse gastric cancer.

Importance of Total Gastrectomy:

The IGCLC emphasized the importance of prophylactic total gastrectomy against subtotal gastrectomy and pathologically identifying both the duodenal and esophageal mucosa in the surgical specimen. Also, there can be multiple foci of malignant cells distributed throughout the specimen. Therefore, leaving a residual stomach further exposes the at-risk patient to the development of gastric cancer.

Familial Adenomatous Polyposis:

Approximately 85% of patients with familial adenomatous polyposis have fundic gland polyps, and over 50% of them contain a somatic adenomatous polyposis coli mutation and up to 40% of these having some type of dysplasia which places these patients at risk of developing gastric cancer. These polyps, along with duodenal polyps, need upper gastrointestinal surveillance for malignant change.

Li-Fraumeni syndrome:

This is an autosomal dominant disorder caused by a mutation in the p53, tumor suppressor gene. These patients are at risk for many cancers including gastric cancer.

Lynch Syndrome:

Hereditary nonpolyposis colorectal cancer, or the Lynch syndrome, which is responsible for 2% to 3% of all colon and rectal cancers, is associated with microsatellite instability. This is also associated with an increased risk of gastric and ovarian cancers.

Other Risk Factors:

Pernicious anemia:

These patients are at increased risk for developing gastric cancer. The defining feature of this condition is Achlorhydria and it occurs when the chief and parietal cells are destroyed by an autoimmune reaction. The mucosa becomes atrophic and develops intestinal metaplasia. The patient with pernicious anemia has a relative risk of up to 5.6 for developing gastric cancer compared to the general population.

Polyps:

Adenomatous polyps carry a definite risk of malignancy in the polyp. Mucosal atypia is frequently seen and progression to carcinoma in situ has been observed. In approximately 10% to 20%, carcinoma develops in the polyp and the risk increases with increasing size of the polyp. Pedunculated polyps can be removed endoscopically and is sufficient if the polyp is completely removed and there are no foci of invasive cancer. Operative excision is warranted for a polyp more than 2 cms, sessile polyp or a polyp with a proven focus of invasive carcinoma.

Fundic gland polyps are benign lesions that result from glandular hyperplasia and decreased luminal flow. They are associated with proton pump inhibitor use and occur in up to a third of patients by one year of usage. But, dysplasia has only been described as individual case reports in these polyps. These do not require excision, surveillance or cessation of therapy.

Proton Pump Inhibitors (PPIs):

The PPIs usage has risen dramatically for patients with upper gastrointestinal complaints. They are often the first-line treatment for

dyspepsia and reflux disease. The impact of PPIs on the incidence of gastric cancer has not been fully elucidated.

PPIs block the hydrogen-potassium pump within the parietal cells, thereby blocking all the acid secretion in stomach. As a result, patients on PPIs develop hypergastrinemia, which reverses on PPI withdrawal. In patients on long-term PPIs associated with H. pylori infection, the low-acid environment allows the bacteria to colonize, leading to corpus gastritis.

Up to a third of patients with corpus gastritis develop atrophic gastritis. This atrophic gastritis quickly resolves after eradication of H. pylori. Currently, there is no study demonstrating the association of atrophic gastritis in this subset of patients with an increased risk of cancer. However, atrophic gastritis in general, is considered a major risk factor for the development of gastric cancer. Hence, in patients with persistent symptoms after initiation of therapy or who require long-term therapy, surveillance and eradication of H. pylori is warranted. [8]

MECHANISMS OF MALIGNANT TRANSFORMATION IN GASTRIC CANCER

Several molecular pathways interact in a complex way to ultimately produce the cancer. The most common and the most well studied pathways include the chromosomal instability, microsatellite instability and germline E-cadherin mutation. These pathways lead to oncogenes activation, tumor suppressor genes inactivation, telomerase reactivation, reduction of cellular adhesion, defective regulators of growth regulators and regulator genes of cell cycle and apoptotic genes.

Chromosomal Instability (CIN)

The commonest chromosomal abnormality in stomach cancer is the chromosomal instability. Conspicuous chromosomal malformation with addition or deletion of complete chromosome or part of a chromosome is typical of CIN. Also, translocations and chromosomal amplifications are common. The above abnormalities can influence the oncogenes, growth regulators, tumour suppressor genes, DNA repair genes and cell cycle checkpoint control genes. CIN in sporadic stomach cancers is very common and is present in up to 80% of GI cancers. [12]

Various alterations in the chromosomal numbers have been described and they have been linked to various factors like the tumor differentiation, invasion, lymph node spread and distant metastasis.

Loss of heterozygosity also represents the chromosomal instability. Evidence has shown that the degree of chromosomal loss is of clinical course and outcome. Studies have shown that, high-level LOH is associated with intestinal or mixed type of gastric cancers and low-level LOH is associated with diffuse growth pattern. LOH is associated with cancer advancement and conversion LOH-H from LOH-I indicate tumor progression.

The genetic mechanisms of CIN are largely unknown.

Mechanisms of chromosomal alterations include

1. Chromosome segregation defects

2. Defective DNA repair

3. Defects in cell cycle regulators

4. Dysfunction of telomeres

In genetically vulnerable patients, certain mutagens like H.pylori, tobacco and nitrites affect the normal chromosomal stability. They also

increase the risk of gastric cancer by oxidant free radical mediated tissue damage.

Chromosome Segregation Dysfunction

Segregation is a fundamental process in all cells with frequent mitoses including the mucosal cells of the stomach wall. When the regulatory systems controlling these actions fail, the resultant cells will either have errors in DNA or errors in the spindle. The cells will consequently transmit these mutations or have an abnormal chromosomal number.

Mechanisms for CIN development due to segregation dysfunction:

1.   Expression defects

2.   Genetic defects in segregation

3.   Carcinogens activity on individuals with susceptible genetic background.

Defective DNA Damage Response

Gastric wall mucosal cells are continuously under the influence of cellular and intraluminal carcinogens. These tumorogenic factors cause damage in the DNA by various pathways.

The normal DNA repair systems are as follows:

1. Nucleotide excision for in vivo or oxidative defects

2. Adduct restoration by excising the nucleotide

3. Mismatch restoration

4. Slippage or recombination for restoring breaks in double-stranded
   DNA.

When the above mechanisms fail, CIN and genomic aberrations occur resulting in malignancy.

Helicobacter-pylori induced chromosomal instability

H.pylori induces double stranded breaks in the DNA thereby leading to chromosomal instability.

Helicobacter pylori infection initiates a chronic inflammatory trigger that may lead to carcinogenesis by the following mechanisms:

1. Rapid cell division and mitoses

2. Accelerated mutagenesis

3. Free radicals induced damage

4. Negative regulation of repair mechanisms

The immune cells at the inflammatory area produce oxygen free radicals and reactive nitrogen radicals play an important role in H.pylori associated damage to the DNA. The DNA changes include cross linking of the DNA, single stranded DNA breaks, direct mutation in p53 gene, and inhibition of apoptosis by nitrosylation of caspases, protein damage by nitrosylation, and promotion of angiogenesis. The genetic makeup of an individual has a significant part in modulating the above events.

Microsatellite Instability (MSI)

Microsatellite Instability (MSI) is also commonly recognized in gastric cancers. MSI is a classical chromosomal feature of hereditary stomach cancer, developing with Lynch syndrome and in up to 50% of sporadic cancers.

MSI patients have a higher rate of defects in replication. MMR genes usually identify and restore the defects. Abnormalities in MMR genes, especially *MLH1/MSH2* causes tumor suppressor gene inactivation and LOH, thereby leading to cancer. [5]

Defective mismatch repair can occur by the following ways

1. Inactive MMR genes due to mutation

2. Inactive MMR genes due to epigenetic mechanisms

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In stomach cancer, defective mismatch repair is typically caused by CpG island methylator pathway (CIMP).

Genes that are frequently affected because of defective mismatch repair include cellular cycle genes and apoptotic genes and that are involved in genomic integrity maintenance. These alterations further promote genetic instability and enhance the carcinogenesis.

Mutation in E-cadherin gene (CDH1)

E-cadherin mutations are well documented in younger age stomach tumors and in hereditary tumors. Somatic inactivation of this gene manifests as aggressive tumor with poor prognosis.

IMPORTANT GENE ABNORMALITIES

Members of Tyrosine kinase family

Tyrosine kinase family genes including HER2/neu, K-sam and c-met when amplified indicate stomach cancer advancement. [7]

The c-met oncogene, a member of the hepatocyte growth factor receptor family is more commonly involved in diffuse cancers. The c-met oncogene mutation is associated with advanced tumor stage and poor outlook.

The oncogene K-sam belongs to the fibroblast growth factor receptor family, and is commonly involved in diffuse cancers. Increased expression is seen with 30% of diffuse stomach cancers. The K-sam oncogene mutation is associated with poor outlook.

The HER2/neu glycoprotein is homologous to the epidermal growth factor receptor. Studies demonstrate that increased expression is preferentially found in intestinal type cancers and serve as an indicator for nodal metastasis and invasion. Increased expression of HER2/neu protein is up to 30% of stomach malignancy. Also, increased expression of HER2/neu predicts a poor survival.

RUNX3

RUNX3, is a member of the runt domain-containing family of transcription factors and is expressed in only 40% of gastric cancers. This factor affects cell growth, angiogenesis and apoptosis by having a negative influence on the vascular endothelial growth factor (VEGF) and positive influence on the levels of p21. Low levels of RUNX3 are associated with poor survival.

Vascular Endothelial Growth Factor (VEGF)

The pro-angiogenic VEGF is commonly seen in increased levels in stomach cancer. P53 gene has a negative influence on the VEGF in normal conditions and may be a factor in the increased level of VEGF. VEGF levels correlate with nodal spread as well as hepatic spread. Increased levels are associated with a poor outlook.

Cyclooxygenase 2(COX2)

This is a key enzyme in prostaglandins production in the stomach mucosa and has a part in the stomach cancer pathogenesis. Increased expression of COX2 is associated with lymphatic spread, poor cellular differentiation and invasion and hence signifies a poor outlook.

Osteonectin

Osteonectin belongs to the group of matrix cellular proteins. They modulate cellular and matrix relations and influence cellular performance without taking part in the extracellular matrix structure. Overexpression of osteonectin correlates with distant metastasis and poor prognosis.

P53

P53 has a basic role in cellular growth and differentiation. Genetic mutations and loss of heterozygosity are the common factors behind P*53* gene abnormality. P53 abnormalities are seen in well differentiated stomach tumors in up to 35% of cancers. P53 abnormalities are not seen in early stage diffuse type tumors and tumors in young patients. They are found in increased levels in early stage intestinal type tumors and in later stages of the disease.

P21

The *p21* protein is a cyclin dependant kinase inhibitor (CDK I) mediating the cell cycle regulatory function of p53. Survival of gastric cancer patients with p21-positive tumors is significantly longer than p21-negative tumors. The expression of p21 assessed in combination with p53 status contributes to predict the clinical outcome in gastric cancer patients.

p27

P27 is another cyclin-dependent inhibitor, which controls the transition from G1 to S in the cell cycle. Reduced expression of p27 is seen in approximately 45% of gastric cancers. Tumors with a low expression of p27 protein are poorly differentiated and present at an advanced stage. P27 has also been analyzed in combination with p21 and p53, as prognostic markers.

BCL2

BCL2 is involved in the regulation of apoptosis. LOH of the *BCL2* is frequently observed in gastric cancer. BCL2 overexpression reduces cellular proliferative activity and correlates with a less aggressive behavior of the tumor.

BAX

BAX gene encodes a protein of the BCL family members. Decreased expression of BAX has been associated with poor differentiation, lymph node metastasis and a shorter survival.

C-myc

C-myc gene encodes nuclear phosphoprotein that plays an important role in cell cycle progression, cellular transformation and apoptosis. It acts

as a transcription factor that regulates transcription of target genes. The c-myc protein is significantly enhanced in well-differentiated gastric cancers and is associated with a poor prognosis. In gastric cancers, overexpression of the cancerous inhibitor of protein phosphate 2A (CIP2A) stabilizes c-myc. CIP2A overexpression is also associated with reduced overall survival.

Cyclin E

Cyclin E overexpression is a marker of tumor aggressiveness and correlates with invasiveness and proliferation. Reduced expression is associated with invasion and metastasis both diffuse and intestinal type gastric carcinomas.

APC

Inactivation of Adenomatous Polyposis Coli gene leads to activation of the Wnt-frizzled-β-catenin signaling pathway and is frequently seen in gastric carcinoma. LOH of APC gene occurs in approximately 25% of intestinal type gastric carcinomas. Inactivation of APC gene leads to poor differentiation and increased tumor invasiveness.

MUC1

Mucins are high molecular weight glycoproteins that are major components of the mucus and protect the gastric epithelium. Mucin 1 (MUC1) overexpression is linked to accelerated tumor invasion by the impairment of E-cadherin and indicates a poor prognosis in gastric cancer. Survival for gastric cancer patients with abnormal E-cadherin and MUC positive expression is shorter than others.

ARIDI1A

ARIDI1A, the short form for AT-rich interactive domain 1A, are mutated in 90% of patients with MSI, 70% of Epstein-Barr virus infected patients and in 10% of microsatellite stable and non EBV infected patients.

ARIDI1A controls genetic expressions by attaching itself to the AT-rich sequence of the DNA. It is involved in repair of the DNA and plays a controlling part in cellular division. ARIDI1A abnormalities predict a comparatively better outlook in high MSI and EBVpositive stomach cancers. ARIDI1A loss indicates a large tumor, invasiveness, nodal spread and poor outlook in MSS and EBV negative stomach tumors. ARIDI1A abnormalities are also inversely related to the P53 gene mutations and directly related with the PIK3CA genetic abnormalities..

EGFR

EGFR, or ErbB1, is a member of the tyrosine kinases family and functions as a transmembrane receptor. It has an intracellular cytoplasmic domain, a transmembrane portion and an extracellular binding domain. Binding of a ligand to the extracellular domain leads to tyrosine kinase phosphorylation and activation. The primary intracellular pathways that are activated following the phosphorylation of EGFR include:

1. Phosphoinositol-3-kinase (PI3K)/Akt pathway

2. RAS/mitogen activated protein kinase (MAPK) pathway.

The PI3K pathway is involved in apoptotic and survival signalling. The RAS/MAPK pathway is involved in cancer cell proliferation and gene transcription.

EGFR inhibitors

Two classes of EGFR inhibitors are available:

1. Monoclonal antibodies

2. Small molecule tyrosine kinase inhibitors.

Monoclonal antibodies include cetuximab and panitumumab. Small molecule tyrosine kinase inhibitors include erlotinib and gefitinib.

FGFR2

FGFR2 is another member of the tyrosine kinases family and functions as a transmembrane receptor. It has an intracellular cytoplasmic tyrosine kinase domain, a transmembrane hydrophobic segment and an extracellular, three immunoglobulin-like domains. The extracellular portion interacts with fibroblast growth factors, triggering a cascade of downstream signals, which ultimately influence mitogenesis and cellular differentiation. The FGFR signalling axis plays a very important role in normal skeletal, organ and vascular development. Germline mutation of the FGFR gene is implicated in a variety of congenital disorders. In gastric cancer, FGFR2 amplification occurs more frequently in undifferentiated and diffuse cancers.

Inhibition of FGFR signaling can result in anti-proliferative and pro-apoptotic effects.

Survival in Gastric Cancer Patients

The prognosis of gastric cancer varies enormously across the world, remaining dismal in the west whereas it has become favourable in Japan. The 5-year survival on an average is only 25% in the west. In contrast, the 5-year survival is around 60% in patients with gastric cancer in Japan.

The good prognosis of carcinoma stomach in Japan is the result of aggressive screening strategy and detection of early stage cancers. The lack of improvement in survival in other areas has been attributed to aggressive and advanced stage gastric cancers. Hence, controlling the stage of the disease and modifying the risk factors are the factors that will lead to improved patient outcomes.

Prevention of Gastric Cancer

Gastric cancer prevention can be done by two strategies:

1. Primary Prevention

    Helicobacter pylori eradication

    Modifying other risk factors

2. Secondary Prevention

    Screening

Targeted molecular therapies in Gastric Cancer

The prognosis of gastric cancer patients in Indian scenario is very bleak. Primary and secondary prevention strategies are not viable on large scale and effective treatment, once the disease has occurred is the only practical way at present. As far as the treatment is concerned, vast majority of our patients present at a late and very advanced stage and curative surgery is not feasible for them. Effective chemotherapy is the only hope for them.

The chemotherapy regimens at present are not uniformly effective and the response is highly variable. Targeted molecular therapy can be of great help in such patients. Also, the targeted therapies can be used in early stage disease also as adjuvant therapy.

The candidate genes/pathways that are actively investigated for molecular target therapy in gastric cancer includes [11]

1. Epidermal growth factor (EGFR)

2. Vascular endothelial growth factor (VEGF)

3. P13K/AKT/mTOR pathway

4. Insulin-like growth factor receptor (IGFR)

5. MET pathways

6. FGFR

Various target agents are under study and the agents that are most promising and in phase III clinical trials include

1. EGFR inhibitors

2. VEGF inhibitors

EGFR (Epidermal Growth Factor Receptor) inhibitors

Trastuzumab

Trastuzumab is a targeted agent against HER2 (c-erbB2) proto-oncogene that encodes a tyrosine kinase receptor. HER2 (c-erbB2) is overexpressed in many cancers, including gastric cancers. The ToGA trial compared trastuzumab with chemotherapy and chemotherapy alone in HER2-positive advanced gastric cancer patients. The study demonstrated that adding trastuzumab to conventional chemotherapy is superior in patients with HER2- positive advanced gastric cancer than chemotherapy alone.

Lapatinib is another HER2 targeted agent which inhibits both EGFR and HER2. It is actively investigated for trastuzumab- resistant gastric

VEGF (Vascular Endothelial Growth Factor) inhibitors

Bevacizumab

Bevacizumab is a humanized monoclonal antibody against VEGF. Bevacizmab is proved effective in colorectal, renal and lung malignancy as well as recurrent glioblastoma.

The phase III study, the AVAGAST trial evaluated bevacizumab combined with chemotherapy and chemotherapy alone in unresectable gastric cancers. There was improvement in progression free survival and overall response rate in bevacizumab treated patients.

Sunitinib and Sorafenib

These are also tyrosine kinase inhibitors with multi target activity, recently introduced against VEGF.

AIM OF THE STUDY

To investigate the mRNA expression levels of *EGFR, FGFR2 & C-Myc genes* in the gastric cancers and their clinical significance.

MATERIALS AND METHODS

Study Centre

Department of Surgical Gastroenterology

Center of Excellence for Upper G.I Surgery

Madras Medical College &

Rajiv Gandhi Government General Hospital

Chennai – 3

&

Department of Genetics

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University of Madras

Taramani, Chennai – 600113

Study Period

August 2010 to December 2012

Study Design

Genetic Analysis

Sample Size

25

Inclusion Criteria

Patients who underwent Gastrectomy (Subtotal or Total) for Carcinoma stomach

Exclusion Criteria

Patients with Locally advanced disease

Patients with Metastatic disease

Analysis Plan

Comparing the gene levels of the putative genes with the reference gene

METHODOLOGY

The study was approved by the ethics committee of Madras Medical College and Rajiv Gandhi Government General Hospital. Twenty five patients fulfilling the study criteria and willing to give written and informed consent were recruited for the study.

Cancerous and paired normal mucosa was collected after informed consent from patients who undergo surgical resection. The sample is then taken for genetic analysis.

The steps of genetic analysis can be summarized as follows:

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┌─────────────────────────────────────────────────────┐
│   Paired Cancerous & Normal mucosa from 25 Patients   │
└─────────────────────────────────────────────────────┘
                         ⇩
┌─────────────────────────────────────────────────────┐
│           RNA isolation (TRI reagent method)          │
└─────────────────────────────────────────────────────┘
                         ⇩
┌─────────────────────────────────────────────────────┐
│         UV Spectro - photometric Quality Check        │
└─────────────────────────────────────────────────────┘
                         ⇩
┌─────────────────────────────────────────────────────┐
│                   Amplified by PCR                    │
└─────────────────────────────────────────────────────┘
                         ⇩
┌─────────────────────────────────────────────────────┐
│            Converted to cDNA (By RT- PCR)             │
└─────────────────────────────────────────────────────┘
                         ⇩
┌─────────────────────────────────────────────────────┐
│ Electrophoresis & Semi-quantitative Analysis (UV band method) │
└─────────────────────────────────────────────────────┘
```
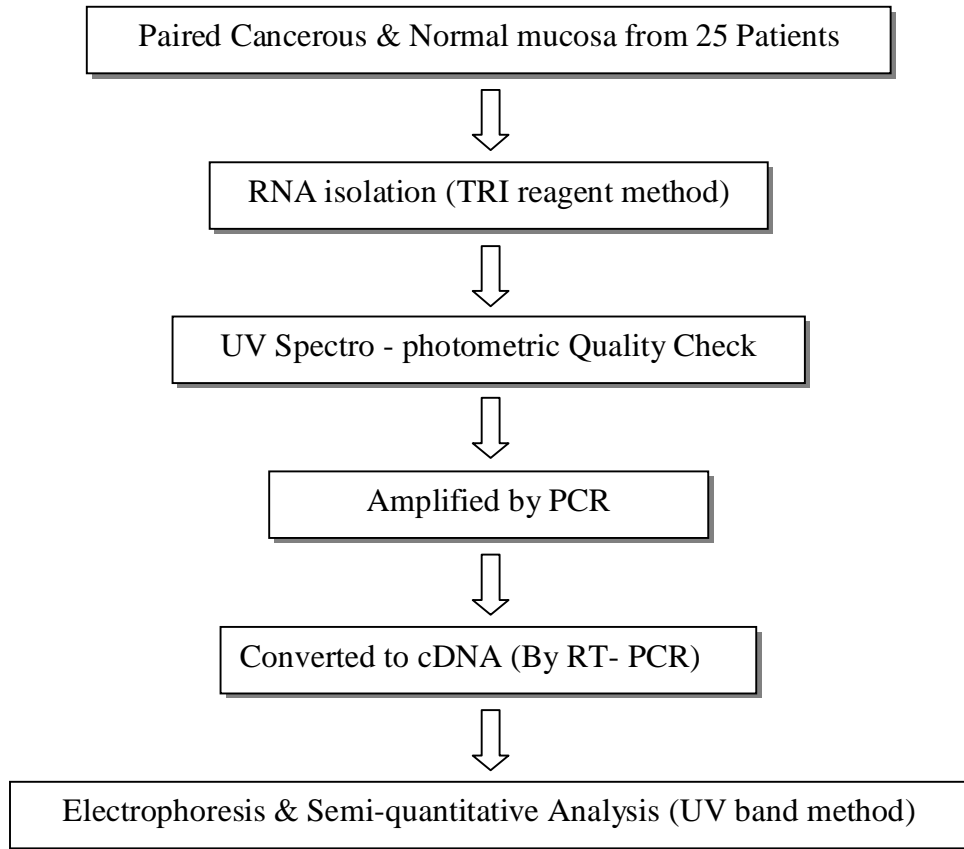
Sample collection and Transport

The tumor as well as normal tissue specimens were collected from the fresh gastrectomy (Total / Sub-total) specimen removed for curative intent treatment of cancer stomach.

The tumor samples were collected in RNA*later®,* a dedicated solution to maintain RNA stable in the tissue when sample transport is performed in between the hospital and laboratory. All the tumor samples obtained from the patients were identified clinically and confirmed by pathological biopsy report.

Total RNA isolation

Total RNA was isolated using TRI® reagent method. 50 mg of tumor and normal tissue were taken in separate tubes and homogenized with 1 ml of TRIzol. After homogenization, the homogenate was transferred into fresh tube and 200 µl of chloroform was added. The tubes were then, vigorously shaken for 15 min and centrifuged at 12,000 rpm for 15 min at $4°C$. After centrifugation the aqueous phase was transferred into fresh tube and 500 µl of Isopropanol was added. Then the tubes were frozen at -80 $°C$ for 3 hours. Later the tubes were taken out and thawed over ice and immediately centrifuged at 12,000 rpm for about 15 min at $4°C$. The supernatant was decanted and with the pellet 750 µl 75% Ethanol was added. The sample tubes were centrifuged at 12,000 rpm for 15 min at $4°C$. After centrifugation, the supernatant was discarded and pellet was air dried at room temperature. Then the pellet was resuspended in 50 µl of RNase-free water and stored at -80$°C$ for later usage.

UV Spectrophotometric quantification

After the isolation of RNA, 1 µl of RNA was used to analyze the quality using the UV spectrophometer (Nanodrop, Thermo).The A_{260} and A_{280} Values were obtained .Based upon the A_{260}/A_{280} value, samples between 1.8 to 2.1 were chosen as RNA samples with good yield and free from other impurities.
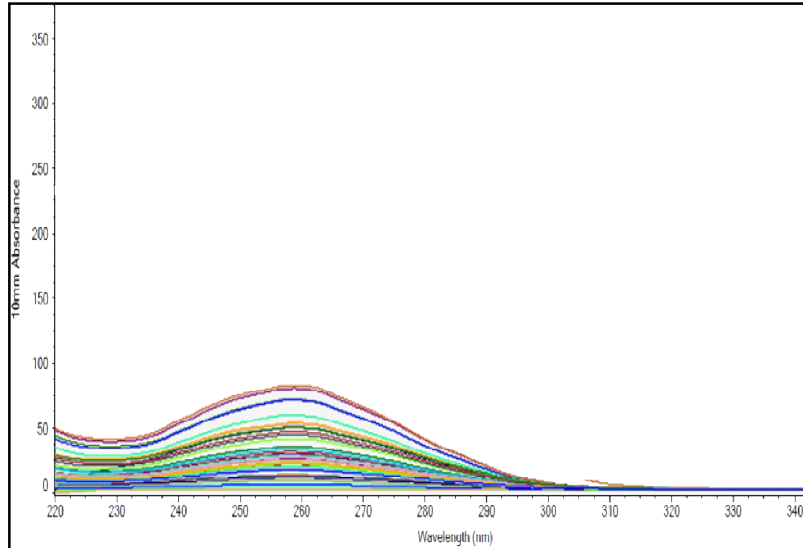
cDNA Synthesis

Based upon the RNA quality samples were selected for the Reverse transcriptase Polymerase chain reaction to convert the RNA into cDNA molecules. 5 µg of RNA was taken from each sample and diluted with DEPC treated water and Random haxamer (100 µM) for the final volume 12 µL. Then the mixture was kept at 65°C for 20 Minutes in PCR machine. After incubation the tubes were transferred on ice. The Pre-PCR mix was mixed with RT buffer (1X); DTT (0.01M), dNTP (0.5mM).Finally, Superscript III (Invitrogen Inc, USA) reverse transcriptase enzyme (200U) was added separately into each reaction tube. The Final Volume was adjusted to 20.0 µL and the tubes were kept at 50°C for 90 Minutes in PCR machine. The reaction cycle was programmed as follows: 70°C for 15 Minutes then 4°C for 15 Minutes. After the completion of PCR, reaction

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the tubes were stored at -20°C. Then, 1:24 dilution was prepared with PCR grade water for downstream application.

PCR using cDNA template

Polymerase chain reaction (PCR) methodology was standardized using the laboratory working protocols. The concentration of reagents and enzymes are given as below:

Reagents	Stock	Final	Per 10 µL Rxn	Volume Required (µL)	Total No.Rxn (X=?)
Template DNA	100ng/µL	*	*	1	9
Taq Pol U /µL	5	*	0.25U	**0.05**	0.45
10 X PCR Buffer	10	1	10	**1**	9
MgCl2 mM	25	2.5	10	**1**	9
dNTP µM	2500	100	10	**0.4**	3.6
F Primer nM	2000	80	10	**0.4**	3.6
R Primer nM	2000	80	10	**0.4**	3.6
DD.H2O	*	*	10	**5.75**	51.75
Master Mix					81
For 10 µL Rxn					9

All PCR primers were designed using bioinformatic tools available in the internet. The annealing temperature of the primers was normalized to 60°C.The sequences of oligos have been given below:

EGFR: Forward 5'-**AGGGACTGCGTCTCTTGCCG**-3'

EGFR: Reverse 5'- **CCTGGCCCAGTGCATCCGTAG** -3'

FGFR2: Forward 5'- **AGCGGCTGTACTGCAAAAACGG** -3'

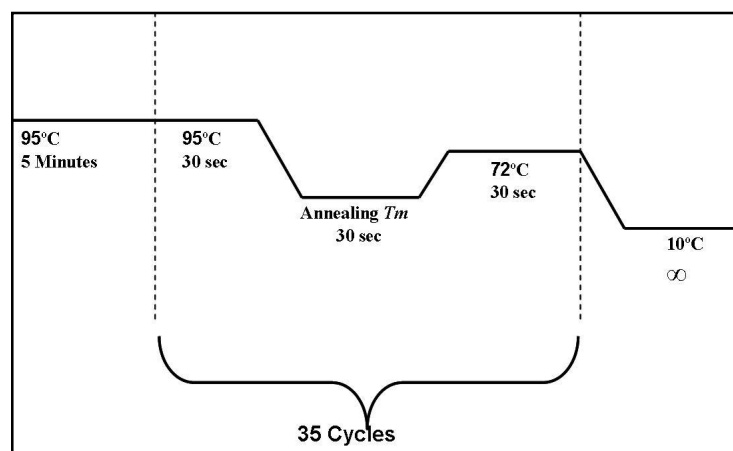FGFR2: Reverse 5'- **AGCCAGGTAACGGTTAGCACAC** -3'

c-Myc: Forward 5'- **AGCGAATAGGGGGCTTCGCC** -3'

c-Myc: Reverse 5'- **GGGAGGCTGCTGGTTTTCCAC** -3'

GAPDH: Forward **5'-TTCGACAGTCAGCCGCATCTTCTT-3'**

GAPDH: Reverse **5'-CAGGCGCCCAATACGACCAAATC-3'**

The amplification of cDNA was carried out in ABI GeneAmp 9700 thermal cycler. The reaction condition has been given as below:

The PCR amplicons obtained from different gene targets namely GAPDH and EGFR, FGFR2 and C-Myc. 10 µl of PCR product was mixed 2 µl of 6X gel loading dye. The samples were resolved in 1 % agarose TAE gel containing Ethidium bromide. The Electrophoresis was carried out at 100V for 20 Minutes. After the visual observation of loading dye migration, the gels were UV photographed with the aid of UV-transilluminator. Finally the photographs were saved for further gel quantification analysis.

Semi quantification Gene expression level

Amplified PCR products were resolved in 1 % TAE agarose gel stained with Ethidium Bromide. The gel image was captured in UV-Transilluminator and subsequently the band intensity of the PCR products were calculated using UVI band software available with gel imaging system. The band intensity of reference GAPDH was compared with band images of EGFR, FGFR2 and C-Myc. The comparative signals were then tabulated to generate a bar diagram summarizing the gene expression pattern.

RESULTS

The analysis demonstrates that the genes *EGFR, FGFR and C-Myc* are highly expressed in tumor mucosa when compared with the normal mucosa.

We found that C-Myc and EGFR are expressed more abundantly when compared to FGFR2.

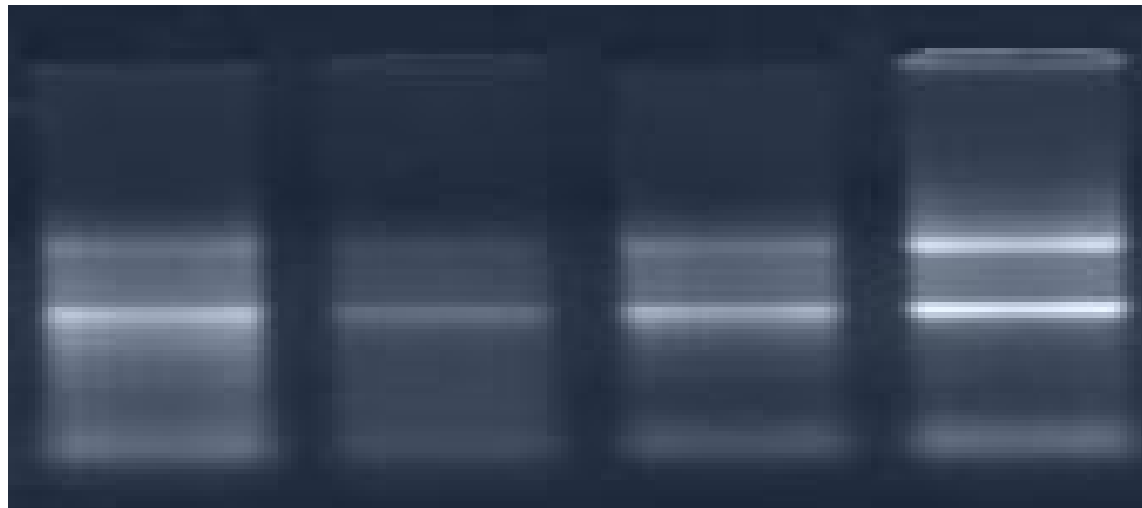The results of the study are pictorially depicted as follows:

Total RNA isolated from Gastric tumor Mucosa

Agarose Gel Electrophoregram of PCR product of GAPDH mRNA

100bp **T1** **T2** **T3** **T4**

→ 327 bp
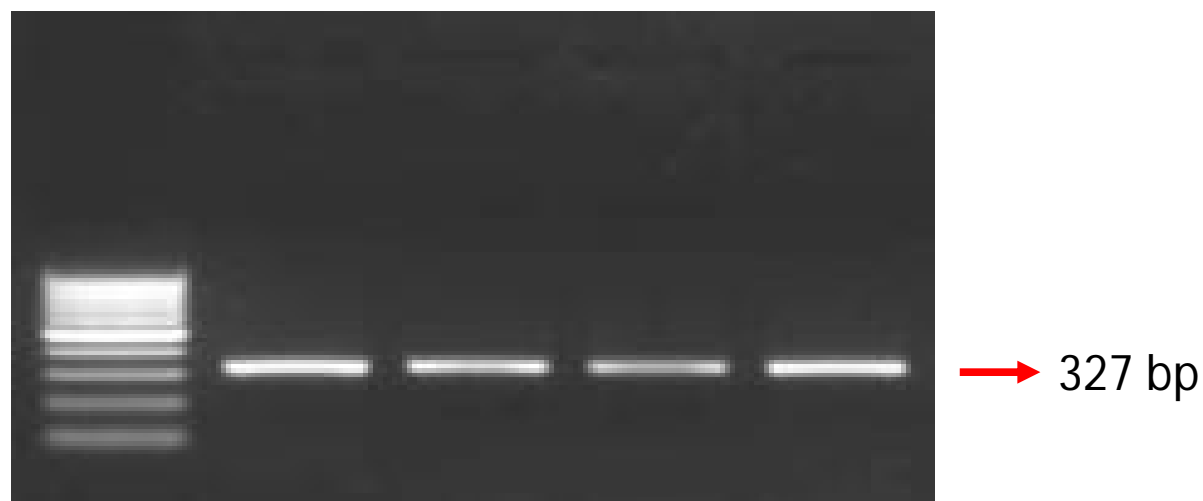
Sample Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Tumor	98.73	92.023	94.18	96.2	97.66	98.4	93.19	99.54	98	93.98	94.67	94.29	97.9	96.9	97.32

Sample Number	16	17	18	19	20	21	22	23	24	25
Tumor	98.642	94.83	92.96	95.8	97.3	96.74	96.82	98.34	96.39	98.47

GAPDH mRNA Expression Level
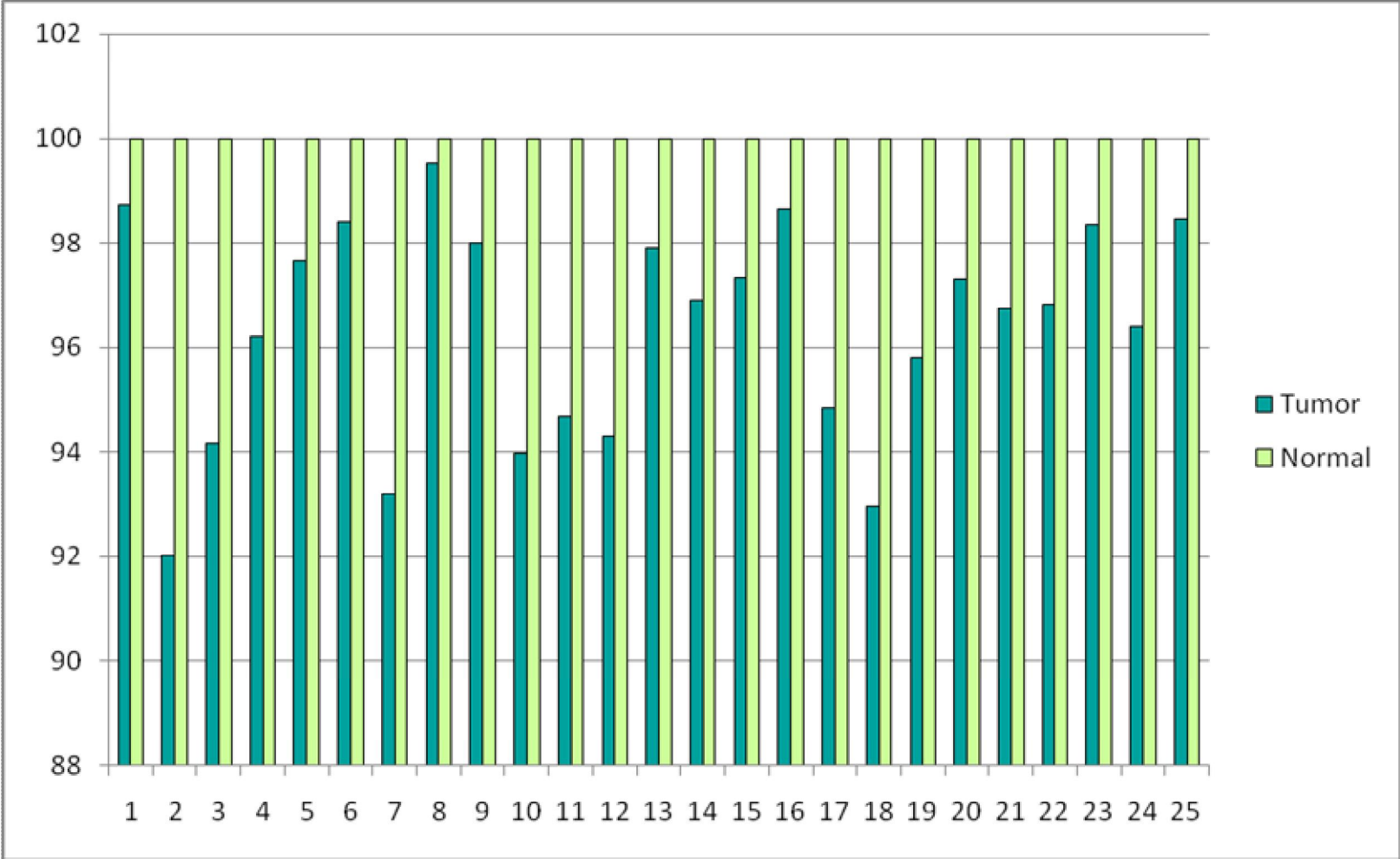
Agarose Gel Electrophoregram of PCR product - EGFR mRNA
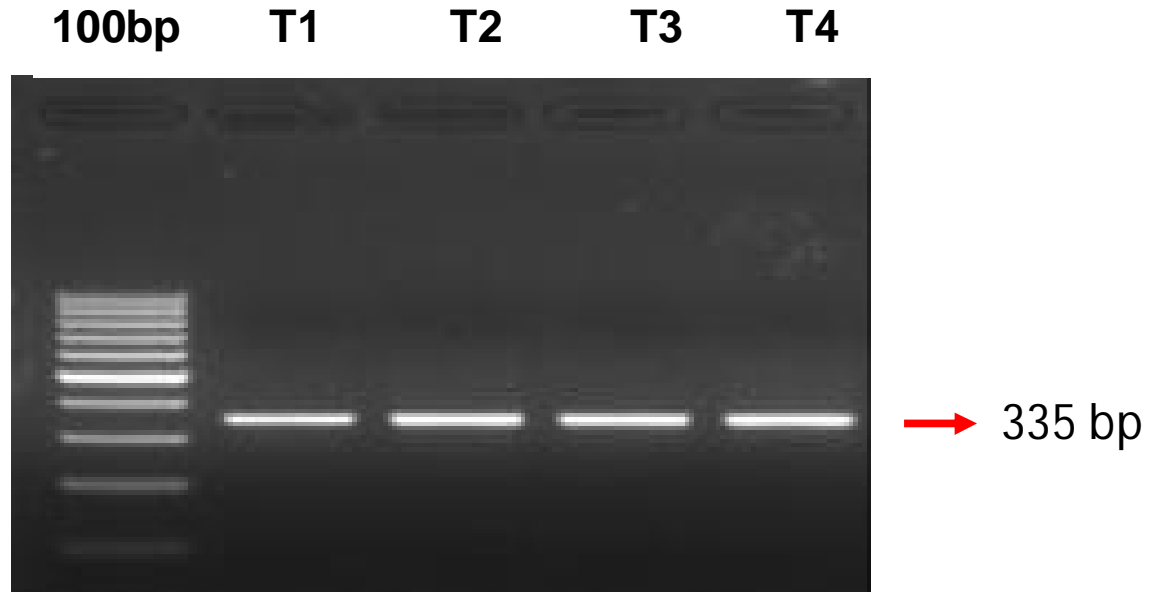
Sample Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Tumor	118.56	102.06	104.66	117.3	92.5	99.2	108.65	114.58	125.4	148.71	152.51	76.96	106.036	112.936	120.736

Sample Number	16	17	18	19	20	21	22	23	24	25
Tumor	120.42	141.08	131.02	126.7	128.23	102.72	83.16	114.8	111.21	134.23

EGFR mRNA Expression Level
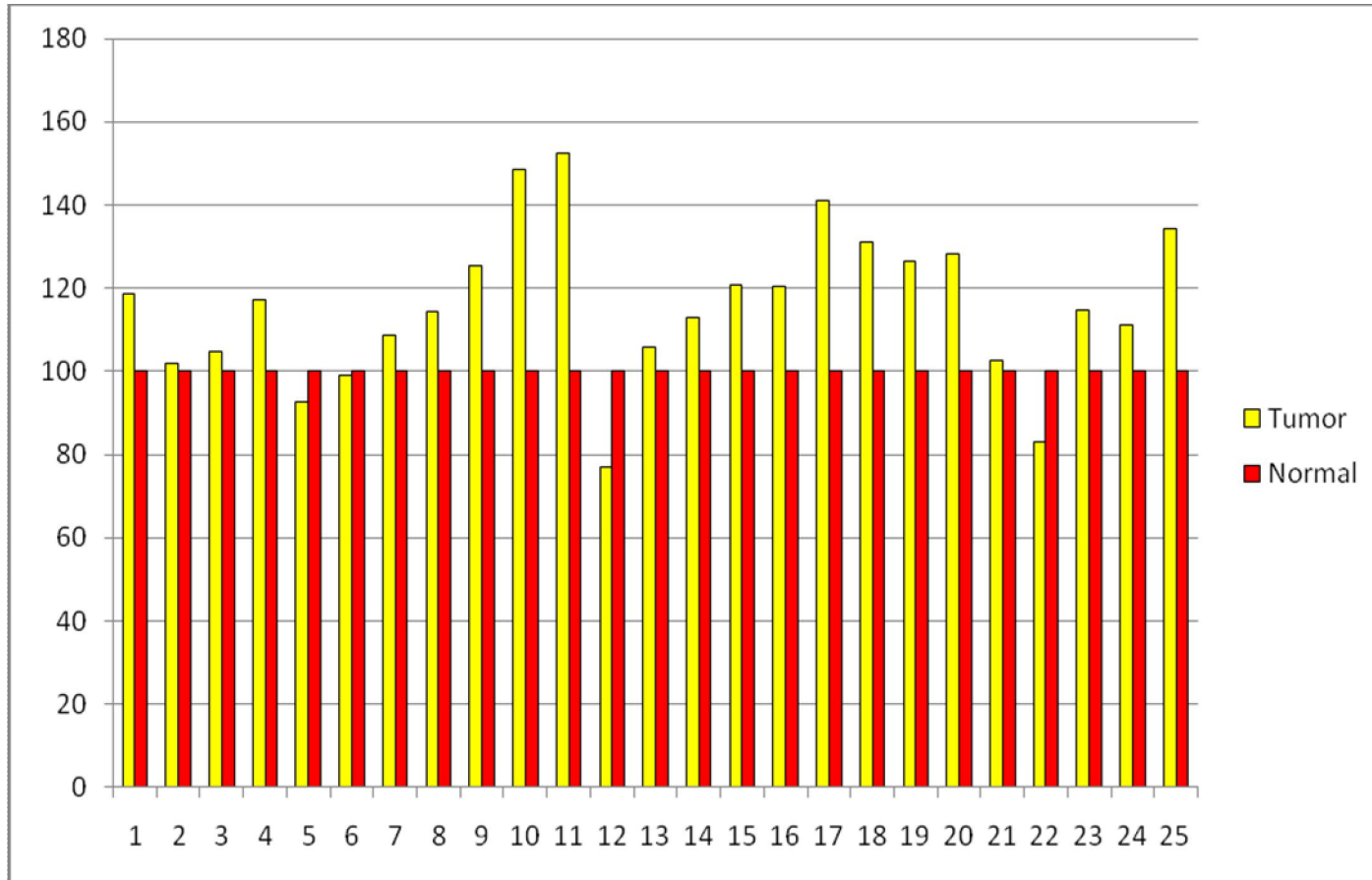
Agarose Gel Electrophoregram of PCR product - FGFR2 mRNA

100bp   T1   T2   T3   T4

→ 164 bp
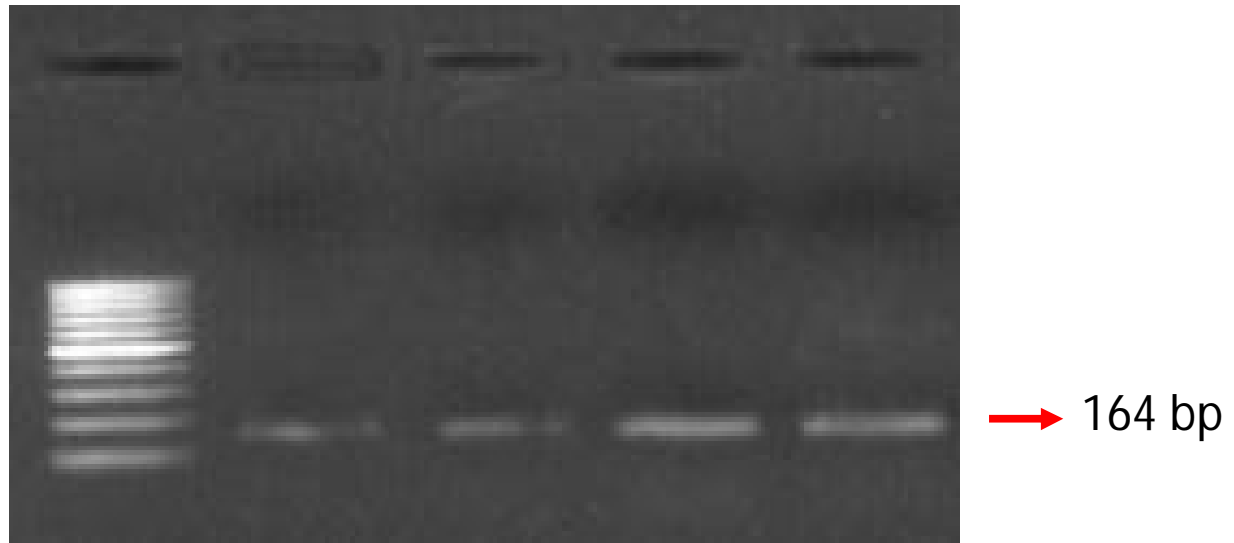
Sample Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Tumor	89.73	78.93	83.8	75.2	132.2	125.07	127.87	119.57	111.27	102.97	94.67	76.37	73.37	50.07	98.37

Sample Number	16	17	18	19	20	21	22	23	24	25
Tumor	90.07	109.07	124.37	116.07	150.37	99.47	91.17	82.87	104.17	107.77

FGFR2 mRNA Expression Level
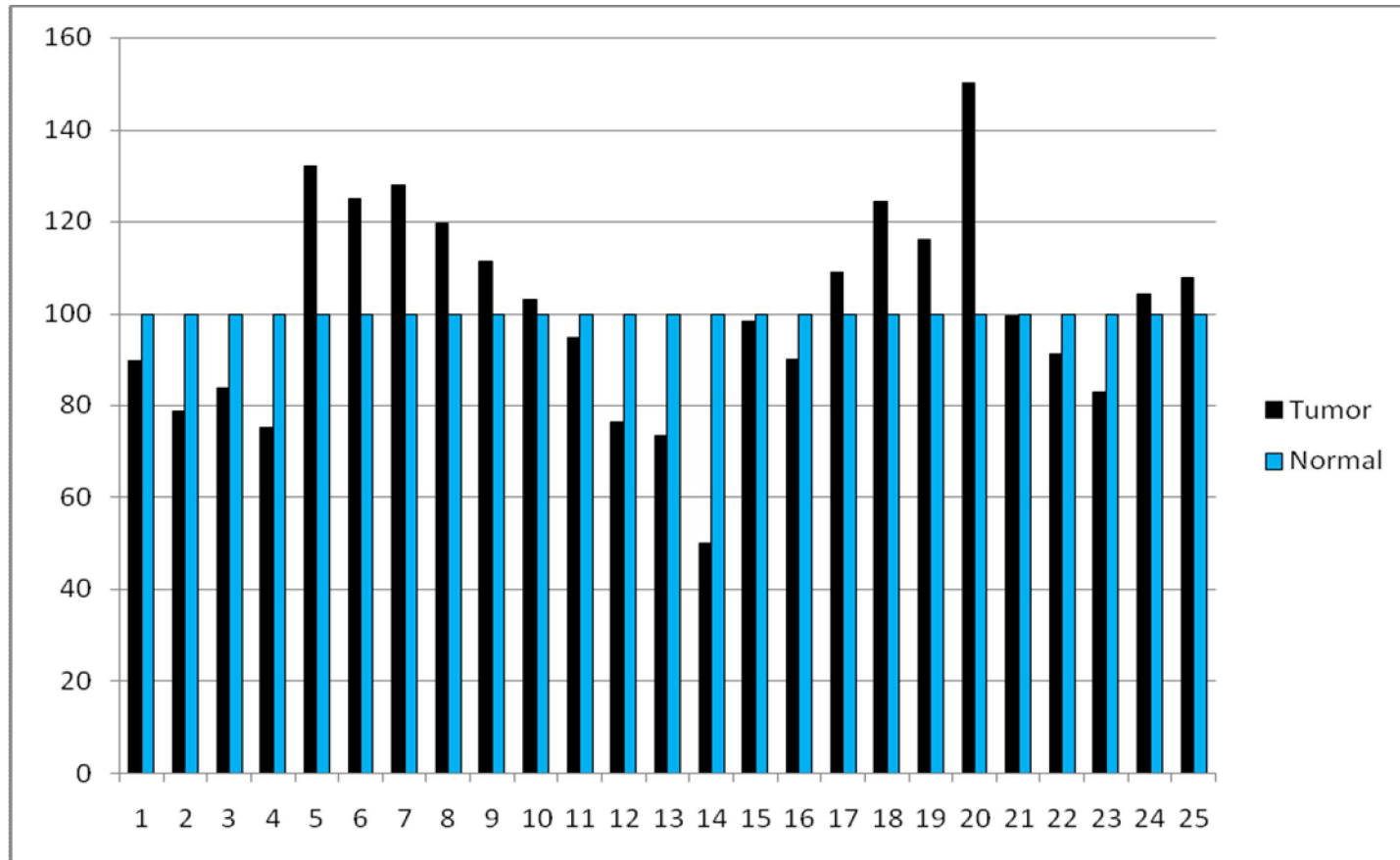
Agarose Gel Electrophoregram of PCR product - c- Myc mRNA

→ 228 bp
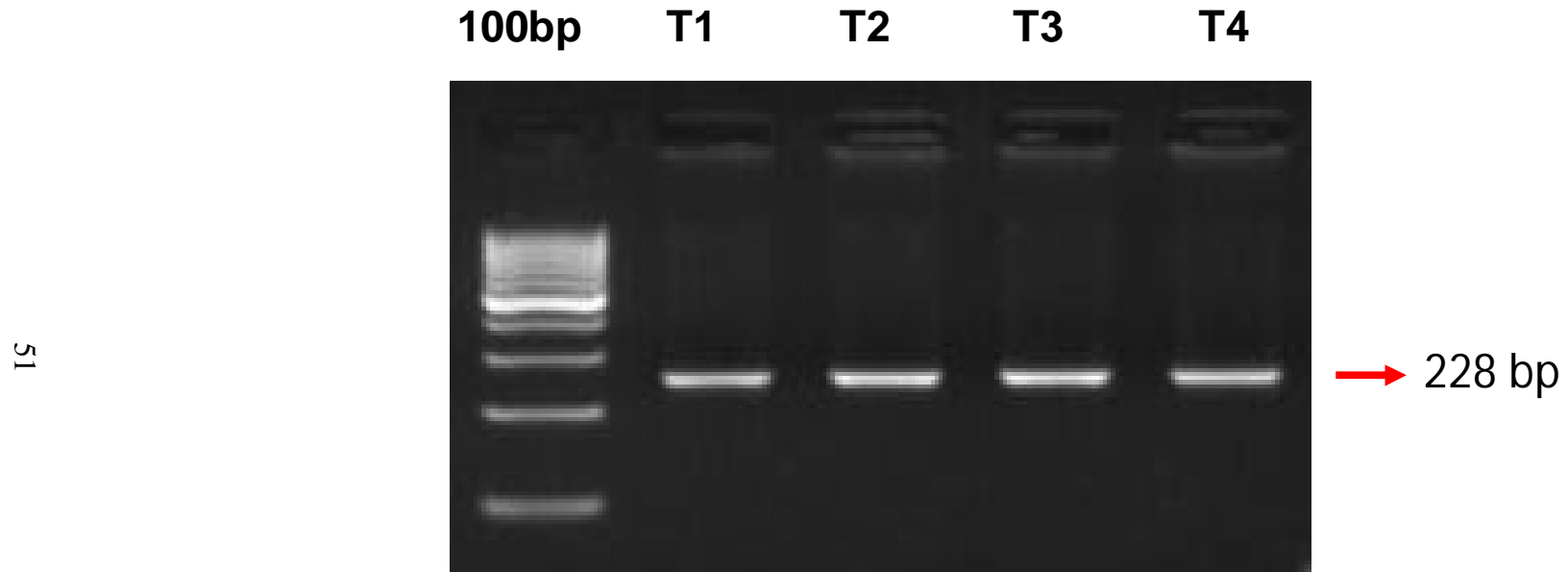
Sample Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Tumor	132.73	133.47	138.21	128.37	105.91	111.65	98.945	118.685	103.72	112.854	113.594	119.914	122.714	130.454	121.714

Sample Number	16	17	18	19	20	21	22	23	24	25
Tumor	109.674	128.414	93.684	111.504	112.244	112.984	113.724	114.464	115.204	115.944

C-Myc mRNA Expression Level
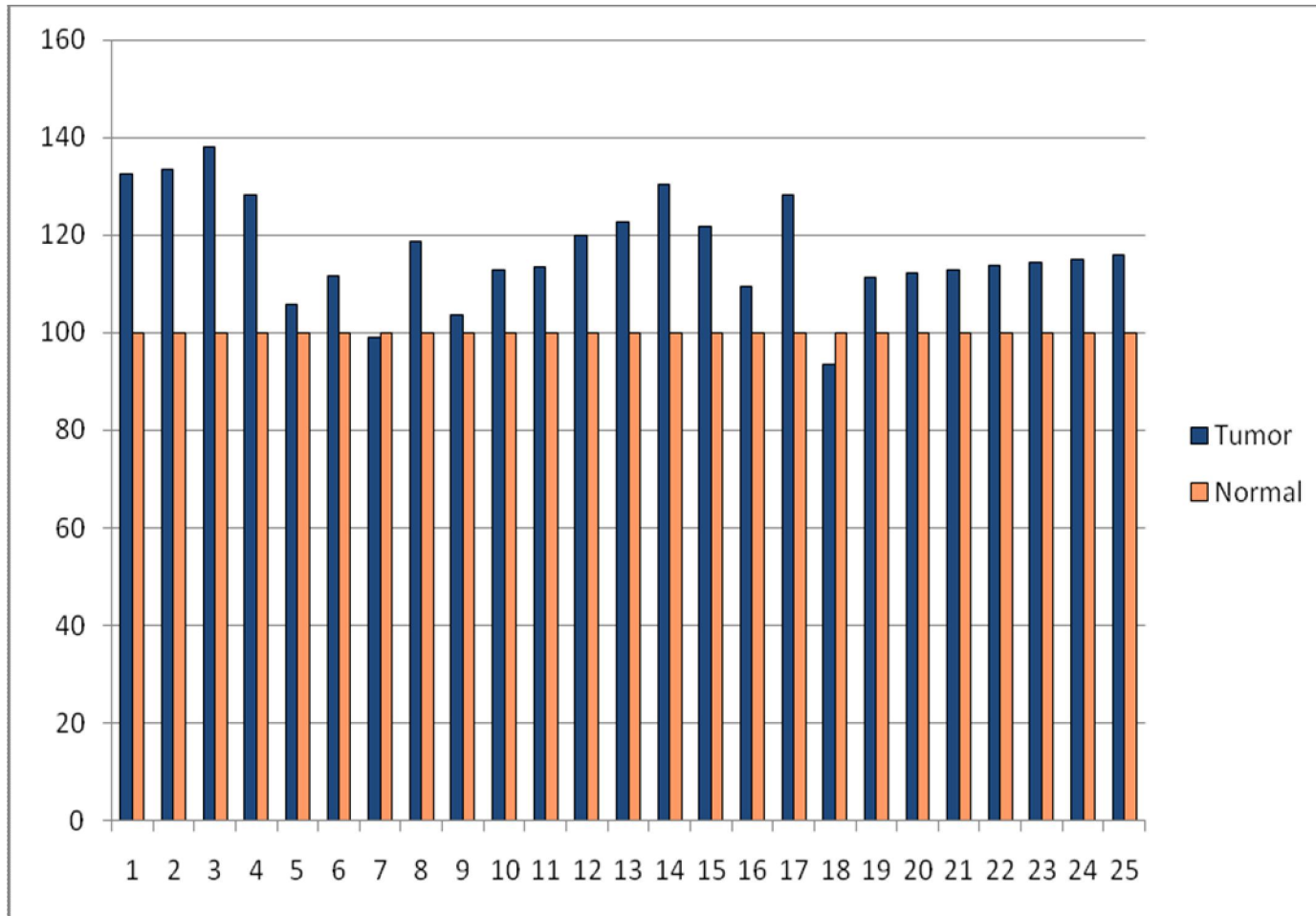
DISCUSSION

Stomach cancer is a major age associated cancer. Surgical therapy is the most promising treatment modality for the management.

Identification of early stage cancers and recurrences by unique molecular signature is possible in these tumors. A novel non-invasive method towards the early diagnosis of Gastric cancer remains a global hunt for decades.

Levels of *EGFR, FGFR2 and C-Myc* genes are frequently overexpressed in glandular epithelial cancers, including Gastric cancer. The mRNA level of tumor specific genes *EGFR, FGFR2 & C-Myc* is often associated with gastric carcinogenesis by triggering a cascade of molecular events.

This is the first study of expression of the above three genes in Indian patients. This study analyzes the mRNA expression levels of *EGFR, FGFR2 & C-Myc genes* in the gastric cancers and their clinical significance. This study was a prospective genetic analysis study done during the period August 2010 to December 2012.

This is a pilot study and hence, only 25 patients were recruited for genetic analysis after written informed consent and ethical committee

approval. The positive result of this study has prompted us to extend the study with large sample size and advanced experimental set up to prove the usefulness of these molecular markers in the gastric cancer patient management.

Also, further correlation of the levels of these mRNAs with clinic-pathological features is planned as an extension of this study.

This study is based on the study by HK Kim et al, [14] from the National Cancer Institute, Bethesda, MD, USA. HK Kim et al analysed the transcriptional profiles of stomach cancer patients to assess the effects of chemotherapy (fluorouracil and cisplatin). They concluded that combined expression of C-MYC, FGFR2 and EGFR in metastatic gastric cancer is predictive of poor survival in chemotherapy treated patients.

Based on the above study, we analysed the above three gene signature in our patients with cancer of stomach cancer who underwent gastrectomy with curative intent.curative gastrectomy. The reference gene in our study was also GAPDH (Glyceraldehyde-3-phosphate dehydrogenase), one of the most common housekeeping genes used to normalize gene expression data in genetic studies.

Our results show that the three gene signature (*EGFR, FGFR2 & C-Myc)* is significantly overexpressed in the gastric cancers even in the early

stage of the disease. This is the only study in the world literature, demonstrating the usefulness of this gene signature in early stage gastric cancers.

This opens up a whole new window of exciting possibilities directed against these genes in early stage gastric cancers including

1. Diagnosis of early cancers

2. Targeted molecular therapy

3. Predicting the survival after surgery

4. Predicting tumor response to chemotherapeutic drugs

But, our study results need confirmation in a larger clinical trial with adequate sample size to prove the role of this gene signature conclusively.

REVIEW OF LITERATURE

The study by HK Kim et al, [14] from the National Cancer Institute, Bethesda, MD, USA is the only other literature available regarding this three gene signature.

They have studied the transcriptional profiles of gastric cancer patients to predict the usefulness of chemotherapy (fluorouracil and cisplatin) in patients with metastatic disease.

The study was done at the National Cancer Center Hospital, Korea after Institutional Ethical Committee approval.

Their inclusion criteria included:

1. Age: More than 18 years

2. Biopsy proved adenocarcinoma of stomach

3. Distant metastasis – Clinically demonstrated

4. No other malignancy

5. No chemotherapy history before

6. Normal organ functions

Their exclusion criteria included:

1. Patients who didn't complete chemotherapy

The primary endpoint of their study was overall survival. The training set for expression profiling were the patients who underwent chemotherapy – a total of 96 patients. Validation and training samples underwent the same tissue procurement and processing.

Chemotherapy was continued until the patients had severe toxicities or the disease was progressive inspite of chemotherapy.

Genetic analysis was done using transcriptional profiling and Comparative Genomic Hybridization (CGH). Transcriptional profiling identified 917 genes that were correlated with poor patient survival after chemotherapy. Making use of the genes identified within the genomic amplicons, a risk predictor for survival was constructed.

The three genes (C-MYC, EGFR and FGFR2) when expressed together, independently predicted a poor overall survival. Thus, when expressed together, C-MYC, EGFR and FGFR2 were predictors of poor survival in metastatic stomach cancer cases treated with chemotherapy.

These three genes did not predict the prognosis and only predicted the chemotherapy response.

CONCLUSION

Our results show that the three gene signature (*EGFR, FGFR2 & C-Myc)* is significantly overexpressed in the gastric cancers and this opens up exciting possibilities in

1. Diagnosis of early gastric cancers

2. Targeted molecular therapy

3. Predicting the survival after surgery

4. Predicting tumor response to chemotherapeutic drugs

But, our study results need confirmation in a larger clinical trial with adequate sample size to prove the role of this gene signature conclusively.

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13. Rajini Seevaratnam et al, A systematic review of the indications for genetic testing and prophylactic gastrectomy among patients with hereditary diffuse gastric cancerGastric Cancer (2012) 15 (Suppl 1):S153–S163.

14. HK Kim et al, Three-gene predictor of clinical outcome for gastric cancer patients treated with chemotherapy, The Pharmacogenomics Journal (2010), 1–9.

CASE RECORD FORM

Name: **Age / Sex:**   **IP NO:**

Occupation: **Address:**

Contact Number:

LAB ID:

GENETIC ANALYSIS RESULT:

HISTOPATHOLOGY REPORT:

    Type of Malignancy:

    Grade:

PATIENT DETAILS

Lab ID	Gender/Age	Type of Malignancy	Pathological Status
GC001	F/52	Adenocarcinoma	Poorly Differentiated
GC002	F/35	Adenocarcinoma	Poorly Differentiated
GC003	M/43	Adenocarcinoma	Poorly Differentiated
GC004	M/50	Adenocarcinoma	Poorly Differentiated
GC005	M/62	Adenocarcinoma	Poorly Differentiated
GC006	F/62	Adenocarcinoma	Poorly Differentiated
GC007	M/48	Adenocarcinoma	Poorly Differentiated
GC008	F/28	Adenocarcinoma	Poorly Differentiated
GC009	F/50	Adenocarcinoma	Poorly Differentiated
GC010	M/73	Adenocarcinoma	Moderately Differentiated
GC011	M/80	Adenocarcinoma	Poorly Differentiated
GC012	M/60	Adenocarcinoma	Moderately Differentiated
GC013	M/60	Adenocarcinoma	Moderately Differentiated
GC014	M/35	Adenocarcinoma	Poorly Differentiated
GC015	M/50	Adenocarcinoma	Moderately differentiated
GC016	F/65	Adenocarcinoma	Poorly Differentiated
GC017	M/40	Adenocarcinoma	Well Differentiated
GC018	M/60	Adenocarcinoma	Poorly Differentiated
GC019	M/45	Adenocarcinoma	Poorly Differentiated
GC020	F/45	Adenocarcinoma	Moderately differentiated
GC021	M/65	Adenocarcinoma	Poorly Differentiated
GC022	M/60	Adenocarcinoma	Moderately differentiated
GC023	M/48	Adenocarcinoma	Poorly Differentiated
GC024	M/62	Adenocarcinoma	Moderately differentiated
GC025	M/67	Adenocarcinoma	Well Differentiated

INFORMED CONSENT FORM – ENGLISH

Title of the study – Analysis of Three gene signature in Gastric Cancer Patients

Name of the participant: _____

Name of the Principal/Co-Investigator: SATHEESH KUMAR M

Name of the Institution: MADRAS MEDICAL COLLEGE AND RAJIV

                             GANDHI GOVERNMENT GENERAL

           HOSPITAL

Name and address of the sponsor / agency (ies), if any: Nil

I,_____(name of participant), have read the information in this

form (or it has been read to me).

I was free to ask any questions and they have been answered. I am over 18 years

of age and, exercising my free power of choice, hereby give my consent to be

included as a participant in **"Analysis of Three gene signature in Gastric**

Cancer Patients"

1. I have read and understood this consent form and the information
   provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study.
4. I have been explained about my rights and responsibilities by the
   investigator.
5. I have informed the investigator of all the treatments I am taking or
   have taken in the past _____ months including any native
   (alternative) treatments.

6. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Government agencies, and ethics committee. I understand that they may inspect my original records.

7. I understand that my identity will be kept confidential if my data are publicly presented.

8. I have had my questions answered to my satisfaction.

9. I consent voluntarily to participate as a participant in this research study.

I am aware, that if I have any questions during this study, I should contact the investigators. By signing this consent from, I attest that the information given in this document has been clearly explained to me and understood by me. I will be given a copy of this consent document.

Name and signature / thumb impression of the participant:

(Name) _____(Signature)_____

Date:

Name and signature of the Investigator:

(Name) _____(Signature)_____

Date:

INFORMED CONSENT FORM – TAMIL

ஆராய்ச்சி தகவல் தாள்

இரைப்பை புற்றுநோய் கட்டியிலிருந்தும் அருகில் உள்ள பாதிக்கப்படாத இடத்தில் இருந்தும் எடுக்கப்படும் குறிப்பிட்ட மூன்று மரபனுக்கள் பற்றிய ஆய்வு

பங்கேற்பாளர் பெயர்   :

ஆராய்சியாளர் பெயர்   :

சென்னை அரசு பொதுமருத்துவமனையில் குடல் இரப்பை அறுவைசிகிச்சை துறைக்கு வரும் இரைப்பை புற்றுநோயாளிகளிடம் இந்த ஆய்வு செய்யப்படுகிறது.

இவ்வாராய்சியின் நோக்கம் யாதெனில் அறுவை சிகிச்சையின் மூலம் அகற்றப்படும் இரைப்பை புற்றுநோய் கட்டியிலிருந்தும் அருகில் உள்ள பாதிக்கப்படாத இடத்திலிருந்தும் ஒரு சிறிய பகுதியை எடுத்து குறிப்பிட்ட மூன்று மரபனுக்கள் அளவை ஒப்பிட்டுப் பார்த்தல்.

நீங்களும் இந்த ஆராய்ச்சியில் பங்கேற்க நாங்கள் விரும்புகிறோம். இதனால் உங்களது உடல்நலமோ, மனநலமோ பாதிக்கப்படாது.

முடிவுகளை அல்லது கருத்துகளை வெளியிடும்போதோ அல்லது ஆராய்ச்சியின் போதோ தங்களது பெயரையோ அல்லது அடையாளங்களையோ வெளியிடமாட்டோம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்த ஆராய்ச்சியில் பங்கேற்பது தங்களுடைய விருப்பத்தின் பேரில் தான் இருக்கிறது. மேலும் நீங்கள் எந்நேரமும் இந்த ஆராய்ச்சியிலிருந்து பின்வாங்கலாம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்த சிறப்பு அறுவைசிகிச்சையின் பலன்களை/ முடிவுகளை ஆராய்ச்சியின்போது அல்லது ஆராய்ச்சியின் முடிவின் போது தங்களுக்கு அறிவிப்போம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

_____   _____

ஆராய்ச்சியாளர் கையொப்பம்   பங்கேற்பாளர் கையொப்பம்

தேதி:   தேதி:

INFORMATION TO PARTICIPANTS

Title: ANALYSIS OF THREE GENE SIGNATURE IN GASTRIC CANCER PATIENTS

Principal Investigator/Co-Investigator: SATHEESH KUMAR M

Name of Participant: _____

You are invited to take part in this research.The information in this document is meant to help you decide whether or not to take part. Please feel free to ask if you have any queries or concerns.

What is the purpose of research?

Stomach cancer is a major age associated cancer.

Surgical therapy is the most promising treatment modality for the management. Identification of early stage cancers and recurrences by unique molecular signature is possible in these tumors.

A novel non-invasive method towards the early diagnosis of Gastric cancer remains a global hunt for decades.

Levels of three genes, EGFR, FGFR and C-Myc genes are frequently over expressed in glandular epithelial cancers, including Gastric cancer.

We want to investigate the mRNA expression levels of EGFR, FGFR2 & C-Myc genes in the gastric cancers and their clinical significance.

We have obtained permission from the Institutional Ethics Committee.

Study Procedures

The study involves Cancerous and paired normal mucosa is collected after informed consent from patients who undergo surgical resection for gastric cancer. The sample is then taken for genetic analysis.

Possible risks to you

There is absolutely no risk to you from the study procedure per se as the mucosal samples are collected only from the surgically resected specimen.

Possible benefits to you

This study will not benefit you as this is a genetic research study and not recommended in clinical practice.

Possible benefits to other people

The results of the research may provide benefits to the society in terms of advancement of medical knowledge and/or therapeutic benefit to future patients.

Confidentiality of the information obtained from you

You have the right to confidentiality regarding the privacy of your medical information (personal details, results of physical examinations, investigations, and your medical history). By signing this document, you will be allowing the research team investigators, other study personnel, Institutional Ethics Committee and any person or agency required by law to view your data, if required.

The information from this study, if published in scientific journals or presented at scientific meetings, will not reveal your identity.

How will your decision to not participate in the study affect you

Your decision not to participate in this research study will not affect your medical care or your relationship with the investigator or the institution. You will be taken care of and you will not loose any benefits to which you are entitled.

Can you decide to stop participating in the study once you start

The participation in this research is purely voluntary and you have the right to withdraw from this study at any time during the course of the study without giving any reasons.

Signature of Investigator   Signature of Participant

Date: Date:

ETHICAL COMMITTEE APPROVAL LETTER

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI -3

Telephone No : 044 25305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To

Dr.M.Satheesh Kumar,
Final Year M.Ch Surgical Gastroenterology,
Department of Surgical Gasteroenterology,
Madras Medical College & RGGGH, Chennai -3

Dear Dr.M.Satheesh Kumar,

The Institutional Ethics committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled "Analysis of a three gene signature in Gastric Cancer Patients" No.13022013.

The following members of Ethics Committee were present in the meeting held on 05.02.2013 conducted at Madras Medical College, Chennai -3.

1. Dr.SivaKumar, MS FICS FAIS --- Chairperson
2. Prof. R. Nandhini MD -- Member Secretary
   Director, Instt. of Pharmacology ,MMC, Ch-3
3. Prof. Shyamraj MD -- Member
   Director i/c , Instt. of Biochemistry , MMC, Ch-3 .
4. Prof. P. Karkuzhali. MD -- Member
   Prof., Instt. of Pathology, MMC, Ch-3
5. Prof. A. Radhakrishnan MD -- Member
   Prof of Internal Medicine, MMC, Ch-3
6. Prof. S. Deivanayagam MS -- Member
   Prof of Surgery, MMC, Ch-3
7. Thiru. S. Govindsamy. BABL -- Lawyer
8. Tmt. Arnold Soulina MA MSW -- Social Scientist

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

Sd/ Chairman & Other Members

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

R.Nadi 22/2/13
Member Secretary, Ethics Committee

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INTRODUCTION

Incidence of Gastric Cancer

Stomach cancer is the 2[nd] most common cause of cancer related mortality in both men and women aged 30–69 years in India next only to oral cancer in men and cervical cancer in female. [1]

Worldwide, stomach cancer is the 2[nd] leading cause of cancer death and 4[th] most common cancer type. Gastric cancer is more common in East Asia and South America than other parts of the world. Gastric cancer is increasing in developing countries and the rates have been decreasing in the developed countries. Among developed countries, Korea and Japan have the highest rates of incidence. But, the mortality rate has dropped by 50% in the above countries as a result of the screening programme. Distal