Immunoexpression of p53 as an indicator of invasiveness in esophageal carcinoma:- a tertiary care hospital study from Punjab, India

Rahul Mannan[1], Mridu Manjari[2], Harleen Kaur[3], Tejinder Singh Bhasin[4], Sonam Sharma[5,*]

[1]Associate Professor, [2,4]Professor, [3]Ex-Resident, Sri Guru Ram Das Institute of Medical Sciences & Research, Amritsar, Punjab, [5]Senior Resident, Dept. of Pathology, Vardhman Mahavir Medical College & Safdarjung Hospital, New Delhi, India

*Corresponding Author:
Email: drsonamsharma@gmail.com

Abstract

Background: Esophageal carcinoma (EC) is an upcoming major health challenge with 4,07,000 deaths related to it reported globally in the year 2008 alone. Its risk factors apart from tobacco and alcohol vary according to various geographical areas which include ethnicity, dietary and other socio-economic factors. This can have a bearing on its various prognostic factors in which Immunohistochemistry (IHC) has emerged as a major tool for predicting the same.

p53 has proved to be a particular important molecule in development of many tumors including esophageal carcinoma as it has a role in both tumor immunity and cell cycle.

The present study was conducted in Punjabi population where tobacco usage is low because of the societal and religious taboos and where life style and developmental indicators are high in comparison to rest of India. The aim was to analyze epidemiologically the cases suffering from esophageal carcinoma, to study p53 expression in histologically proven cases of esophageal carcinoma and also to correlate p53 positive expression with the type and grade of the tumor along with other clinico-pathological parameters.

Material and Methods: The study was conducted on 50 cases of EC diagnosed in the Department of Pathology, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India. The 50 cases (either endoscopic biopsies or esophagectomy specimen) were independently verified for sub classification and histopathological grading. In all the cases patient's complains, age, gender and other important clinical parameters were recorded. All the cases were taken up for IHC study for p53 expression.

The results obtained were tabulated and statistically analyzed. A p value less than 0.05 was considered statistically significant.

Result: In the present study, maximum cases (60%) were observed in the age group of 41-60 years where female patients outnumbered male patients in a ratio of (1.3:1). Lower segment (56%) was the most common segment involved. Histopathologically, most of the cases were of the squamous cell type (constituting 94% of the total cases) followed by adenocarcinoma with most of these cases graded as G2 (moderately differentiated). In 20 cases of esophagectomy specimen included in the present study; both lymph node metastasis and adventitial layer involvement (full thickness) was identified in 13 cases each. p53 expression was observed in 50% cases (percentage of positive cells varying from 5-89% with mild, moderate and strong staining intensity) with positivity seen in 48.9% cases of squamous cell carcinoma (ESCC) and 66.7% in adenocarcinoma (EAC). Of the cases included in the study; no significance between grading, lymph node status and p53 expression was elicited. However, correlation of p53 and adventitial involvement was noted and this finding was statistical significant.

Conclusion: The present study is the first such study done on North Indian Punjabi population. The finding of significant association of p53 expression with adventitial involvement (full thickness involvement) is an important prognostic factor to comment upon the invasiveness of the disease in an individualized manner.

Keywords: Esophageal Carcinoma, Immunohistochemistry, India, p53, Punjab

Introduction

Carcinoma of the esophagus poses a considerable medical and public health challenge in many parts of the world including resources challenged countries. Esophageal carcinoma (EC) is the eighth most common cancer around the world. In the year 2008, an estimated 4,82,000 new esophageal cancer cases were diagnosed and 4,07,000 related deaths occurred globally.[1]

In India, in addition to the usual reported associations additional factors play a significant role in causation of EC such as malnutrition (vitamin A, E, C, riboflavin and niacin along with deficiency of micronutrients such as molybdenum and zinc) and betel quid chewing (relative risk 1.5 to 3.5).[2,3]

In recent years, Immunohistochemistry (IHC) in esophageal cancers has emerged as an important tool for diagnosis and predicting prognosis. Various IHC markers are used as a research tool in cases of EC such as p53, CK 6, CK 7, pRB amongst others.[4]

p53 has proved to be a particular important molecule in development of many tumors including EC as it has an important central and vital role in tumor immunity by checking apoptotic mechanisms and also an identifiable role in cell cycle control. It has been hypothesized that its interaction with another important cell cycle proliferative marker pRb leads to oncogenesis of SCC and also can affect its prognosis.[5]

There have been many studies internationally and also in India especially in high incidence reported areas such as Kashmir valley[6] and even in Meghalaya[5] where high p53 immunoexpression has been reported; but no worthwhile study has been conducted in North Indian Punjabi population to investigate for the same. The

present study was conducted in a population where tobacco usage is low because of the societal and religious taboos and where life style and developmental indicators are high in comparison to rest of India.

Aims and Objectives
1. Epidemiological analysis of the cases suffering from EC (age, sex and clinical features).
2. To study p53 expression in histologically proven cases of EC.
3. To correlate p53 positivity with the type and grade of the tumor along with other parameters such as age, sex, lymph node status and adventitial involvement in histologically proven cases of EC.

Material and Methods
The study was conducted on 50 cases of EC diagnosed in the Department of Pathology, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India. The 50 cases (either endoscopic biopsies or esophagectomy specimen) were independently verified for sub classification and histopathological grading. In all the cases patient's complains, age, gender and other important clinical parameters were recorded.

All the cases included in the study were taken up for IHC study for p53 expression. The IHC was done on 3-4 micron thick sections with antigen retrieval done under pressure and using primary antibody for p53 being – CME298BK procured from Diagnostic Biosystem.

The IHC was employed using the standard protocol as practiced universally. Positive and negative controls were run with every batch of the IHC. If the test sections showed positivity at the site of target antigen in the form of coloured end product (brown nuclei), it meant that the tissue had antibody specific antigen. Cytoplasmic brownish colouration was ignored. Three histopathologists in double blinding manner reviewed the IHC slides and the results were taken as agreement of 2 out of 3. Immunoreactivity was classified into the following three categories based on the percentage of tumour cells showing nuclear reactivity: less than 10%, 10% to 50% and more than 50%. Immunointensity was classified as no immunostaining (-), weakly immunostaining (+), weak immunostaining (++) and strongly positive immunostaining (+++). (Fig. 1)

Statistics: The results obtained were tabulated and statistics (Chi-Square (χ2) test and Fischer's exact test) were employed. p valve were calculated to analyze and investigate for significance of p53 with other clinical and histopathological parameters. A p value less than 0.05 was considered statistically significant.

Results
Epidemiological and Anatomical Parameters: In the present study, maximum cases (60%) were observed in the age group of 41-60 years where female patients outnumbered male patients in a ratio of (1.3:1). (Table 1)

Most of the patients presented with clinical symptoms of short duration (3-6 months) with dysphagia being the commonest complaint.(Table 2) The commonest anatomical segment recorded in both esophagectomy as well as endoscopic biopsies was found to be the lower segment (56%). Grossly, the most common observed growth pattern was polypoidal type which was seen in 28 cases (56%) followed by proliferative and ulcerative type. On histopathology, most of the cases were of the squamous cell type (constituting 94% of the total cases) followed by adenocarcinoma.

On grading, the commonest pattern identified was moderately differentiated seen in 37 cases (74.0%) followed by 7 cases of well differentiated and 6 cases of poorly differentiated carcinoma.

Table 1: Age and Gender Wise Distribution

Age (Group)	Females	Males	Total
21-40 Yrs	2	3	5
41-60 Yrs	17	13	30
61-80 Yrs	8	6	14
81-100 Yrs	1	0	1
Total	28	22	50
Percentage	56	44	100

Table 2: Clinical Presentation
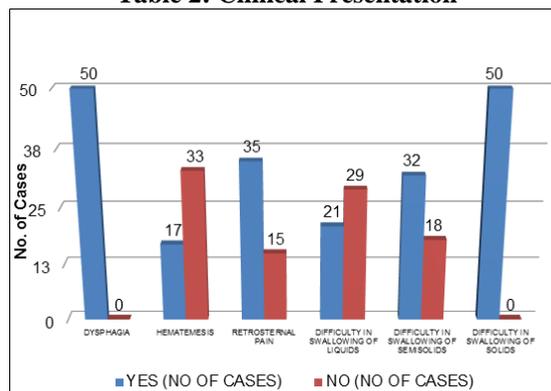
In 20 cases of esophagectomy specimen included in the present study; both lymph node metastasis and adventitial layer involvement (full thickness) was identified in 13 cases each.

Immunohistochemical Analysis: In the present study, the p53 expression was observed in 50% cases (percentage of positive cells varying from 5-89% with mild, moderate and strong staining intensity) with positivity seen in 48.9% cases of squamous cell carcinoma and 66.7% in adenocarcinoma.

Of the cases included in the study; no significance relationship between grading and p53 expression was observed.(Table 3)

Table 3: Correlation of p53 Expression with Tumor Grade

Grade	p53 Expression		Total
	Negative	Positive	
Well	05	02	07
Moderate	19	18	37
Poor	01	05	06
Total	25	25	50

A point of interest was regarding p53 expression in lymph nodes recovered. Of the 7 cases, which showed reactive hyperplasia of the lymph nodes the immunoexpression, was elicited in 4 cases. In 13 esophagectomy cases where there was evidence of metastatic deposits; 9 cases showed evidence of expression of p53 molecule. The finding however was not found statistically significant (p = 0.568). (Table 4)

Table 4: p53 Expression Correlation with Lymph Node Status

Lymph node status	p53 Expression		Total
	Negative	Positive	
Reactive	3	4	7
Metastatic	4	9	13
Total	7	13	20

An attempt was also made in the current study to delineate immunoexpression in 12 cases in which adventitial involvement was noted and this finding was statistical significant (p = 0.03).(Table 5)
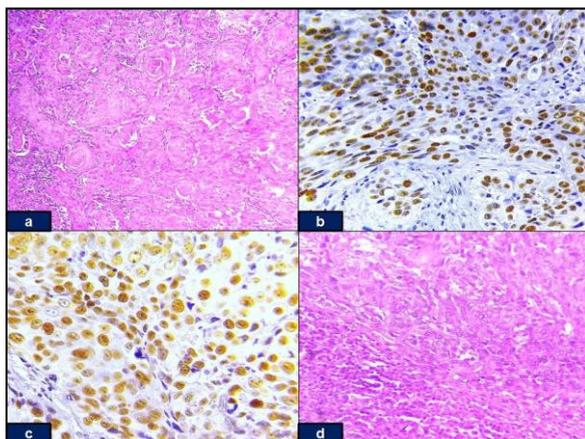

Fig. 1: a) Moderately differentiated squamous cell carcinoma [H & E; 200 X]
b) Tumor cells exhibiting p53 expression of 3 + intensity [IHC; 200X]

c) **Tumor cells showing 2 + intensity p-53 expression [IHC; 200X]**
d) **Poorly differentiated squamous cell carcinoma [H & E; 200 X]**

Table 5: p53 Expression with Adventitial Involvement

Adventitial involvement	p53 Expression		Total
	Negative	Positive	
Absent	5	2	7
Present	3	10	13
Total	8	12	20

Discussion

It is now well documented that despite the availability of good screening tools and multifactorial measures, there is requirement of identification of newer molecular markers, which can predict the survival or even lead to a tailor made individualized therapy. Despite advances in screening and multimodal management of EC, overall survival for both the squamous cell type and adenocarcinoma remains poor.[7] There is a need to identify tumor markers as prognostic indicators and as targets for new therapeutic strategies which still remains a major challenge in EC research.[8]

The maximum incidence in present study seen in age group of 41-60 years concurred with the work done by other research study from China.[9]

In the present study although most of the carcinomas histologically were squamous cell type and this is on similar lines with Asian studies such as the one by Cherian *et al*[10] but its incidence has been declining over the past decades, with a concomitant increase in incidence of adenocarcinoma (6 fold from 1975 to 2000) due to increase in incidence of gastroesophageal reflux (most importantly associated with Barrett's esophagus).[11,12]

Just like the other oncological important sites; lymph node involvement is the single most important prognostic factor in cases of EC. The rate of metastasis in the lymph nodes recovered from the esophagectomy specimen in the present study was only 65% which was comparatively lower than the reported rate in literature.[13]

p53; the molecule under investigation in the present study is perhaps the most referenced and well-know tumor suppressor gene thought to have a central role in various human neoplasia and also associated with Li–Fraumeni and related syndromes. It is associated with various carcinomas such as lung, colon and breast and also with sarcomas, hematological malignancies and brain tumor.

The p53 protein is a DNA-binding protein product of the p53 tumor suppressor gene encompassing 16-20 kb of DNA on the short arm of chromosome 17 at

position 17-13.1. Wild-type p53 protein exerts growth-inhibitory activity. Its up-regulation in human hematopoietic cells in response to certain types of DNA damage prolongs the G1 phase of the cell cycle and gives the cell time to repair that damage before entering the S phase.[14]

A major thrust of the study was an attempted correlation of p53 with various epidemiological and histopathological parameters.

p53 positivity rate was 45% which was slightly lower than the reported range by other researchers outside India (47%- 87.2% cases).[15,16] In Indian context, 2 studies done in high incidence of EC areas – Meghalya[5] and Kashmir valley[17] have reported the rates of 83.3% and 77.7% respectively. However, lower p53 immunoexpression rates have been reported from Japan by Ikeguchi M et al.[4] The high variance of p53 expression is thought to be related to the ethnic, religious, geographic and dietary (including life style factors) differences.[18] In the present study; tobacco was not such a big factor as tobacco intake is a religious taboo in Sikh religion of which most of the patients were and also the dietary intake of western diet (highly saturated with alcohol intake) makes this area markedly different from the rest of India.

In the present study, no significant correlation between p53 positivity with patient's age and gender were observed as also noted by Coggi G et al.[19]

Although many researchers have corroborated the association of p53 expression with increasing grade of EC; in the present study no significance was derived between p53 expression and grading. Other researchers such as Sarbia M et al have also found no correlation between p53 expression and histological grade.[20]

The literature is varied for the expression of p53 in metastatic lymph nodes and a possible association between the two. Immunoexpression shows a spectrum of variation from as low as 43 % by Casson AG et al[21] and as high as 65.5 by Han U et al.[16] Many researchers have observed a very strong relation between p53 positivity and lymph node metastasis.[5,13] In our study the expression of p53 with lymph node metastasis was however; statistically not significant just like the work conducted by Sarbia M et al.[20]

The p53 expression was however, significantly associated with invasion into the adventitia (full thickness involvement). This is in accordance with the work done by Dey B et al.[5] This association there by suggests that protein end products related to the dysregulated p53 expression has a bearing on the final prognostication of the patients suffering from EC where the cases with expression of p53 should be kept under constant follow up and should follow a rigorous cancer regime as these patients will be expected to suffer from higher rates of invasiveness and thereby can have a bearing on both local and distant metastasis (leading to lower survival rates).

Conclusion

The study documents the first such study done on North Indian Punjabi population as very limited work is done in India regarding the same. The finding of significant association of p53 expression with adventitial involvement (full thickness involvement) is an important prognostic factor to comment upon the invasiveness of the disease in an individualized manner. However, an attempt is being made to increase the sample size in our institute in future so that further interpretations can be derived regarding the invasiveness of EC with p53 expression.

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