A DISSERTATION

ON

TUMOR SUPPRESSOR GENE p53 STATUS

in

UPPER GASTRO INTESTINAL MALIGNANCIES

Submitted for

M.S. GENERAL SURGERY
BRANCH - I
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KILPAUK MEDICAL COLLEGE

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CERTIFICATE

This is to certify **Dr. G. MAHENDRAN,** a bonafide M.S. General Surgery Post Graduate Student, from Government Royapettah Hospital, Kilpauk Medical College, Chennai - 600 010 has submitted the dissertation on **TUMOUR SUPPRESSOR GENE P53 STATUS IN UPPER GASTRO INTESTINAL MALIGNANCIES** in partial fulfilment 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 September 2006.

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CONTENTS

PREFACE

The overall incidence of esophageal cancer and gastric cancer is increasing. The vast majority of these cancers have inactivation of the p53 and p16 genes at an early stage followed by defects in genes such as Apc, Rb and Cyclin D, at later stages of progression.

There is also mounting evidence that numerous, specific regions throughout the genome are frequently lost in these cancers. The study of the p53 tumor suppressor gene has blossomed into a field of its own. With the abundance and intensity of research continuing in the field of molecular biology on carcinogenesis, we begin to understand the importance of p53 tumor suppressor in human cells.

The accumulating knowledge about the inactivation of the p53 tumor suppressor gene could ultimately provide us with objective diagnostic tools, more accurate markers for prediction of malignant transformation from premalignant epithelium and facilitate the introduction of novel therapeutic options for the management of esophageal and stomach cancers.

INTRODUCTION

Esophageal cancer may develop as either squamous cell cancer or adenocarinoma. Squamous cell carcinoma is the predominant subtype of esophageal cancer worldwide. It is the third most common gastro intestinal malignancy. Incidence in India is between 10 to 50 in 1,00,000 individuals. Though cure is possible when detected early, most often it is advanced at the time of presentation.

Gastric Cancer remains second only to lung cancer as a leading cause of cancer mortality worldwide. Adenocarcinoma of the cardia and GE junction appears to have increased in the past two decades. The incidence of intestinal type gastric cancer is increasing whereas that of diffuse type has remained constant overtime. There is a consistent predominance of gastric cancer in males, worldwide (2 : 1 male : female ratio). Most cases of the disease present at advanced stages and effective therapy is limited.

Survival rates are dismal even in the best of centres. Five year survival rates ranges between 5-12% according to the International Tumour Registry. 75% of the therapy is palliative and less than a fifth of patients with gross tumor resection survive more than 5 years.

The present multimodality therapy is showing encouraging results in mortality but the morbidity of undergoing it remains very high. Hence these epidemiologic variables provoke intense efforts to identify new features in depth and strategies of molecular biology for better understanding the pathogenesis and / or management of esophageal and gastric malignancies (3).

Human esophageal carcinogenesis is a multi stage progressive process which involves the conversion of normal epithelium to that with Basal cell hyperplasia, dysplasia or carcinoma in situ and then to invasive squamous cell carcinoma (SCC) (11-15).

Development of intestinal type of gastric cancer is probably a multi-step process whereas it remains tentative whether the diffuse type of malignancy follows an analogous progression. Hence the intestinal type comprise gastric mucosal metaplasia - dysplasia - carcinoma sequence.

The accumulation of multiple genetic alterations leading to

(1) Oncogene over expression

(2) Tumor suppressor loss and

(3) Defective DNA mismatch repair

is associated with malignancies of esophagus and stomach.

The gene that has garnered the most attention is the tumor suppressor p53. The most frequent genetic abnormalities found in these cancers tend to be loss of Heterozygosity (LoH) of TSG. Early studies reported that LoH (60-70%) and mutations (38-71%) of p53 gene are quite frequent. In addition, p53 mutations are also observed in intestinal metaplasia (38%) and gastric dysplasia (58%) suggesting that p53 mutations may be an early event in carcinogenesis.

p53 gene product regulates cell-cycle progression, DNA repair, apoptosis and neo-vascularisation in normal and malignant tissue via highly complex DNA and protein interactions.

Studies have shown that restoration of p53 expression by gene therapy techniques results in cell cycle arrest and apoptosis as well as enhanced sensitivity to chemotherapy and radio-therapy in esophageal and gastric cancer cells.

In this study we have experimentally identified the presence of mutant p53 status in esophageal and gastric cancer patients.

TUMOUR SUPPRESSOR GENE -- p53

Cell cycle is a term used to describe the orderly sequence of events which ensure the faithful duplication of all the cellular components in their correct sequence and partitioning of these components into daughter cells.

Two classes of genes and their protein products are employed for this process

(1) genes whose products are obligatory for progress through the cell cycle G_1 to S – to G_2 to M phases and

(2) genes whose proteins act as checkpoints and stop the progression through the cell cycle if conditions are not satisfactory.

The crucial differences between normal cell and cancer cells stem from discrete changes in specific genes controlling proliferation and tissue homeostasis.

p53 gene have become the centre of intensive study because slightly more than 60% of human cancers contain mutations in this gene. p53 gene was originally discovered in 1979 by Arnold Levine of Princeton University, David Lane of the University of Dundee, Scotland

and William old of the memorial sloan - kettering cancer centre in New York City (5, 6, 17).

In 1989, Bert Vogelstein, Ray white and their colleagues showed that p53 is actually a tumor suppressor. Since then mutant forms of p53 have cropped up in so many tumors and aroused so much interest that science hailed the p53 protein as the "Molecule of the Year" in 1993.

p53 is located at chromosome region 17 p13. Normally, in a cell the p53 protein is kept at low concentration by its relatively short half life of about 20 minutes. DNA damage, hypoxia and fall in ribonucleoside triphosphate pools below a critical threshold will results in rapid increase in the level of p53 in the cell and activation of p53 as a transcription factor (11, 17). This activated p53 acts to stop cell division by cell cycle arrest thus giving the cell a chance to repair the DNA before the errors are duplicated and passed on to daughter cells or initiate apoptosis if the damage is irreparable. But when mutated, it loses its protective function which allows mutations to accumulate in other genes and leads to more than 60 percent of all human cancers including cancers of esophagus, stomach, colon, lungs, bladder, brain and liver (9, 10, 21).

Immunohistochemistry (IHC) detects the presence of mutant p53 in the tumor cells (29). Presence of immunoreactive p53 correlates the

presence of p53 mutation especially when the percentage of immuno-reactive cells exceeds 10-20% (12).

This is because transcription of mutated p53 gene usually results in the production of a protein that is more stable than the wild type and therefore it accumulates in the nucleus, which can be detected by IHC (32).

There have been a plethora of studies correlating p53 IHC with clinicopathologic parameters in a variety of cancers. In head and neck squamous cancers presence of abnormal p53 expression correlates with an increased risk of loco-regional recurrence, resistance to radiotherapy, and risk of developing a second primary tumor.

Nuclear accumulation of aberrant p53 is an independent indicator of poor prognosis (36). Similarly, in invasive breast cancers, aberrant p53 is associated with many aggressive biological factors and poor clinical outcome. Finally in melanoma, p53 expression is associated with an increase in the depth of invasion in the primary tumor, and the presence of metastatic disease. These, and other studies have convincingly validated the use of p53 IHC as a reliable assay for prognostication purposes (25).

In brief

Expression

- The p53 protein is located in the nucleus and is very labile. Agents which damage DNA, induce p53 to become very stable by a post-translational mechanism, allowing its concentration in the nucleus to increase dramatically.

Cellular Functions

- Causes cell cycle arrest in response to DNA damage thereby allowing DNA repair and hence prevents the transmission of damaged genetic information to next generation.

- Initiate apoptosis if the cell damage is severe and so loss of p53 function is a key step in the neoplastic cascade.

- Often as a tumor suppressor : p53 mutations can cause cells to become oncogenically transformed (33).

- p53 may play a role in monitoring developmental factor dependence, in the haematopoietic systems.

- p53 can also initiate apoptosis in response to the expression of a viral or cellular oncogene or the absence of a critical tumor suppressor gene product (Rb).

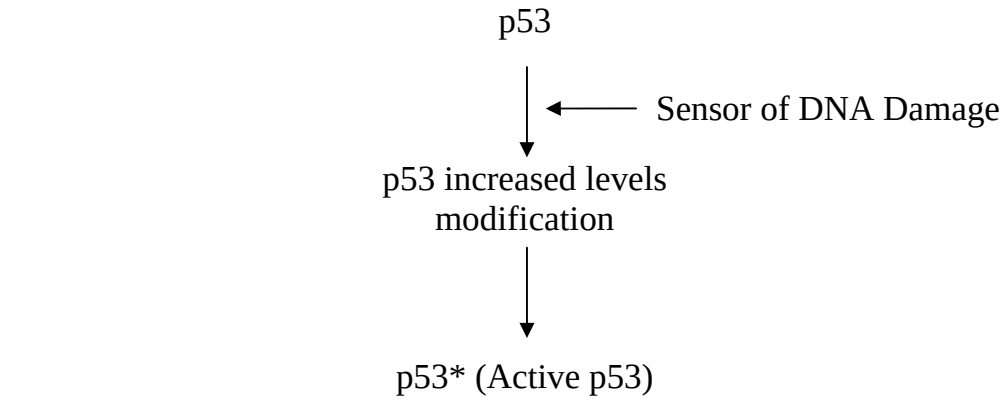
- In addition to p53 mutations, some tumors inactivate p53 by the amplification of the MDM2 gene or by the localisation of p53 in the cell cytoplasm.

- Humans who are heterozygous for wild type allele of p53 develop cancer with a very high frequency (> 90-95%) and often at an early age.

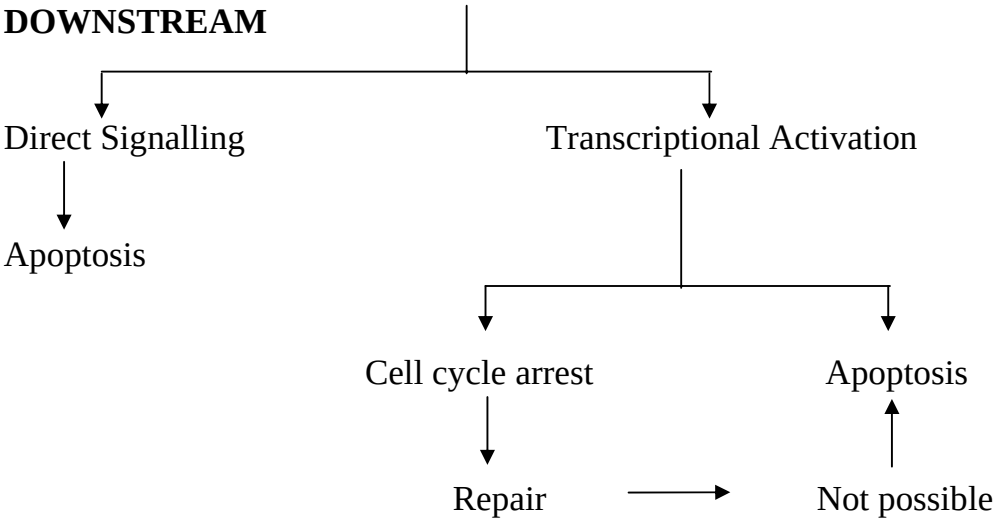
- p53 plays a more central role in the surveillance of gene amplifications, abnormal recombination processes and the control over ploidy.

- The function of p53 is critical to the way that many cancer treatment kill cells, since radiotherapy and chemotherapy act in part by triggering cell suicide in response to DNA damage. So tumors that frequently contain p53 mutations often respond poorly to radiation or chemotherapy.

UPSTREAM



DOWNSTREAM



Due to above reasons, p53 is terms as "Guardian of Human Genome" or "Cellular Gatekeeper" (17).

PURPOSE OF THE STUDY

This study is done to analyse the p53 status of our patients with esophageal and gastric malignancies. For planning a gene therapy, genetic assessment of the tumor is the mandatory entry criterion.

As most of these malignancies are diagnosed at advanced stage, curative therapy may not be possible in most of the situations and hence identification of mutant genes can help in genetically manipulating the tumor.

This will help us in identifying the patients who will respond to ionising radiation and in choosing appropriate chemotherapeutic agents. For example ESCCs expressing mutant p53 will have augmented response for vinorelbine, taxanes and irinotecan (31,45). This study will form the basis for initiating adenoviral vector mediated gene therapy.

The likely ways to increase p53 levels are by impairing Mdm2 function and by inducing the accumulation of the p53 in the nucleus by nuclear export inhibitor leptomycin B (LMB).

This study on p53 will be contributing new ideas to the development of novel non-genotoxic activators of p53 and stimulate researchers to keep on discovering new targets of the p53 pathway.

LACUNAE IN CURRENT KNOWLEDGE

Studies on mutant p53 analysis in esophageal and gastric malignancies principally come from Western World (2), Middle East (19), Japanese (26) and Chinese(1) subcontinents. There are not many studies from this part of India. It is prudent to have a local data about expression of mutant p53 in our patients, so that a comparative analysis of etiological risk factors could be done. With the present multimodal therapy, the results obtained are not very effective and is principally palliative (30, 31). The modern therapies with adenoviral vector and other non-genotoxic agents have been promising in Japanese studies.

To give the benefit of these advanced therapies we need to have a base line data based on the Indian population.

The tumorigenesis of Esophageal and gastric malignancies is still elusive to the researchers. So far, the epidemiology has advanced to tell us about the possible association with smoking and ethanol usage (27, 42). But still there are patients who succumb to the type of malignancy without the above risk factors (23). Till date we are not in a position to explain the etiology for occurrence of malignancy in different

levels of esophagus and stomach. Understanding the molecular basis of these cancers will remove the lacunae in the current knowledge about these malignancies.

More closer look into the carcinogenesis is needed to identify other synergistic and dysynergistic factors which determine the tumor progression.

HYPOTHESIS

Cell cycle abnormalities are said to be the cause for initiation and maintenance of any malignancy. Normally several key proteins are essential for maintenance of this homology. When there is aberrance in expression of any of the key proteins, abnormal cell cycling sets in and malignancy ensures. Point mutations, occurring frequently in the gene coding for the p53 protein, results in the accumulation of mutant protein. India is experiencing one of the highest cancer incidence rates in the world for esophageal and gastric malignancy. We have attempted here to elucidate the mutant p53 status in these malignancies.

REVIEW OF LITERATURE

Early studies based on mutant p53 status in esophageal and gastric cancers dates back to 1990.

- During Dec. 1990, the research team consisting of Holsteich MC et al., (36) of the international agency for research on cancer, cyon, france published their work on this subject. They identified mutant p53 in one third of the ESCCs studied. They were the early team to sequence the p53 and studied about the mutational status in exon 5-9. However, the study was not able to suggest further insight possibly due to technical limitations.

- Meltzer S.J. et al., (37) from Univ of Maryland, Baltimore elucidated the loss of heterozygosily (LoH) affecting the chr. 17p. They established the allelic loss in nearly half of the tumors studied and hypothesised that inactivation of p53 was involved in the pathogenesis of ESCCs.

- In 1994, Wang DY et al., (43) from Hamamatsu Medical School, Japan did a larger study from ESCC patients from the Linxian province of China, which still remains the endemic area. He related the accumulation of mutant p53 to the invasiveness and capability

for metastasis of cancer cells. He was amongst the early researchers to prognosticate ESCC patients with respect to mutant p53 expression.

- In 1997, Annals of surgical oncology presented a publication by Baron PL (46) from Medical University of S. Carolina, Charleston, USA. He established the presence of p53 mutation on both ESCC tumor tissues and in other normal looking tissues. He concluded that they inherited a defect in the p53 expression. He again raised an important issue why esophagus is the targeted tissue when there is a generalized mutant p53 expression. The scientists at p53 lab, unit of carcinogenesis. International Agency for research on. Cancer, Lyon, France went into the molecular pathway of p53 mechanism, from the eso. cancer cell lines. They were able to induce the malignancy in the natural tumor cells when they exposed the non-tumor esophageal mutant p53 expressing cells to genotoxic stress.

- In 2001, Koide et al., (53) studied the co-expression of VEGF and p53 protein in ESCC. His results confirmed the association of the expression of mutant p53 with angiogenesis and distal metastasis in ESCC and established the role of VEGF in p53 associated

angiogenesis. Aloia et al., from Duke University Medical School, North Carolina, USA established the prognostic value of the p53, TGF - α in ESCC patients. Putz et al., based on his extensive study concluded "Although difficult because of the documented co-exposure to various life style risk factors in most patients of this series, the hypothesis is proposed that besides smoking and alcohol drinking the commonly consumed hot mate tea in this high risk area for ESCC is responsible this different pattern of TP 53 mutations because of chronic hyperthermia, irritation and inflammation in the esophagus with an endogenous formation of radicals or carcinogenic factors that lead to a higher prevalence of transition mutations.

- In 2002, Wys P. et al., from the MRC Centre, South Africa and Shimada et al., (55) from Chiba University, Japan started the first human trials on Ad5 CMV - p53 based gene therapy. He conducted his trials safely without complications. He also presented the data based on serum p53 antibodies is ESCC patients. It was useful to detect esophageal cancer, identify those who will have early recurrence and those with poor prognosis. Simultaneously Raja et al., from Pittsburgh, USA published his review on tumor

suppressor genes in ESCC. He highlighted the accumulating knowledge about the inactivation of the TSG could ultimately provide us with objective diagnostic tools and information which will facilitate the researchers to work on novel therapeutic avenues.

- In 2003, Zuo LF et al., (57) from the provincial tumor institute, Hebei province, China investigated the alteration of molecular events and the early carcinogenesis mechanism of esophageal epithelial cells. His data showed almost 100% mutant p53 expression in the tissue studied from the endemic Hebei province. He used advanced DNA flow cytometry and correlated the DNA ploidy and various other TSG. He concluded increased DNA content and heteroploid rate, accumulation of p53 protein and over-expression of p21, telomerase and cyclin D proteins were early molecular events during the development of esophageal cancer.

- In 2003, Matsubara H (58) published the update from the ongoing therapy for advanced ESCC. He devised a newer protocol for improving the efficacy of gene therapy by using invivo electroporation. Moreover, electric pulse to established solid tumors increases intracellular concentrations of chemotherapeutic agents. He inferred electrochemo-gene therapy is a relatively

simple method and can produce a better therapeutic effect. Xing Y et al., published his famous study "Expressions of PCNA, p53, p21 (WAF-1) and cell proliferation in fetal esophageal epithelia." Comparative study with adult esophageal lesions from subjects of high-incidence area for esophageal cancer in Henon, North China. He inferred that p53 may play an important role in growth and differentiation of fetal esophageal epithelium.

STUDY DETAILS

Type of study : Prospective descriptive experimental study

Study Duration : January 2004 to December 2005

Collaborating Institutions :

1. Department of surgery, Government Royapettah Hospital, Kilpauk Medical College, Chennai -10, India.

2. Division of immunohistochemistry, R & D path lab, Mylapore, Chennai, India.

3. Department of Surgical, Gastro-enterology, Government Royapettah Hospital, Chennai - 14, India.

DETAILS OF MATERIAL AND EXPERIMENTAL DESIGN

TISSUE SPECIMENS

Formalin fixed, paraffin embedded tissue from endoscopic biopsy of esophageal and gastric cancer patients were used for the study. Histological sections were studied by the collaborating pathologists at immuno histochemistry division of R & D Histopath Lab, Mylapore, Chennai.

TECHNIQUE OF DETECTION

IHC is a multi-step procedure that requires specialised processing of the tissue, the selection of appropriate reagents and interpretation of the stained tissue sections. In general, IHC staining techniques allow for the visualisation of antigens by sequential application of a specific antibody to the antigen, a secondary antibody to the primary antibody, an enzyme complex and a chromogenic substrate. The enzymatic activation of the chromogen results in a visible reaction product at the antigen site. We have used this method in identifying mutant p53 in esophageal and gastric malignancies in our study.

MATERIALS AND METHODS

The primary antibody mouse anti-p53, clone-PAb 1801 was obtained from Zymed laboratories INC, South Sanfrancisco, CA 94080, USA. The necessary reagent, buffers, and humidifying chambers were utilised from the IHC division R & D histopath lab; Mylapore, Chennai-4. The primary monoclonal antibody is purified from mouse ascites and diluted in Phosphate buffered Saline (PBS), pH 7.4, and 1% bovine serum albumin (BSA) with 0.1% sodium azide (NaN_3) as preservative.

Tissue section of 5 microns was 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 & sections were brought to water through graded alcohol. They were subjected to microwave antigen retrieval in citrate buffer of pH 6 for 30 mins. 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 p53 protein 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 10 mm 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.

The IHC stained positive cells, look brown and negative cells look blue.

EVALUATION OF STAINING

INTENSITY OF STAINING

The staining were graded as per the UICC guidelines.

Grade 1	:	Weak Staining (< 10%)
Grade 2	:	Staining in the peripheries alone (10 - 40%)
Grade 3	:	Intense staining in most of the tumor cells
Grade 4	:	Intense staining in all tumor cells.

DATA ANALYSIS

STATISTICAL METHODS

The significance of the association between variables was tested by the χ^2 test. The variables included in univariate statistical analysis were gender, age, degree of differentiation, location of the tumor, intensity of staining of mutant p53 protein. P values reported are for a two-sided test and the level of significance was set at 0.05.

SPSS version 14 software was used for the statistical analysis. This was done with help of the statistical analyst, the Institute of Social Paediatrics, Stanley Medical College, Chennai - 600 003.

The basic data collected during endoscopy regarding the age, sex, and the location of the tumor and degree of differentiation in the esophagus and stomach were analysed with regards to the staining of mutant p53 protein obtained after immunostaining.

STATEMENT OF LIMITATIONS

The study is based on a limited number of patients. A detailed study of more subjects and follow-up for a longer duration will augment the validity of the data.

The immunoexpression of mutant p53 comprises of all the mutations. To study the specific type of mutation, one needs to analyse the individual exon. Exon based analysis need more refined techniques in the form of PCR or DNA vector array. Use of these advanced tools will help one to locate the exact location and type of the mutation in the specific chromosome studied.

ETHICAL ISSUES INVOLVED

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

PATIENT CHARACTERISTICS

n = 80

	Characteristics		No. of Patients
1.	**Gender**		
	Carcinoma esophagus	=	55
	Male	=	38
	Female	=	17
	Carcinoma Stomach	=	25
	Male	=	18
	Female	=	7
2.	**Age**		
	Ca esophagus	=	30 - 87 years
	Ca stomach	=	37 - 83 years
3.	**Site**		
	Ca Esophagus	=	55
	Ca Stomach	=	25

4. HPE

Squamous Cell Carcinoma	=	53
Adeno Carcinoma	=	27

5. Differentiation

Ca esophagus

Well differentiated	=	20
Moderately differentiated	=	15
Poorly differentiated	=	13
undifferentiated	=	7

Ca Stomach

Well differentiated	=	11
Moderately differentiated	=	10
Poorly differentiated	=	4
Undifferentiated	=	0

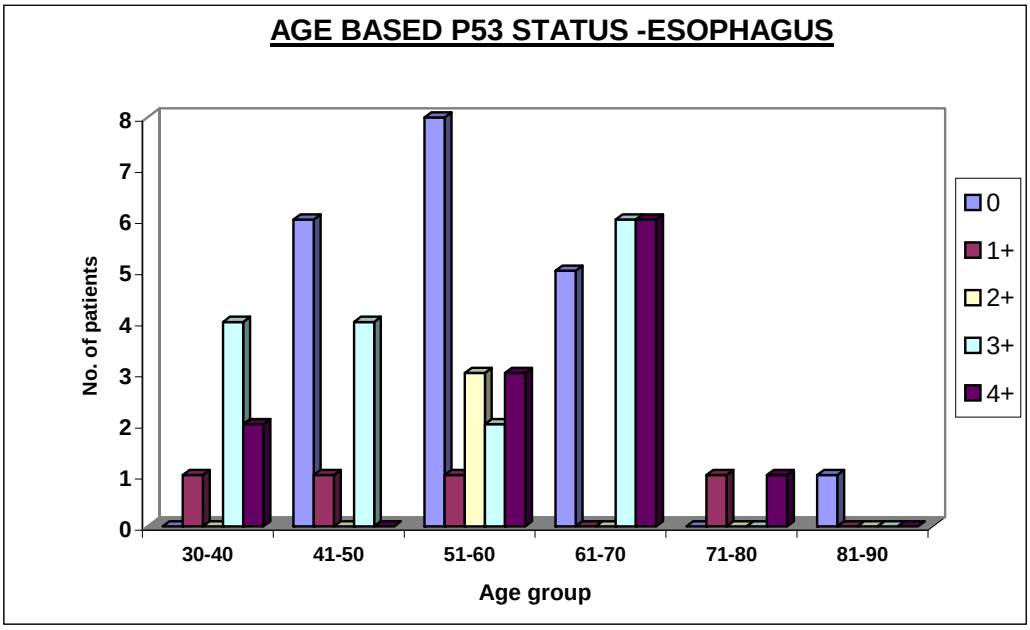
OBSERVATIONS

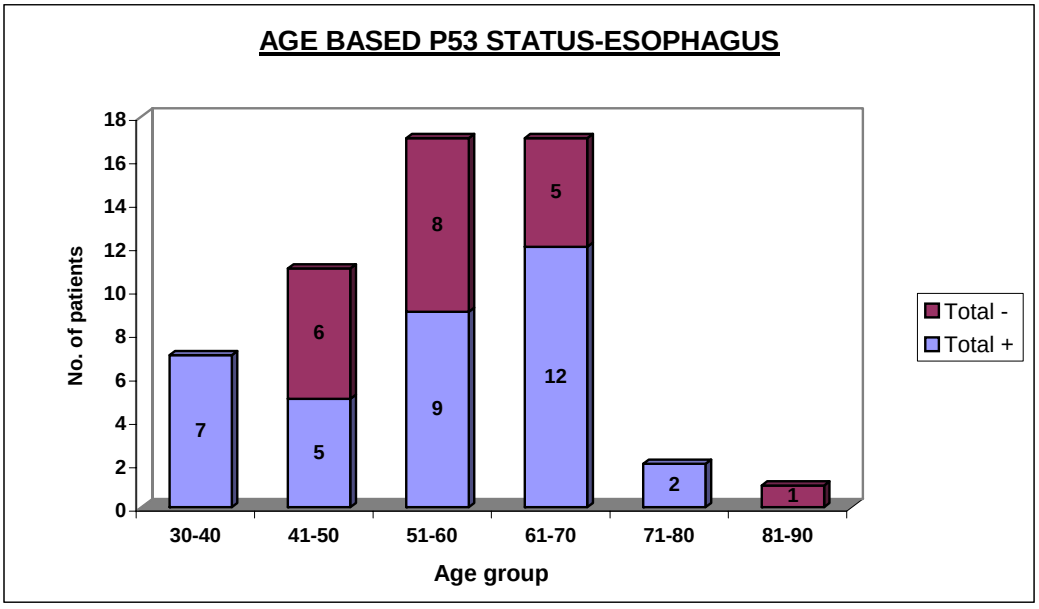
AGE BASED OBSERVATION

CARCINOMA ESOPHAGUS

AGE	INTENSITY OF STAINING						
	0	**1+**	**2+**	**3+**	**4+**	**TOTAL PT**	**TOTAL +**
30-40	0	1	0	4	2	7	7
41-50	6	1	0	4	0	11	5
51-60	8	1	3	2	3	17	9
61-70	5	0	0	6	6	17	12
71-80	0	1	0	0	1	2	2
81-90	1	0	0	0	0	1	0
TOTAL	**20**	**4**	**3**	**16**	**12**	**55**	**35**

Age	Total +	Total -
30-40	7	0
41-50	5	6
51-60	9	8
61-70	12	5
71-80	2	0
81-90	0	1





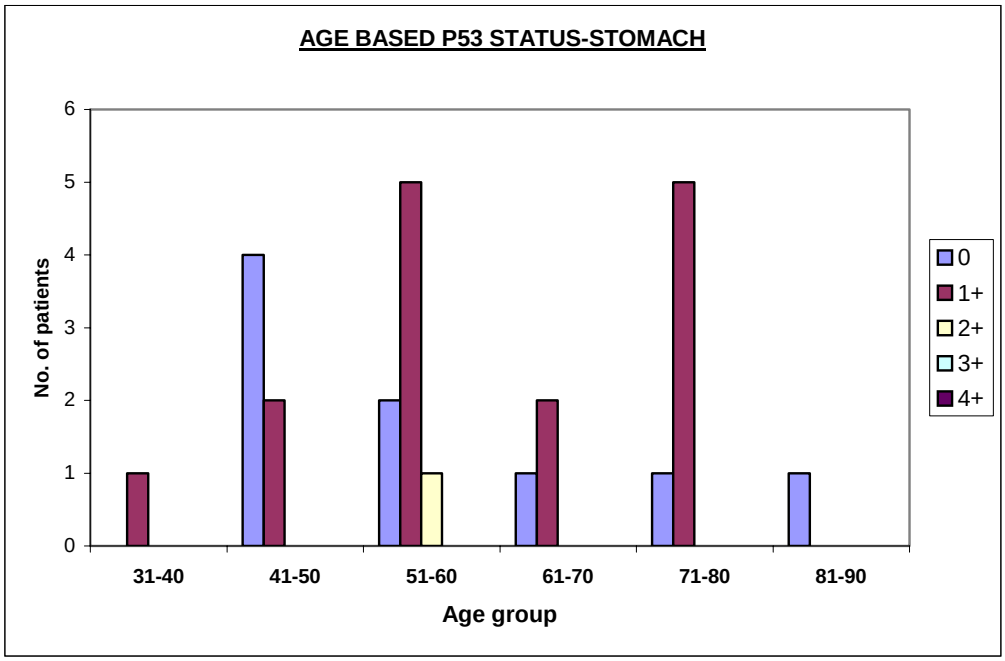
The above data was statistically analysed, Pearson Chi-square test χ^2 = 9.66, P-value = 0.08. The study patients predominantly belonged to the 4th to 6th decade. The positivity was more appreciated in fifth and sixth decade.

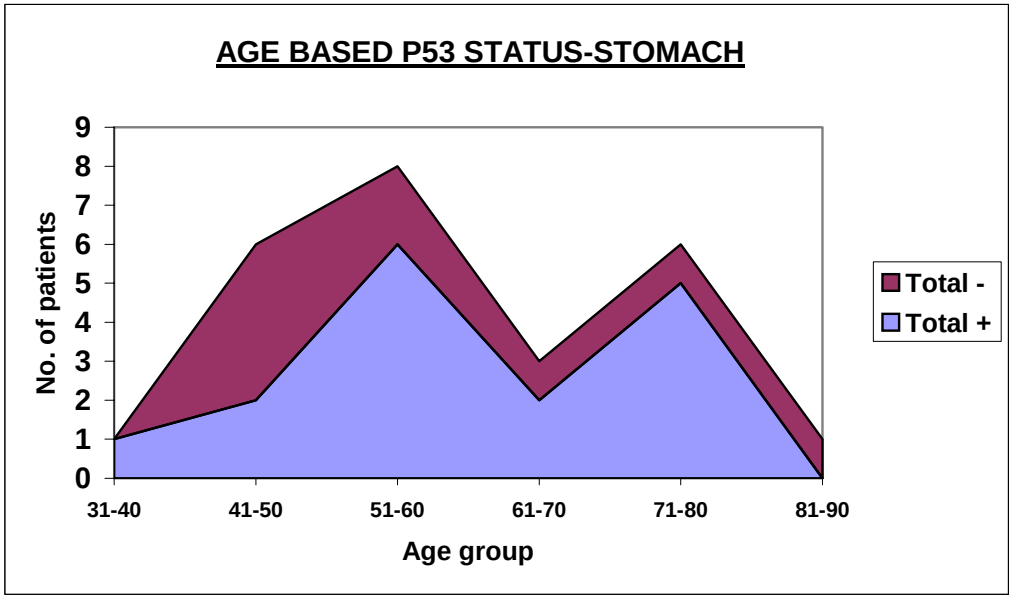
AGE BASED OBSERVATION

CARCINOMA STOMACH

AGE	INTENSITY OF STAINING						
	0	**1+**	**2+**	**3+**	**4+**	**TOTAL PT**	**TOTAL +**
31-40	0	1	0	0	0	1	1
41-50	4	2	0	0	0	6	2
51-60	2	5	1	0	0	8	6
61-70	1	2	0	0	0	3	2
71-80	1	5	0	0	0	6	5
81-90	1	0	0	0	0	1	0
TOTAL	**9**	**15**	**1**	**0**	**0**	**25**	**16**

Age	Total +	Total -
31-40	1	0
41-50	2	4
51-60	6	2
61-70	2	1
71-80	5	1
81-90	0	1



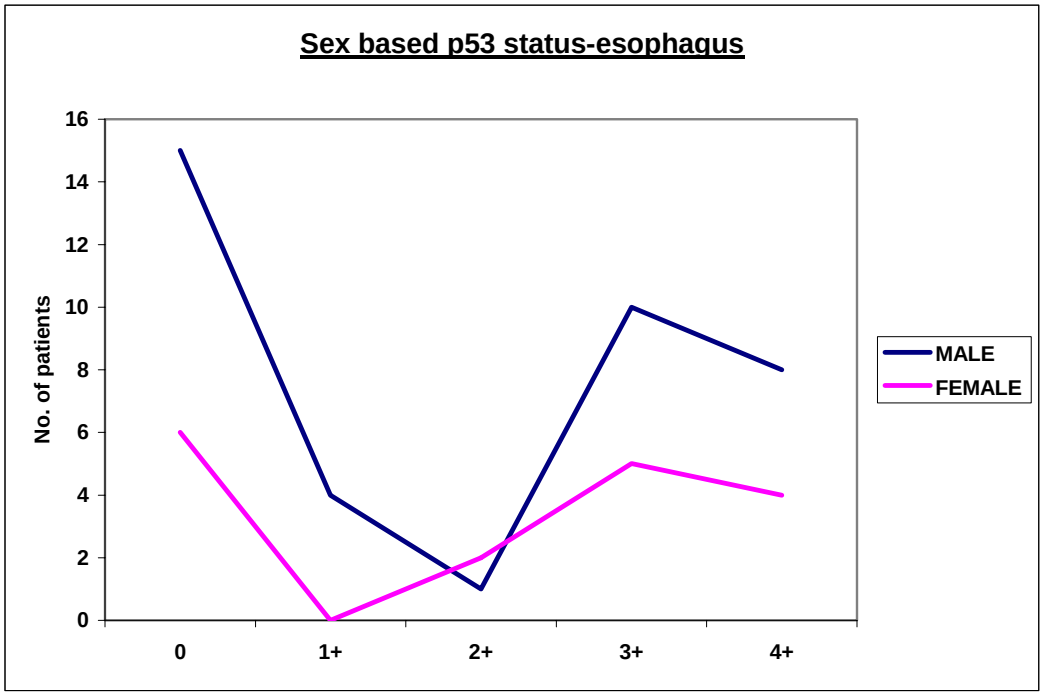


The above data was statistically analysed, χ^2 test = 6.19 and P-value = 0.28. The study patients predominantly belonged to the 4th to 5th decade. The positivity was more appreciated in the fifth decade.

SEX BASED P53 STATUS

ESOPHAGEAL CARCINOMA

SEX	INTENSITY OF STAINING						
	0	**1+**	**2+**	**3+**	**4+**	**TOTAL PT**	**TOTAL +**
MALE	15	4	1	10	8	38	23
FEMALE	6	0	2	5	4	17	11
TOTAL	**21**	**4**	**3**	**15**	**12**	**55**	**34**



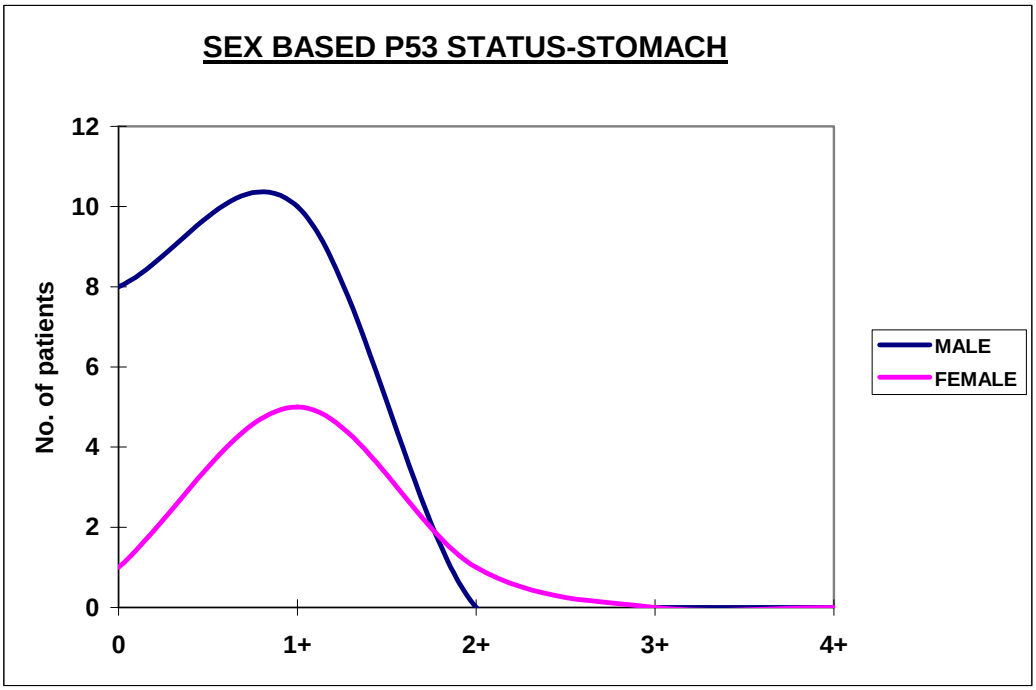
The above data was statistically analysed, χ^2 test = 0.09, P-value = 0.77. The percentage of positivity of p53 had no difference among sexes in upper gastrointestinal malignancies.

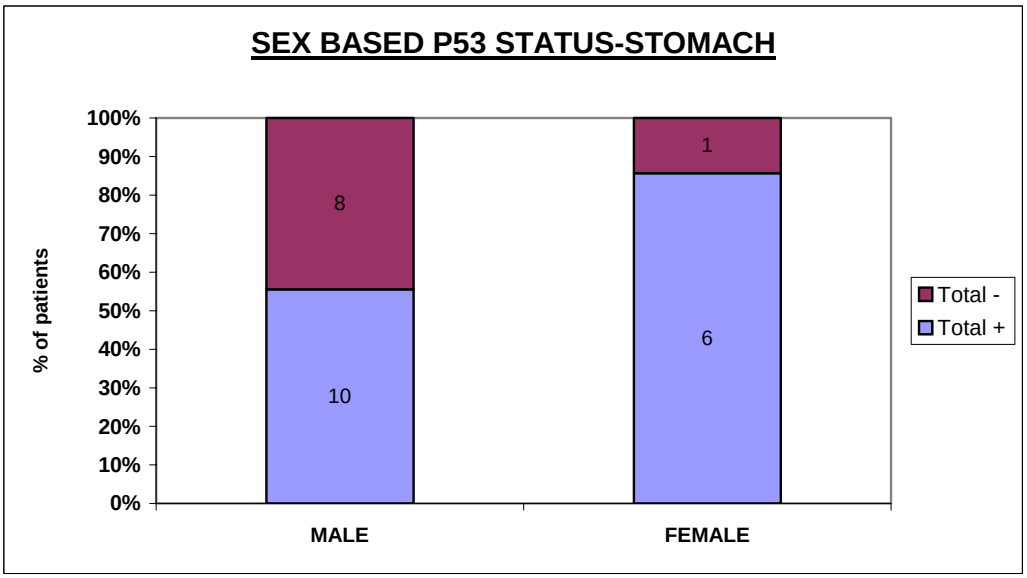
SEX BASED p 53 STATUS

CARCINOMA STOMACH

SEX	INTENSITY OF STAINING						
	0	**1+**	**2+**	**3+**	**4+**	**TOTAL PT**	**TOTAL +**
MALE	8	10	0	0	0	18	10
FEMALE	1	5	1	0	0	7	6
TOTAL	**9**	**15**	**1**	**0**	**0**	**25**	**16**

Sex	Total +	Total -
MALE	10	8
FEMALE	6	1



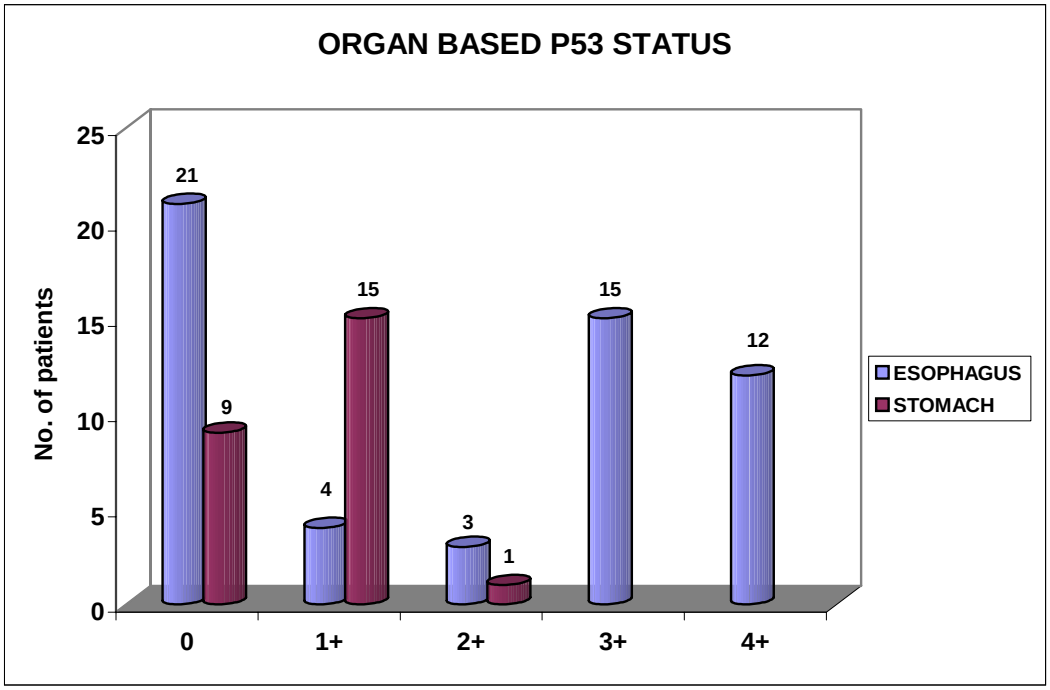


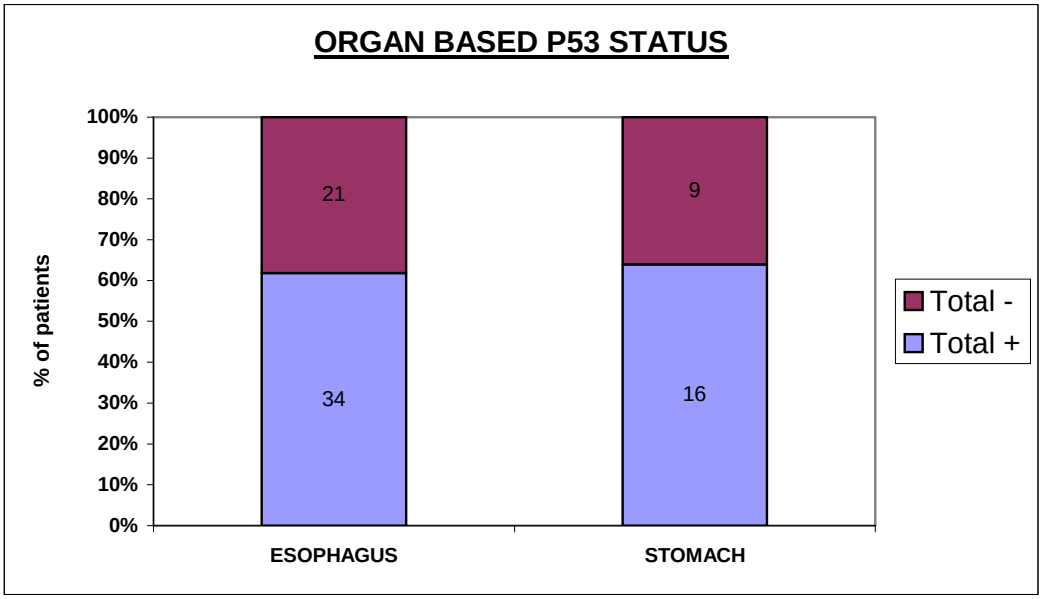
The above data was statistically analysed, χ^2 test = 0.90, P-value = 0.34. The percentage of positivity of p53 is more in females when compared to males.

ORGAN BASED p53 STATUS

ORGAN	INTENSITY OF STAINING						
	0	**1+**	**2+**	**3+**	**4+**	**TOTAL PT**	**TOTAL +**
ESOPHAGUS	21	4	3	15	12	55	34
STOMACH	9	15	1	0	0	25	16
TOTAL	**30**	**19**	**4**	**15**	**12**	**80**	**50**

ORGAN	Total +	Total -
ESOPHAGUS	34	21
STOMACH	16	9





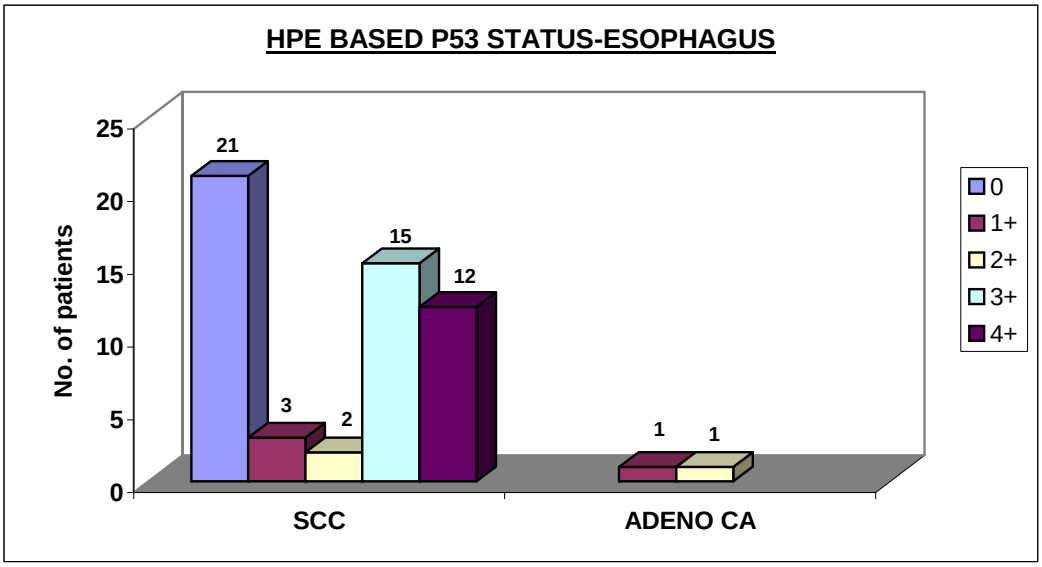
The above data was statistically analysed, χ^2 test = 0.03, P-value = 0.85. The intensity of positivity is more in carcinoma esophagus.

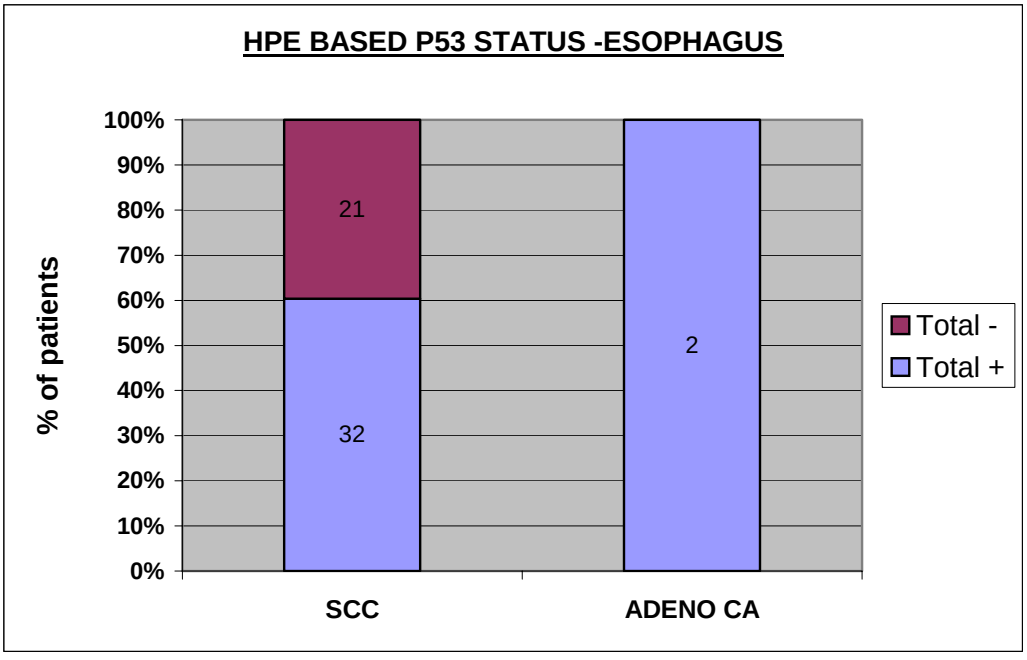
HISTOPATHOLOGY BASED p53 STATUS

CARCINOMA ESOPHAGUS

HPE	INTENSITY OF STAINING						
	0	**1+**	**2+**	**3+**	**4+**	**TOTAL PT**	**TOTAL +**
SCC	21	3	2	15	12	53	32
ADENO CA	0	1	1	0	0	2	2
TOTAL	**21**	**4**	**3**	**15**	**12**	**55**	**34**

HPE	Total +	Total -
SCC	32	21
ADENO CA	2	0



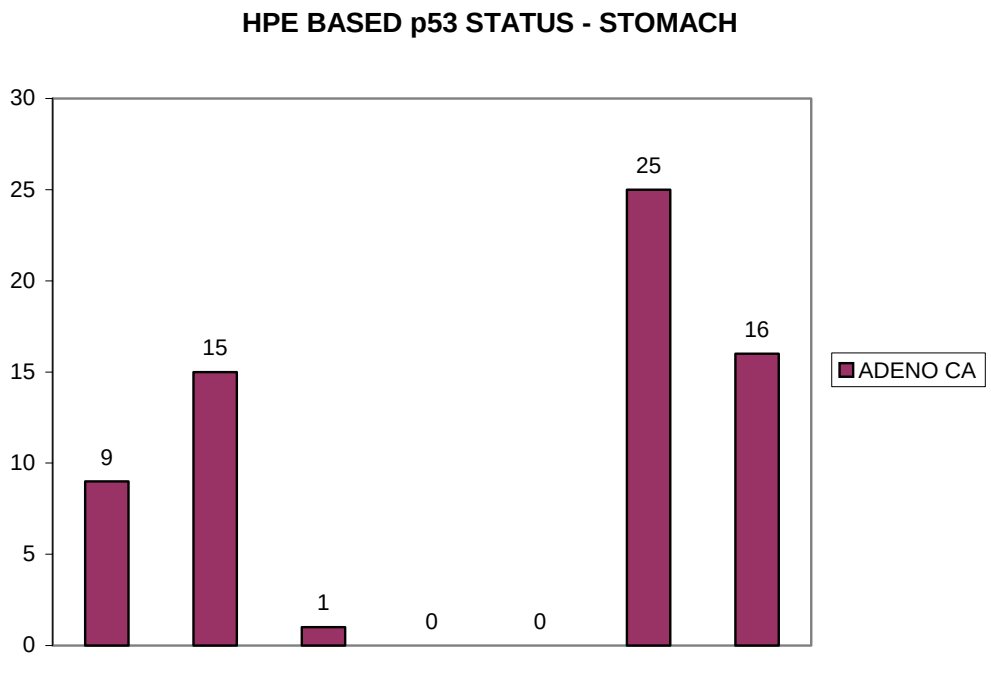


The above data was statistically analysed, χ^2 test = 0.15, P-value = 0.70. There is no significant difference in positivity for both histopathological types in carcinoma esophagus.

HISTOPATHOLOGY BASED p53 STATUS

CARCINOMA STOMACH

HPE	INTENSITY OF STAINING						
	0	**1+**	**2+**	**3+**	**4+**	**TOTAL PT**	**TOTAL +**
ADENO CARCINOMA	9	15	1	0	0	25	16



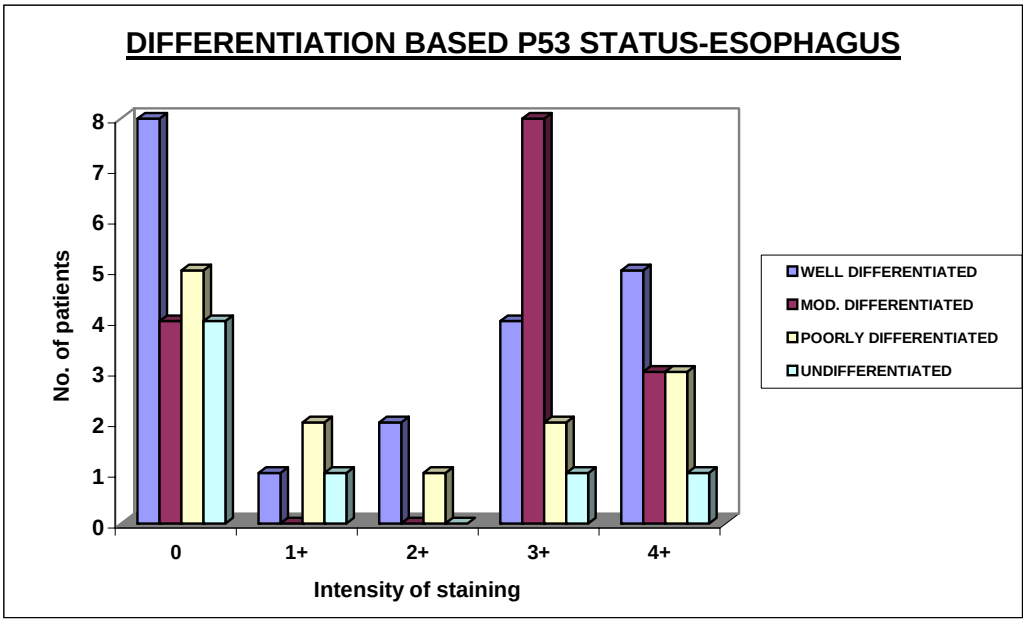
p53 positivity is 64% in gastric carcinoma.

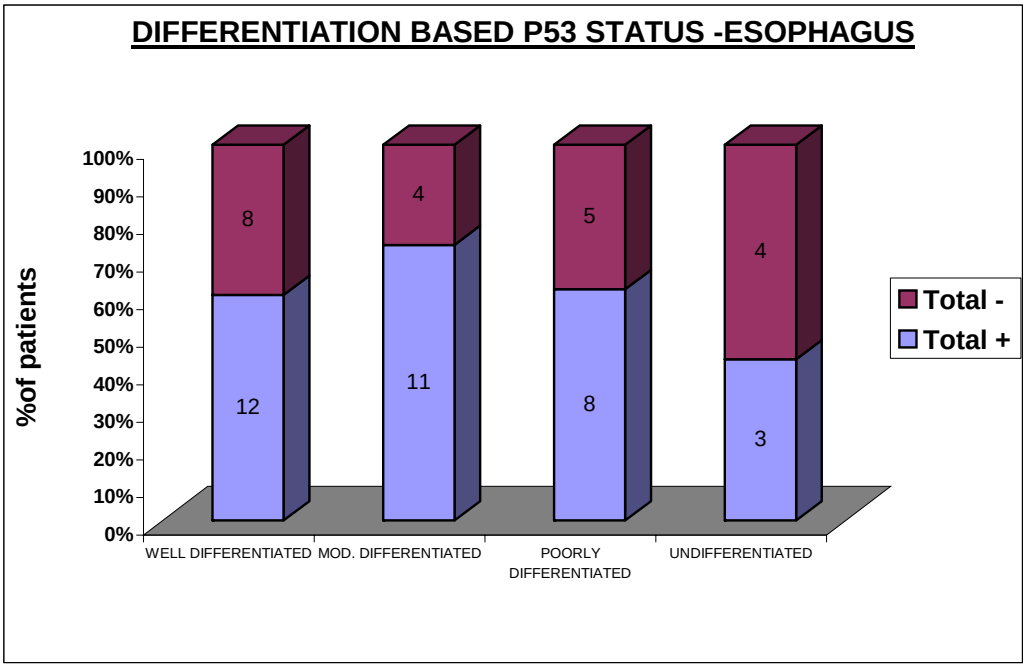
DIFFERENTIATION BASED p53 STATUS

CARCINOMA ESOPHAGUS

DIFFERENTIATION TYPE	INTENSITY OF STAINING						
	0	**1+**	**2+**	**3+**	**4+**	**TOTAL PT**	**TOTAL +**
WELL DIFFERENTIATED	8	1	2	4	5	20	12
MOD. DIFFERENTIATED	4	0	0	8	3	15	11
POORLY DIFFERENTIATED	5	2	1	2	3	13	8
UNDIFFERENTIATED	4	1	0	1	1	7	3
TOTAL	21	4	3	15	12	55	34

DIFFERENTIATION TYPE	Total +	Total -
WELL DIFFERENTIATED	12	8
MOD. DIFFERENTIATED	11	4
POORLY DIFFERENTIATED	8	5
UNDIFFERENTIATED	3	4





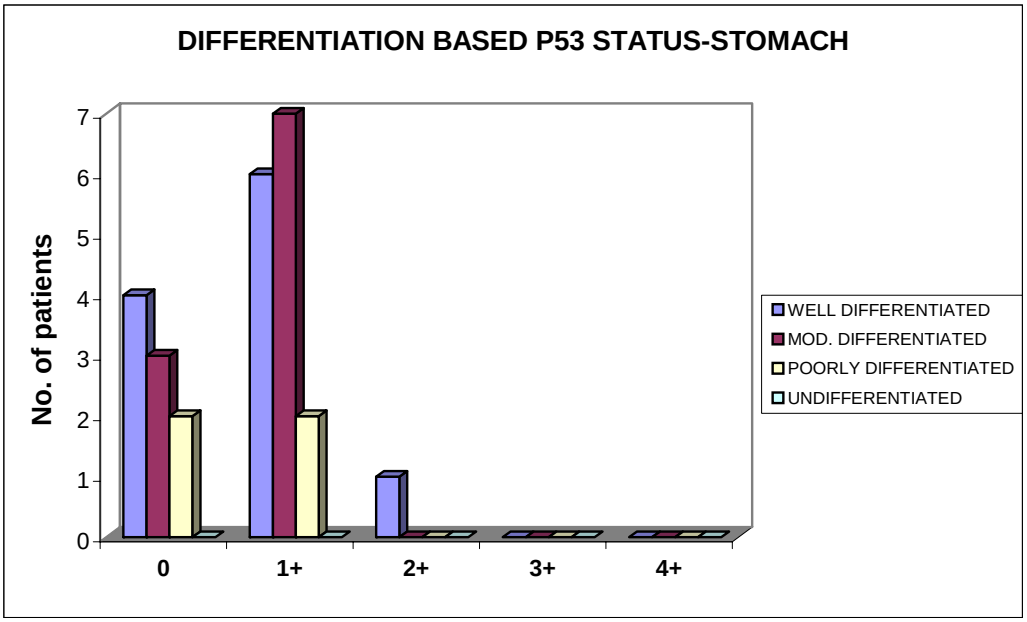
The above data was statistically analysed, χ^2 test = 1.94, P-value = 0.59. There is no significant difference in positivity depending upon the differentiation of the tumor.

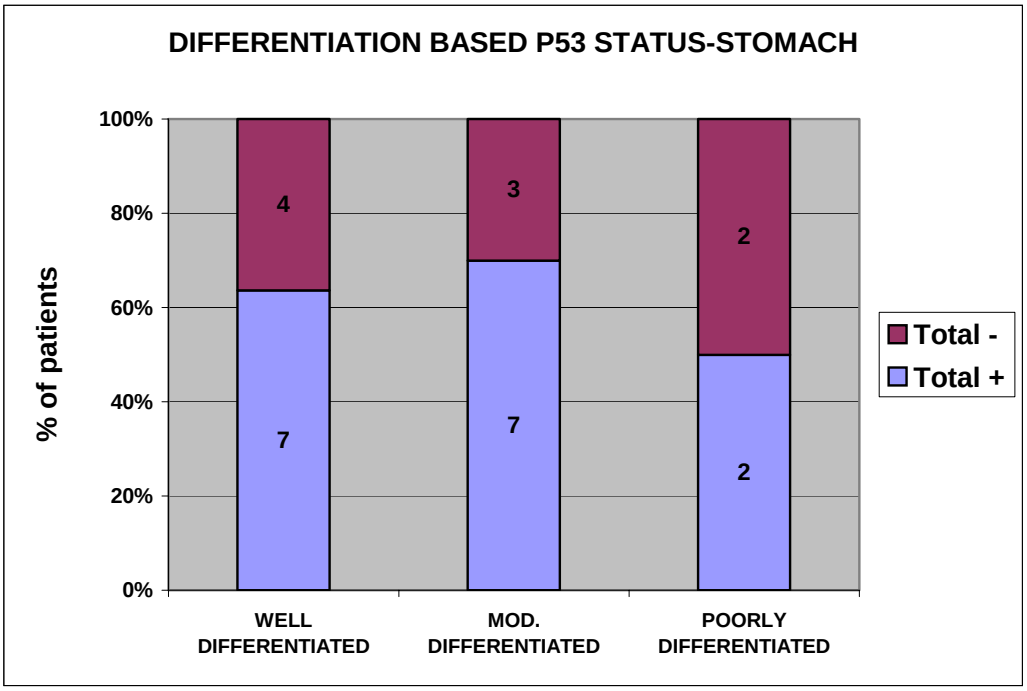
DIFFERENTIATION BASED p53 STATUS

CARCINOMA STOMACH

DIFFERENTIATION TYPE	INTENSITY OF STAINING						
	0	**1+**	**2+**	**3+**	**4+**	**TOTAL PT**	**TOTAL +**
WELL DIFFERENTIATED	4	6	1	0	0	11	7
MOD. DIFFERENTIATED	3	7	0	0	0	10	7
POORLY DIFFERENTIATED	2	2	0	0	0	4	2
UNDIFFERENTIATED	0	0	0	0	0	0	0
TOTAL	**9**	**15**	**1**	**0**	**0**	**25**	**16**

DIFFERENTIATION	Total +	Total -
WELL DIFFERENTIATED	7	4
MOD. DIFFERENTIATED	7	3
POORLY DIFFERENTIATED	2	2
UNDIFFERENTIATED	0	0





The above data was statistically analysed, χ^2 test = 0.50, P-value = 0.77. p53 positivity is more in the poorly differentiated tumors.

DISCUSSION

Observation of this study and other similar studies

Study Group	Immuno-Positivity - p53
Baron P.L. et al., (24, 46)	67%
Kuwano et al., (66)	56%
Coggi et al., (25)	55%
Novoc et al., (26)	51%
Okuda et al., (71)	75%
Robert et al., (68)	84%
Stephanie et al., (35)	70%
Nan Hu et al., (65)	75%
Miyata et al., (70)	72%
Biramijamal et al., (19)	65%
Mizobuclu et al., (67)	65%

The positive expression obtained in our study 64%, is comparable with Western, Japanese and middle east literature. The published literature have initially depended on IHC based detection of p53. As technology advanced, the recent research are based on PCR, Vector array mediated analysis, yeast cell culture assays. But the incidence of immunopositivity, 51-84% has been uniformly reproduced across various studies in esophageal and gastric cancer. The higher immunoexpression in few later studies might be due to the technical improvements in the detection methods. The possibility of unexplained factors controlling the expression of mutant p53 must be elucidated with more sophisticated diagnostic tools.

UNANSWERED QUESTIONS

In this study, p53 immunoreactivity was considered to be attributable to the accumulation of abnormal p53 protein. Although this idea is generally accepted, we should remember that negative immunoreactivity of p53 does not always reflect normal p53 function, because there may be homozygous detection, stop codon mutation or acceleration of protein degradation. We do not know the type of mutation from the results obtained in this study.

In fact, a functional assay of p53 using fission yeast showed that 70-90% of cancers display loss of the p53 function in various tumors, including SCC of the head and neck and uterus (59). In contrast, radiation therapy is clinically effective in more than half of these cancers (45). This strongly suggests the existence of an alternative pathway independent of p53, which regulates radiation sensitivity. The relationship between cell cycle and radiation effect is not fully understood. Prolonged cell cycle arrest is considered to induce terminal differentiation and loss of proliferative activity, and those cells would undergo cell death through a different mechanism from checkpoint. Detailed study of p53 in relation to the radiation and chemotherapy may assess this discrepancy in esophageal and stomach cancer.

We are not able to establish from this study the real implication of the intensity of staining and role of p53 in the carcinogenesis.

The published data available do not explain the reason for the mutant p53 expression in various sites like esophagus and stomach. We feel that the coexisting risk factors also play a role in determining the location of the tumor.

Hence one should always keep in the mind regarding the possibility of a potent non-p53 based mechanism creating its control over the esophageal and gastric carcinogenesis.

The reagents and the primary antibody used in this study came from the western world. The expression of mutant p53 in our cancers may be different if we use the locally developed primary antibodies.

NEW QUESTIONS

It is evident from this study that the presence of mutant p53 expression is almost 100% in the early onset cancers. Again a periodic p53 screening is mandatory in the patients who have the predilection and risk factors for the esophageal and gastric malignancies. Already research is on in analysing the expression of p53 in the fetal esophageal and gastric tissues. We do not know the role of p53 in the development of fetal esophagus and stomach.

In this study we are able to establish the presence of the mutant p53 in the tumor tissue in about 64%. A detailed exon based analysis of mutations will help us to understand the above questions. So far we do not know about the existence of other factors which control the tumor pathway based on the type of mutations. We do not know whether there will be difference in the malignant cascade based on the type of mutations.

SUMMARY AND CONCLUSION

Esophageal cancer represents the third most common gastrointestinal malignancy and the gastric cancer remains second only to lung cancer as a leading cause of cancer mortality worldwide. Most of these cancers are advanced and often unresectable at diagnosis and resistant to chemotherapy. Recent advances in the world of molecular genetics have helped us in understanding the biology of the cancer (16, 17, 32, 33, 41).

p53 is an important tumor suppressor gene, which plays a definite role in the upper gastro intestinal carcinogenesis (5-15, 24-36). We have done this study to elucidate the mutant p53 expression in upper gastro intestinal malignancies. The tissue obtained with endoscopic biopsy were utilised for this study. The histopathology was confirmed and typing was done. The tumor tissues were subjected to immunohistochemical examination for identification of mutant p53. The evaluations of the stained tissue were based on the UICC guidelines. Data obtained were based on various parameters. Statistical analysis was done before the data interpretation.

Of the 80 cases of upper gastro-intestinal malignancies, 55 were esophageal cancers and the remaining 25 were gastric malignancies. Of the 55 cases of esophageal SCC, 34 (61.8%) of them stained positive for mutant p53. Among the positive, 23 were male and 11 were female. Of the 25 cases of gastric malignancies, 16 (64%) of them stained positive for mutant p53. Among the positive, 10 were male and 6 were female.

81.8% of the patients with esophageal malignancy studied belonged to the age group of 41 to 70 yrs. 92% of the patients with gastric malignancy studied belonged to the age group of 41 to 80 yrs. Decade wise distribution was equally shared, except for the extremes. Expression of mutant p53 and intensity of staining were different in different sites. An alternative strategy should be searched for the patients who stain negative for mutant p53 as the clinical efficacy of the available therapy is not favourable for these patients.

Mutant p53 expression is closely associated with the carcinogenesis of upper gastro intestinal malignancy. Expression of p53 and other apoptotic markers could be used as an important biological index for planning therapy. As the role of p53 expression and response to chemotherapy and ionising radiation is evident from the upcoming large trials, this identification of p53 will help us to segregate patients to

different multimodal therapy (30). This mutant p53 expression is clearly implicated for the prognosis of upper gastrointestinal malignancy patients (45). This will help us to understand the degree of efficacy of various therapies in our patients. Apoptosis promoters such as bortezomib are at the forefront of current clinical development.

Recombinant adenoviral vector mediated gene therapy for advanced upper gastrointestinal malignancies have been safely given and the response obtained are encouraging. A baseline data of the mutant p53 expression is mandatory for planning gene therapy in our patients. We have established the presence a mutant p53 in about two-thirds of our patients.

With the available technology, the reason for the findings could not be explained properly. More detailed analysis, say in the form of mutational analysis based on the exon level will help us to understand the mutations in a better way. Mapping of the entire genome of these cancers will help us in elucidating the pathway of carcinogenesis.

This study and similar studies will probably form the basis for progression towards the recombinant viral vector mediated gene therapy.

BIBLIOGRAPHY

1. Yang, C.S. (1980) Research on esophageal cancer in China : a review. *Cancer Res.*, 40, 2633-2644.

2. Correa, P. (1982) Precursors of gastric and esophageal cancer. *Cancer*, 50, 2554-2565.

3. Qiu, S.L. and Yang, G.R. (1988) Precursor lesions of esophageal cancer in high-risk populations in Henan Province, China. *Cancer*, 62, 551-557.

4. Wang, L.D., Lipkin, M., Qiu, S.L., Yang, G.R., Yang, C.S. and Newmark, H.L. (1990) Labeling index and labeling distribution of cells in the esophageal epithelium in individuals at increased risk for esophageal cancer in Huixian China. *Cancer Res.*, 50, 2651-2653.

5. Bennett, W.P., Hollstein, M.C., Metcalf, R.A., *et al.*, (1992) p53 Mutation and protein accumulation during multistage human esophageal carcinogenenesis. *Cancer Res.*, 52, 6092-6097.

6. Wang, L.D., Hong, J.Y., Qiu, S.L., Gao, H.K. and Yang, C.S. (1993) Accumulation of p53 protein in human esophageal precancerous lesions: a possible early marker for carcinogenesis. *Cancer Res.*, 53, 1783-1787.

7. Wang, L.D., Shi, S.T., Zhou, Q., Goldstein, S., Hong, J.Y., Shao, P., Qiu, S.L. and Yang, C.S. (1994) Changes in p53 and cyclin D1 protein levels and cell proliferation in different stages of human esophageal and gastric-cardia carcinogenesis. *Int. J.* Cancer, 59, 1-6.

8. Gao, H., Wang, L.-D., Zhou, Q., Hong, J.-Y., Huang, T.-Y. and Yang, C.S. (1994) p53 Tumor suppressor gene mutation in early esophageal precancerous lesions and carcinoma among high-risk population in Henan, China. *Cancer Res.*, 54 : 4342-4346.

9. Jaskiewicz, K. and De Groot, K. (1994) p53 gene mutants expression, cellular proliferation and differentiation in esophageal carcinoma and non-cancerous epithelium. *Anticancer Res.*, 14, 137-140.

10. Bennett, W.P. (1995) p53 alterations in progenitor lesions of the bronchus, esophagus, oral cavity, and colon. *Cancer Detect. Prev.*, 19, 503-511.

11. Parenti, A.R., Rugge, M., Frizzera, E., Ruol, A., Noventa,F., Ancona, E. and Ninfo, V. (1995) p53 over expression in the multistep process of esophageal carcinogenesis. *Am. J. Surg. Pathol.*, 19, 1418-1422.

12. Volant, A., Nousbaum, J.B., Giroux, M.A., Roue-Quintin, I., Metges, J.P., Ferec, C., Gouerou, H. and Robaszkiewicz, M. (1995) p53 protein accumulation in esophageal squamous cell carcinomas and precancerous lesion. *J. Clin. Pathol.*, 48, 531-534.

13. Itakura, Y., Sasano, F., Date, F., Kato, K., Sekine, H., Mori, S. and Nagura, H. (1996) DNA ploidy, p53 expression, and cellular proliferation in normal epithelium and squamous dysplasia of non-cancerous and cancerous human esophagi. *Anticancer Res.*, 16, 201-208.

14. Kitamura, K., Kuwano, H., Yasuda, M., Sonoda, K., Sumiyoshi, K., Tsutsui, S., Kitamura, M. and Sugimachi, K. (1996) What is the earliest malignant lesion in the esophagus? *Cancer*, 77, 1614-1619.

15. Greenblatt, M.S., Benentt, W.P., Hollstein, M. and Harris, C.S. (1994) Mutations in the p53 tumor suppressor gene : clues to cancer etiology and molecular pathogenesis. *Cancer Res.*, 54, 4855-4878.

16. Hollstein, M., Sidransky, D., Vogelstein, B. and Harris, C.C. (1991) p53 mutations in human cancers. Science, 253, 49-53.

17. Levine, A.J. (1997) p53, the cellular gatekeeper for growth and division. *Cell*, 88, 323-331.

18. El-Deiry, W.S., Tokino T., Velculescu, V.E., Levy, D.B., Parsons, R., Trent, J.M., Lin, D., Mercer, W.E., Kinzler, K.W. and Vogelstein, B. (1993) WAF1, a potential mediator of p53 tumor suppression. *Cell*, 75, 817-825.

19. Firouzeh Biramijamal et al., Unusual Profile and High Prevalence of p53 Mutations in Esophageal Squamous Cell Carcinomas from Northern Iran. *Cancer Research* 61, 3119-3123, April 1, 2001.

20. Zhou Q., Wang L.D., Gao S.S., Li YX, Zhao X, Wang LX p53 immunostaining positive cells correlated positively with S phase cells as measured by BrdU in the esophageal precancerous lesions from the subjects at high incidence area for esophageal cancer in northern China *World J Gastroenterol,* 1998; 4 (Suppl 2) : 106-107.

21. Harris CC et al., Clinical implications of the p53 tumor suppressor gene. *N. Engl. J. Med.* 329 : 1318; 1993.

22. Souza R.F. Department of medicine, Dallas VA Medical Center, Dept. of GI, Harold C. Simmons Comprehensive Cancer Center, University of Texas - Southwestern Medical Center at Dallas, TX, USA. Molecular and biologic basis of upper gastrointestinal malignancy - esophageal carcinoma. *Surg. Oncol. Clin. N. Am.* 2002. Apr; 11 (2) : 257-72, viii.

23. Uys P, van Helden PD., MRC Centre for Molecular and Cellular Biology and Department of Medical Biochemistry, University of Stellenbosch, Tygerberg, On the nature of genetic changes required for the development of esophageal cancer. South Africa. *Mol. Carcinog* 2003 Feb; 36(2) : 82-9.

24. Baron PL, Gates CE, Reed CE, Dikeman RL, Drosieko JJ, Passmore RN, Bromberg JS, Willingham MC. Department of Surgery, Medical University of South Carolina, Charleston 29425, USA. p53 overexpression in squamous cell carcinoma of the esophagus. *Ann. Surg. Oncol* 1997 Jan; 4(1) : 37-45.

25. Coggi G, Bosari S, Roncalli M, Graziani D, Bossi P, Viale G, Buffa R, Ferrero S, Piazza M, Blandamura S, Segalin A, Bonavina L, Peracchia, A. Department of Pathology II, Ospedale S. Paolo, Milan, Italy p53 protein accumulation and p53 gene mutation in esophageal carcinoma. A molecular and immunohistochemical study with clinicopathologic correlations. *Cancer* 1997 Feb. 1; 79(3) : 425-32.

26. Nozoe T, Kuwano H, Toh Y, Watanabe M, Kitamura M, Sugimachi K Department of Surgery II, Faculty of Medicine, Kyushu University, Fukuoka 812, Japan. Significance of p53 protein expression in growth pattern of esophageal squamous cell carcinoma. *Oncol Rep.* 1998 Sep-Oct; 5(5) : 1119-23.

27. Putz A, Hartmann AA, Fontes PR, Alexandre CO, Silveira DA, Klug SJ, Rajbes HM. Institute of Pathology, Ludwig TP53 mutation pattern of esphageal squamous cell carcinomas in a high risk area (Southern Brazil) : role of life style factors. *Int. J Cancer* 2002, Mar 1 : 98 (1) : 99-105.

28. Polyak, K., Xia Y., Zweier,J.L., Kinzler,K.W. and Vogelstein, B.A. (1997) Model for p53 induced apoptosis. *Nature*, 389, 300-305.

29. Stephanie T. Shi et al. Role of p53 gene mutations in human esophageal carcinogenesis : results from immunohistochemical and mutation analysis of carcinomas and nearby non-cancerous lesions *Carcinogenesis*, Vol. 20, No.4, 591-597, April 1999.

30. Hamada M., Fujiwara T., Hizuta A., Gochi A., Naomoto Y., Takamura N., Roth J.A., Tanaka N., Orita K. The p53 gene is a potent determinant of chemosensitivity and radiosensitivity in gastric and colorectal cancers. *J. Cancer Res. Clin. Oncol.,* 122 : 360-365, 1996.

31. Bergh J., Norberg T., Sjogren S., Lingren A., Holmberg L. Complete sequencing of the p53 gene provides prognostic information in breast cancer patients, particularly in relation to adjuvant systematic therapy and radiotherapy. *Nat. Med.,* 1 : 1029-1034,1995.

32. Hollstein M., Sidransky D., Vogelstein B., Harris C. C. p53 mutations in human cancers. *Science* (Washington DC), 253 : 49-53, 1991.

33. Vogelstein B., Kinzler K.W. p53 function and dysfunction. Minireview, *Cell,* 70 : 523-526, 1992.

34. Kashiswazakii H., Tonoki H., Tada M., Chiba I., Shindoh M., Totsuka Y., Iggo R., Moriuchi, T. High frequency of p53 mutations in human oral epithelial dysplasia and primary squamous cell carcinoma detected by yeast functional assy. *Oncogene,* 15 : 2667-2674, 1997.

35. Shi et al. Role of p53 gene mutations in human esophageal carcinogenesis : results from immunohistochemical and mutation analyses of carcinomas and nearby non-cancerous lesion *Carcinogenesis,* Vol. 20, No.4, 591-597, April 1999.

36. Hollstein MC, Metcalf RA, Welsh JA, Montesano R, Harris CC. Frequent mutation of the p53 gene in human esophageal cancer. International Agency for Research on Cancer, Lyon, France. *Proc Natl. Acad Sci, USA* 1990 Dec; 87 (24) : 9958-61.

37. Meltzer SJ et al. Reduction to homozygosity involving p53 in esophageal cancers demonstrated by the polymerase chain reaction. *Proc Natl. Acad. Sci. USA* 1991 Jun 1; 88(11) : 4976-80.

38. Bennett WP et al., Archival analysis of p53 genetic and protein alterations in Chinese esophageal cancer. *Oncogene* 1991 Oct; 6(10) : 1779-84.

39. Casson AG et al., p53 gene mutations in Barrett's epithelium and esophageal cancer. *Cancer Res.* 1991 Aug. 15; 51(6) : 4495-9.

40. Suo Z, Holm R, Nesland JM. Squamous cell carcinomas, an immunohistochemical and ultra structural study. *Anticancer* Res. 1992 Nov. - Dec; 12(6B) : 2025-31.

41. Nishihira T, Hashimoto Y, Katayama M, Mori S, Kuroki T. Molecular and cellular features of esophageal cancer cells : *J Cancer Res. Clin Oncol* 1993; 119(8) : 441-9.

42. Chung KY, Mukhopadhyay T, Kim J, Casson A, Ro JY, Goepfert H, Hong WK, Roth JA Discordant p53 gene mutations in primary head and neck cancers and corresponding second primary cancers of the upper aerodigestive tract. *Cancer Res.* 1993 Apr. 1; 53(7) : 1676-83.

43. Wang DY, Xiang YY, Tanaka M, Li XR, Li JL, Shen Q, Sugimura H, Kino I. High prevalence of p53 protein over expression in patients with esophageal cancer in Linxian, China and its relationship to progression and prognosis *Cancer* 1994 Dec. 15 ; 74 (12) : 3089-96.

44. Nakanishi Y, Noguchi M, Matsuno Y, Saikawa M, Mukai K, Shimosato Y, Hirohashi S. p53 expression in multicentric squamous cell carcinoma and surrounding squamousepithelium of the upper aerodigestive tract. Immunohistochemical analysis of 94 lesions. *Cancer* 1995 Apr 1; 75(7) : 1657-62.

45. Seitz JF, Perrier, H, Monges G, Giovannini M, Gouvernet J. (Multivariate analysis of the prognostic and predictive factors of response to concomitant radiochemotherapy in epidermoid cancers of the esophagus. Value of immunodetection of protein p53) *Gastroenterol Clin. Biol.* 1995 May; 19(5) : 465-74.

46. Devesa SS, Blot WJ, Fraumeni FJF. Changing patterns in the incidence of esophageal and gastric carcinoma in the United States. Cancer 1998; 83: 2049–2053.

47. Webb A, Cunningham D, Scarffe JH et al. Randomized trial comparing epirubicin, cisplatin, and fluorouracil versus fluorouracil, doxorubicin, and methotrexate in advanced esophagogastric cancer. J Clin Oncol 1997; 15: 261–267.

48. Pozzo C, Barone C, Szanto J et al. Irinotecan in combination with 5-fluorouracil and folinic acid or with cisplatin in patients with advanced gastric or esophageal-gastric junction adenocarcinoma: results of a randomized phase II study. Ann Oncol 2004; 15: 1773–1781.

49. Bouche O, Raoul JL, Bonnetain F et al. Randomized multicenter phase II trial of a biweekly regimen of fluorouracil and Leucovorin (LV5FU2), LV5FU2 plus cisplatin or LV5FU2 plus irinotecan in patients with previously untreated metastatic gastric cancer: a Federation Francophone de Cancerologie Digestive Group Study—FFCD 9803. J Clin Oncol 2.

50. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000; 100: 57–70004; 22: 4319–4328.

51. Doi T, Koizumi W, Siena S et al. Efficacy, tolerability and pharmacokinetics of gefitinib (ZD 1839) in pretreated patients with metastatic gastric cancer. Proc Am Soc Clin Oncol 2003; 22: 258.

52. Shah MA, Holen K, Singh D et al. A multicenter, two-stage, phase II study of PS-341 in patients with unresectable or metastatic gastric or gastroesophageal junction (GEJ) adenocarcinoma. Proc Gastrointestinal Am Soc Clin Oncol 2005; 103 (Abstr 41).

53. Ocean AJ, Schnoll-Sussman F, Chen X et al. Phase II study of PS-341 (bortezomib) with or without irinotecan in patients (pts) with advanced gastric adenocarcinomas (AGA). Proc Gastrointestinal Am Soc Clin Oncol 2005; 98 (Abstr 31).

54. Ocean AJ, Lane ME, Xiang Z et al. Genetic análisis of proteasome-related pathways in advanced gastric adenocarcinomas (AGA) treated on a phase II study with the proteasome inhibitor, PS-341 (bortezomib) with or without irinotecan. Proc Gastrointestinal Am Soc Clin Oncol 2005; 117 (Abstr 69).

55. Ishida M, Gomyo Y, Tatebe S, Ohfuji S, Ito H. Apoptosis in human gastric mucosa, chronic gastritis, dysplasia, and carinoma: analysis by terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling. Virchows Arch, 1996;428:229-235.

56. Guo YQ, Zhu ZH, Li JF. Flow cytometric analysis of apoptosis and proliferation in gastric cancer and precancerous lesion. Shijie Huaren Xiaohua Zazhi, 2000;8:983-987.

57. Krajewski S, Blomqvist C, Frarssila K, Krajewska M, Wasenivs VM, Niskanen E. Reduced expression of proapoptosis gene Bax is associated with poor response rates to combination chemotherapy and adenocarcinma. Cancer Res, 1995;55:4471-4478.

58. Sundblad AS, Tamayo R. Expression of MIB-1/Ki-67 and bcl-2 in gastric carcinoma. Relationship with clinic pathological factors. Acta Gastroenterol Latinoam, 1995;25:67-72.

59. Liu HF, Liu WW, Fang DC. Study of the relationship between apoptosis and proliferation in gastric carcinoma and its precancerous lesion. Shijie Huaren Xiaohua Zazhi, 1999; 7 : 649-651.

60. Xu AG, Li SG, Liu JH, Gan AH. Significance of apoptosis and expression of bcl-2 in gastric pre-carcinoma and cancer. Guangdong Yixue, 1998; 19 : 489-490.

61. Gan AH, Xu AG, Liu JH. The apoptosis and expression of c-myc in gastric cancer. Chin J Intern Med, 1999; 38 : 783.

62. Chandramohan et al., Tp53 - Tumor suppressor gene status in esophageal squamous cell carcinoma in India. *Diseases of the esophagus*, Vol. 17, Supplement 1, 2005, Page A-43.

63. Chandramohan et al., Esophageal squamous cell carcinoma patients and p53 status in India. *Japanese Journal of Thoracic and Cardiovascular Surgery*, Vol. 57, Supplement 2, Sept. 2005, p. 274 (Ref. ICE 74).

CUMULATIVE RESULTS

S.No.	PATIENT ENDOSCOPY I/D	AGE	SEX	SITE OF BIOPSY	HPE	DIFFERENTIATION	p53	GRADING
1	442/04	52	M	STOMACH	ADENO CA	Poorly Differentiated	NEGATIVE	
2	404/04	83	M	STOMACH	ADENO CA	Well Differentiated	NEGATIVE	
3	380/04	67	F	STOMACH	ADENO CA	Moderately Differentiated	NEGATIVE	
4	284/04	65	M	STOMACH	ADENO CA	Moderately Differentiated	POSITIVE	1+
5	277/04	45	M	STOMACH	ADENO CA	Moderately Differentiated	NEGATIVE	
6	347/04	59	F	STOMACH	ADENO CA	Well Differentiated	POSITIVE	2+
7	375/04	37	F	STOMACH	ADENO CA	Moderately Differentiated	POSITIVE	1+
8	295/04	73	M	STOMACH	ADENO CA	Well Differentiated	NEGATIVE	
9	231/04	64	M	STOMACH	ADENO CA	Moderately Differentiated	POSITIVE	1+
10	111/04	52	M	STOMACH	ADENO CA	Well Differentiated	POSITIVE	1+
11	220/04	55	M	STOMACH	ADENO CA	Well Differentiated	NEGATIVE	
12	180/04	41	M	STOMACH	ADENO CA	Moderately Differentiated	NEGATIVE	
13	75/04	75	M	OG JN	ADENO CA	Poorly Differentiated	POSITIVE	1+
14	66/04	75	F	STOMACH	ADENO CA	Poorly Differentiated	POSITIVE	1+
15	120/04	58	F	STOMACH	ADENO CA	Moderately Differentiated	POSITIVE	1+
16	56/04	50	M	STOMACH	ADENO CA	Well Differentiated	POSITIVE	1+

S.No.	PATIENT ENDOSCOPY I/D	AGE	SEX	SITE OF BIOPSY	HPE	DIFFERENTIATION	p53	GRADING
17	39/04	75	M	STOMACH	ADENO CA	Well Differentiated	POSITIVE	1+
18	105/04	42	M	STOMACH	ADENO CA	Moderately Differentiated	POSITIVE	1+
19	253/04	51	M	STOMACH	ADENO CA	Moderately Differentiated	POSITIVE	1+
20	182/04	76	F	STOMACH	ADENO CA	Well Differentiated	POSITIVE	1+
21	358/04	79	M	STOMACH	ADENO CA	Well Differentiated	POSITIVE	1+
22	612/04	50	M	STOMACH	ADENO CA	Well Differentiated	NEGATIVE	
23	161/04	47	M	STOMACH	ADENO CA	Poorly Differentiated	NEGATIVE	
24	112/04	76	M	STOMACH	ADENO CA	Poorly Differentiated	POSITIVE	1+
25	610/04	59	M	STOMACH	ADENO CA	Well Differentiated	POSITIVE	1+
26	606/04	58	F	STOMACH	ADENO CA	Moderately Differentiated	POSITIVE	1+
27	584/04	55	F	OG JN	ADENO CA	Well Differentiated	POSITIVE	2+
28	521/04	60	M	ESOPHAGUS	SCC	Well Differentiated	NEGATIVE	
29	467/04	65	F	ESOPHAGUS	SCC	Poorly Differentiated	POSITIVE	4+
30	472/04	55	M	ESOPHAGUS	SCC	Poorly Differentiated	POSITIVE	3+
31	545/04	40	M	ESOPHAGUS	SCC	Undifferentiated	POSITIVE	3+
32	57/04	72	M	ESOPHAGUS	SCC	Well Differentiated	POSITIVE	4+
33	521/04	50	F	ESOPHAGUS	SCC	Poorly Differentiated	NEGATIVE	
34	481/04	62	M	ESOPHAGUS	SCC	Poorly Differentiated	POSITIVE	4+

S.No.	PATIENT ENDOSCOPY I/D	AGE	SEX	SITE OF BIOPSY	HPE	DIFFERENTIATION	p53	GRADING
35	470/04	41	M	ESOPHAGUS	SCC	Well Differentiated	NEGATIVE	
36	531/04	65	M	ESOPHAGUS	SCC	Undifferentiated	NEGATIVE	
37	16/05	48	F	ESOPHAGUS	SCC	Poorly Differentiated	NEGATIVE	
38	22/05	50	F	ESOPHAGUS	SCC	Moderately Differentiated	POSITIVE	3+
39	192/05	55	F	ESOPHAGUS	SCC	Well Differentiated	POSITIVE	2+
40	584/05	68	M	ESOPHAGUS	SCC	Moderately Differentiated	POSITIVE	3+
41	05/05	65	M	ESOPHAGUS	SCC	Poorly Differentiated	POSITIVE	4+
42	49/05	62	F	ESOPHAGUS	SCC	Well Differentiated	POSITIVE	3+
43	479/05	49	M	ESOPHAGUS	SCC	Well Differentiated	POSITIVE	3+
44	24/05	55	M	ESOPHAGUS	SCC	Poorly Differentiated	NEGATIVE	
45	371/05	47	M	ESOPHAGUS	SCC	Poorly Differentiated	NEGATIVE	
46	392/05	56	M	ESOPHAGUS	SCC	Moderately Differentiated	POSITIVE	4+
47	56/05	61	M	ESOPHAGUS	SCC	Moderately Differentiated	POSITIVE	3+
48	104/05	40	M	ESOPHAGUS	SCC	Poorly Differentiated	POSITIVE	3+
49	39/05	70	F	ESOPHAGUS	SCC	Well Differentiated	POSITIVE	3+
50	253/05	50	M	ESOPHAGUS	SCC	Moderately Differentiated	POSITIVE	3+
51	182/05	53	F	ESOPHAGUS	SCC	Well Differentiated	NEGATIVE	
52	358/05	53	F	ESOPHAGUS	SCC	Well Differentiated	NEGATIVE	

S.No.	PATIENT ENDOSCOPY I/D	AGE	SEX	SITE OF BIOPSY	HPE	DIFFERENTIATION	p53	GRADING
53	161/05	55	M	ESOPHAGUS	SCC	Poorly Differentiated	POSITIVE	2+
54	112/05	68	F	ESOPHAGUS	SCC	Well Differentiated	NEGATIVE	
55	612/05	45	M	ESOPHAGUS	SCC	Moderately Differentiated	NEGATIVE	
56	610/05	50	M	ESOPHAGUS	SCC	Undifferentiated	POSITIVE	1+
57	328/05	52	M	ESOPHAGUS	SCC	Undifferentiated	NEGATIVE	
58	66/05	55	M	ESOPHAGUS	SCC	Undifferentiated	NEGATIVE	
59	111/05	60	M	ESOPHAGUS	SCC	Well Differentiated	POSITIVE	1+
60	75/05	66	F	ESOPHAGUS	SCC	Moderately Differentiated	POSITIVE	4+
61	347/05	35	M	ESOPHAGUS	SCC	Moderately Differentiated	POSITIVE	3+
62	180/05	40	M	ESOPHAGUS	SCC	Poorly Differentiated	POSITIVE	1+
63	220/05	60	M	ESOPHAGUS	SCC	Well Differentiated	POSITIVE	4+
64	347/05	58	M	ESOPHAGUS	SCC	Well Differentiated	NEGATIVE	
65	277/05	60	M	ESOPHAGUS	SCC	Moderately Differentiated	POSITIVE	4+
66	404/05	45	F	ESOPHAGUS	SCC	Well Differentiated	NEGATIVE	
67	442/05	50	M	ESOPHAGUS	SCC	Moderately Differentiated	NEGATIVE	
68	380/05	70	M	ESOPHAGUS	SCC	Moderately Differentiated	NEGATIVE	
69	545/05	53	F	ESOPHAGUS	SCC	Well Differentiated	POSITIVE	3+
70	521/05	60	M	ESOPHAGUS	SCC	Moderately Differentiated	NEGATIVE	

S.No.	PATIENT ENDOSCOPY I/D	AGE	SEX	SITE OF BIOPSY	HPE	DIFFERENTIATION	p53	GRADING
71	504/05	40	F	ESOPHAGUS	SCC	Well Differentiated	POSITIVE	4+
72	509/05	30	F	ESOPHAGUS	SCC	Undifferentiated	POSITIVE	4+
73	480/05	63	M	ESOPHAGUS	SCC	Moderately Differentiated	POSITIVE	3+
74	499/05	65	M	ESOPHAGUS	SCC	Well Differentiated	POSITIVE	4+
75	487/05	65	M	ESOPHAGUS	SCC	Poorly Differentiated	NEGATIVE	
76	481/05	87	M	ESOPHAGUS	SCC	Undifferentiated	NEGATIVE	
77	472/05	62	M	ESOPHAGUS	SCC	Well Differentiated	NEGATIVE	
78	470/05	68	F	ESOPHAGUS	SCC	Moderately Differentiated	POSITIVE	3+
79	388/05	40	M	ESOPHAGUS	SCC	Moderately Differentiated	POSITIVE	3+
80	467/05	68	M	ESOPHAGUS	SCC	Well Differentiated	POSITIVE	4+

STUDY FORMAT

Name :

Age :

Sex :

a. Male

b. Female

Patient Endoscopy ID No.

Location of the tumor

a. Esophagus

b. OG Junction

c. Stomach

Histopathology of the tumor

Adenocarcinoma

Squamous cell Carcinoma

Degree of Differentiation

a. **Well Differentiated**

b. **Moderately Differentiated**

c. **Poorly Differentiated**

d. **Undifferentiated**

Immunohistochemistry

a. **Positive**

b. **Negative**

Degree of Staining

a. **Grade 1+**

b. **Grade 2+**

c. **Grade 3+**

d. **Grade 4+**