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CORRELATION BETWEEN P53 GENE POLYMORPHISM AND H PYLORI INFECTION IN ENDOSCOPIC BIOPSIES OF SUSPECTED CASES OF ESOPHAGEAL CANCER

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Conflicts of Interest: **Nil**

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ABSTRACT

BACKGROUND: Esophageal cancer is of two main types (a) Esophageal squamous cell carcinoma (ESCC) and (b) Esophageal adenocarcinoma (EA). Esophageal squamous cell carcinoma accounts for the majority of cases (>90%), especially in the Asian esophageal cancer belt. Helicobacter pylori infection has shown a consistent pattern of association with esophageal adenocarcinoma (EA), especially in the western world. The large majority of studies have found a protective association and the results of recent meta-analyses showed that H. pylori colonization of the stomach is associated with a nearly 50% reduction in the risk of esophageal cancer, especially esophageal adenocarcinoma. The p53 gene is located in human chromosome 17, it suspends the cell cycle when there is DNA damage. The p53 gene is mutated in about 50% of human cancers including breast, lung, colon, prostate, bladder, and skin. It has been suggested that the p53 codon 72 polymorphism may play a role in the susceptibility of esophageal squamous cell carcinoma.

AIM: To study the correlation between p53 gene polymorphism and H. Pylori infection in patients with esophageal cancer. To study the endoscopic findings of suspected cases of esophageal cancer

MATERIAL AND METHOD: The present prospective, cross-sectional study was conducted at the Hospital in the endoscopic unit of the Department of Surgery. The topic was passed by the Committee for Advanced Studies and Research (CASR) of the Faculty of Medicine of the University. A total of 42 patients who were suspected to be patients of esophageal cancer on endoscopic examination were included in the study. Two patients were excluded from the study as they did not fulfill the inclusion criteria for the study so a total of 40 patients were included in the study group. In the study group for the analysis. Adult patients who were referred for upper gastro-intestinal (G.I.) endoscopy to the Endoscopy Unit of the Department of Surgery were the subjects of the study.

RESULTS: A total of 40 patients who were admitted to the surgical wards for non-malignant disorders and gave informed consent were included as controls. In the control group patients' mean age was 24.23±11.65 years with a range of 19-65 years. On histopathological examination of biopsies taken from suspicious lesions from the esophagus, the lesions were malignant, pre-malignant, and benign. Esophageal squamous cell carcinoma was the most common pathology (58%), followed by esophageal adenocarcinoma (22%). Barrett's esophagus (6%), Dysplastic changes (4%), reflux esophagitis (8%), and fungal infection (2%) were the other less common lesions

CONCLUSION: Both the hypotheses were proved correct by the present study, that is, 'The infection with Helicobacter pylori is a risk factor in esophageal cancer in the North Indian population; where the incidence of esophageal squamous cell carcinoma is higher than esophageal adenocarcinoma.' The p53 gene polymorphism is a risk factor in esophageal cancer as in many other cancers. Both Helicobacter pylori infection and p53 gene polymorphism were found directly related (risk factor) in esophageal cancer, however, this positive relationship was more marked in cases of esophageal squamous cell carcinoma as compared to esophageal adenocarcinoma.

KEYWORDS: Esophageal squamous cell carcinoma, Esophageal cancer, esophageal adenocarcinoma, p53 gene polymorphism.

INTRODUCTION

Esophageal cancer (EC) is the 8th most common incident cancer in the world and because of its high fatality rate, ranks 6th among all cancers in mortality.[1,2] The etiology of EC differs by histopathology; it can be classified into two main types: Esophageal Squamous Carcinoma (ESCC) and Esophageal Adenocarcinoma (EA). These two cancers differ not only histologically but also with respect to their incidence trends, the population that they affect, and risk factors. One could call EA "an emerging disease". Until 1970, ESCC constituted the large majority (over 90%) of all EC cases in all parts of the world. Since then, however, incidence rates of EA have sharply increased in many countries in the western world, so this cancer type (EA) now constitutes approximately half of all EC cases in some western countries. In contrast, ESCC continues to be the dominant type in the rest of the world.[3,4] The development of ESCC is a multifactorial process associated with a variety of risk factors. The 2 major risk factors are smoking tobacco and drinking alcohol. Tobacco and alcohol have a synergistic effect and therefore people who smoke and drink have increased rates of esophageal SCC. The principal precursor lesion of ESCC is epithelial dysplasia. ESCC likely develops through a progressive sequence from mild to severe dysplasia, to carcinoma in situ, and finally to invasive carcinoma.[5]

The adenocarcinomas at the esophagus and GEJ differ from those in the stomach. [6] They share epidemiological characteristics with each other and often originate from segments of Barrett's esophagus (BE). It has therefore been proposed that both of them can be called "esophageocardia adenocarcinoma".[7] BE is a well-defined premalignant condition for esophageal adenocarcinoma and most of the adenocarcinomas at GEJ51-[8]. Neoplastic progression of BE has been shown to involve multiple steps with intestinal metaplasia and dysplasia serving as histopathological markers [7]. It is considered that the absence of specialized intestinal metaplasia (IM) in many patients with adenocarcinoma at the GEJ may be due to the complete replacement of the metaplastic epithelium by the tumor, and in these tumors, IM usually is confined to ultrashort segments that may easily be overgrown by the tumor.[7]

The increasing incidence of esophageal cancer is recognized worldwide with high morbidity and mortality. India also comes within the well-recognized 'esophageal cancer belt' as high incidences have been reported from different parts of India, particularly the Northern parts of India.[9,10] With the advent of molecular biology, new strategies are being carried out for the prevention and treatment of cancer. Cumulative evidence indicates that changes in both dominant oncogene and tumor suppressor genes are likely for the malignant transformation of normal cells.[11] Among these genetic abnormalities, the p53 tumor suppressor gene is frequently affected in most human cancers.

The best scientific evidence for the associations of genetic factors with the risk of esophageal cancer will come from large cohort studies that consider simultaneously the different factors potentially involved in esophageal carcinogenesis, including genetic polymorphisms and environmental exposures, such as drinking alcohol and smoking tobacco. Identification of genetic factors that modify the impacts of environmental factors will depend on the direct exploration of interactions between genes and the environment. Furthermore, simultaneous analysis of multiple polymorphic genes should be done to exclude the possibility of identifying gene-gene interactions. The results of these types of studies will allow us to estimate the relative contribution of individual genetic variations to overall esophageal cancer risk. Esophageal cancer is deadly cancer, with an extremely poor prognosis because of late diagnosis, and is a challenge to the medical professionals, engaged in the diagnosis and management of this condition.

Material and Methods

The present prospective, cross-sectional study was conducted at the Hospital in the endoscopic unit of the Department of Surgery. The topic was approved by the Institutional Ethics Committee of the Faculty of Medicine of the University.

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Faculty of Medicine of the University. A total of 42 patients who were suspected to be patients of esophageal cancer on endoscopic examination were included in the study. Two patients were excluded from the study as they did not fulfill the inclusion criteria for the study so a total of 40 patients were included in the study group. In the study group for the analysis.

Patients and methods:

Adult patients who were referred for upper gastro-intestinal (G.I.) endoscopy to the Endoscopy Unit of the Department of Surgery were the subjects of the study.

Inclusion criteria for the study group:

- Age more than 18 years.
- Patients giving informed consent.
- Patients undergoing upper G.I. endoscopy and having lesions/lesions suspicious of esophageal cancer.
- Patients having ASA (American Society of Anesthesiologists) Grade I-III

Exclusion criteria for the study group:

- Patients not giving consent for inclusion in the study.
- Patients having acute gastrointestinal bleeding.
- Patients having ASA (American Society of Anesthesiologists) Grade more than III.

Inclusion criteria for the control group:

- Age More than 18 years.
- Patients admitted to the surgical wards of the Hospital, for non-malignant conditions.
- Patients giving informed consent.

Exclusion criteria for the control group:

- The patient is in shock**.**
- The patient has acute bleeding from any site.
- The patient has any known malignancy.

Sampling:

Tissue for histopathology (Sample A) was kept in 10% Formalin solution and sent for histopathological examination to the pathology department.

Tissue for p53 gene polymorphism (Sample B and C) was collected in a Normal saline vial and transferred to the Biochemistry department to be preserved at (-200C) for the final testing for p53 gene polymorphism by polymerase chain reaction (PCR)

Sample D (Blood Sample): Patients suspected of esophageal carcinoma on endoscopic examination were requested to give a blood sample (2ml) for testing Helicobacter pylori IgG antibodies. The serum was allowed to separate at room temperature. After separation of the serum, the vial was transported to the Department of Biochemistry for storage at (-20°C) for final testing by ELISA technique.

Control group: After obtaining informed consent, 2 ml. of blood was collected from the patients included in the control group. After the separation of the serum, it was transported to the Biochemistry Department for testing for IgG H. pylori antibodies using the Calbiotech H. pylori ELISA KIT.

STATISTICAL ANALYSIS

Records and reports of all the patients enrolled in the study were recorded in the computer database. At the completion of the study, all the individual data were retrieved from the database and analyzed using1- SPSS VERSION 20 SOFTWARE and MINITAB VERSION 17 SOFTWARE

RESULT:

A total of 40 patients who were admitted to the surgical wards for non-malignant disorders and gave informed consent were included as controls. In the control group patients' mean age was 24.23±11.65 years with a range of 19-65 years.

Table 1: Histopathological findings of the endoscopic biopsies in the study group

S. No.	Histopathological diagnosis	N	%
1	Esophageal adenocarcinoma (EA)	10	22
2	Esophageal squamous cell carcinoma (ESCC)	25	58
3	Barrett's esophagus	01	06
4	Reflux esophagitis	02	08
5	Dysplastic changes	01	04
6	Fungal infection	01	02

On histopathological examination of biopsies taken from suspicious lesions from the esophagus, the lesions were malignant (80%), pre-malignant (10%), and benign (10%). Esophageal squamous cell carcinoma was the most common pathology (58%), followed by esophageal adenocarcinoma (22%). Barrett's esophagus (6%), Dysplastic changes (4%), reflux esophagitis (8%), and fungal infection (2%) were the other less common lesions.

Table 2: p53 gene polymorphism in patients with different pathological lesions in the study group

S. No.	Types of lesion	No. of Cases	%	Degree of p53 gene Polymorphism		Overall High/Moderate Polymorphism
				N	%	%
1	Squamous cell Carcinoma	25	52	High – 13 Moderate – 03 Low – 02 Nil – 02 Non-Reacting - 01	55.17 20.68 6.89 10.34 6.89	(75.86%)
2	Adeno Carcinoma	10	17	High – 3 Moderate – 01 Low – 02 Nil – 01 Non-Reacting - 01	27.27 16.16 16.16 16.16 16.16	(45.45%)
3	Reflux esophagitis	02	04	High – 00 Moderate – 02 Low – 00 Nil – 01 Non-Reacting – 01	00 50 00 25 25	(50%)
4	Barrett's Esophagus	01	03	High – 0 Moderate – 01 Low – 01 Nil – 01	00 33.33 33.33 33.33	(33.33)
5	Dysplastic Changes	01	02	High – 01 Moderate – 0 Nil – 01 Low – 0	50 00 50 00	(50%)
6	Fungal Infection	01	02	Moderate - 01	100	(100%)
	Total	**40**	**80**			

High p53 gene polymorphism was observed in 15 out of 40 cases (40%) in the diseased tissues, as compared to only 3 out of 40 specimens of the adjacent normal esophageal tissues. A moderate degree of p53 gene polymorphism was seen in 24% of cases of the diseased tissues as compared to 8% of the normal tissue samples. There was nil p53 gene polymorphism in 16% of the diseased tissues as compared to 44% of the normal esophageal tissues. There was no reaction in 10% of the diseased tissues as compared to 38% of the adjacent normal tissues

Table 3:Degree of p53 gene polymorphism in different disorders

p53 Gene Polymorphism	Esophageal Lesions					
	Malignant (N=30)		Pre-Malignant (N=5)		Benign (N=5)	
	Squamous Cell CA (n=21)	Adeno CA (n=9)	Dysplasia (n=2)	Barrett's (n=3)	Reflux Esophagitis (n=4)	Fungal Infection (n=1)
High	13 (55.17)	04 (27.27)	01 (50)	00	00	00
Moderate	03 (20.68)	02 (18.18)	00	01 (33.3)	02 (50)	01 (100)
Low	02 (06.89)	01 (18.18)	00	01 (33.3)	00	00
Nil	02(10.34)	01 (18.18)	01 (50)	01(33.3)	01 (25)	00
No Result	01 (06.89)	01 (18.18)	00	00	01 (25)	00
Total (n=40)	21	9	02	03	04	01

In patients of esophageal cancer, overall p53 gene polymorphism was observed in 21 (77.5%) cases. Out of these 21 cases, squamous cell carcinoma (82.7%) had p53 gene polymorphism compared to only 63.63% cases in adenocarcinoma, though the number of the cases in the two groups was unequal

Discussion

In India, high incidence of esophageal cancer has been reported in states of Jammu and Kashmir, Assam and Karnataka. **Kotiyar et al.2005**[12] studied 101esophageal tumor biopsy specimens from three different regions of India[13] Assam,[14] Kashmir,[15] New Delhi and made the following observations. The majority of the esophageal cancer patients were males (73%), the age group 40-60 years showed the highest prevalence (67%). More than 61% showed well differentiated squamous cell carcinoma. In the present study also, the majority of patients with esophageal carcinoma were males (60%). The age range was between 30 to 90 years, with a peak incidence between 51 to 60 years. The Male: Female ratio was 1.5: 1. Squamous cell carcinoma (72.5%) was the most common malignant pathology, followed by adenocarcinoma (27.5%).

Kaur et al.2014 [16] in a North Indian study found that out of 136 sporadic esophageal cancer patients, 47 were males and 89 were females, which shows that both sexes are at risk of developing esophageal carcinoma in the North Indian population, a M: F ratio of 1:1.8, which is in contrast to the M: F ratio of 1.5:1 in the present study. According to **McCollum et al.2006**[17] and **Ott et al.2006**[18], Barrett's esophagus is the most important precancerous disease. Patients with Barrett's esophagus have a 50 to 100 times increase in their risk of developing cancer compared to the general population. In a population based cohort study, **Hvid-Jensen et al.2011**[19] reported an annual risk of developing esophageal adeno-carcinoma of 0.12% among patients with Barrett's esophagus. The endoscopic appearance may be suggestive of

malignant lesion, but the biopsy and histopathology provides the true picture. It is also possible that both malignant and premalignant lesions may co-exist, suggesting that some tissues may have become malignant while the adjacent tissues are still in the pre-malignant state, waiting to be transformed into malignancy in due course of time.

Genetic factors as well as environmental factors play a role in the development of esophageal cancer. A variety of genes may be associated with esophageal cancer carcinogenesis including the genes involved in alcohol metabolism, foliate metabolism, carcinogen metabolism, DNA repair and cell cycle control, and the oncogenes. As Aligarh City is located in a high risk 'Cancer Zone', the possibility of genetic mutations because of the exposure to radiation, industrial chemicals and environmental pollutants cannot be ruled out. The p53 tumor suppressor gene is frequently mutated in various human cancers including esophageal squamous cell carcinoma and adenocarcinoma. Since only p53 polymorphism on codon 72 and exclusively on exon 4 was subjected to investigation, the grading of magnitude of such polymorphism was evaluated in the present study depending on the size/intensity of the number of bands expressed/over expressed/under expressed as has been described elsewhere by other investigators including **Lee et al.2006**[20]

Another study from China by **Yuan Hong et al.2005**[21] demonstrated that there was no statistically significant association between the pathologic stage of esophageal squamous cell carcinoma (ESCC) and p53 or MDM2 genotype; similarly, no statistically significant association was detected between these two polymorphisms and tumor grade.

Recently in a study of the sporadic esophageal cancer patients from Northern India, **Kaur et al.2014** [16], observed that among the five TP53 polymorphisms investigated, only P.R72P polymorphism and the RP-AIAL-GG genotype combination may contribute to Esophageal Cancer (EC) susceptibility. They were also of the opinion that the discrepancy among the results of the present and previous studies may be due to ethnic and geographic differences, variable environmental factors and different life styles among the various populations

It is of interest to note that, **Henrik Siman and associates2007**[22] reported that esophageal adenocarcinoma and esophageal squamous cell carcinoma were not significantly associated with H. pylori seropositivity or with CagA seropositivity among H. pylori seropositive subjects. In the conclusion, the authors were of the opinion that CagA seropositivity among H. pylori seropositive subjects is a risk factor for non-cardia gastric adeno-carcinoma but not for esophageal squamous cell carcinoma or adenocarcinoma. **Murakami et al.1999**[23] found that H. pylori infection can induce p53 point mutations in gastric mucosa of gastritis patients that may lead to dysplasia and carcinoma. The p53 point mutations were not found in H. pylori negative gastritis patients. Thus, it was apparent that H. pylori infection and p53 expression might have a synergistic effect on gastric carcinogenesis.

Theodoros Rokkas 2007[24] and associates performed a meta-analysis and concluded that H. pylori has an inverse relationship with esophageal adenocarcinoma and Barrett's esophagus which was statistically significant but no significant relationship was observed with esophageal squamous cell carcinoma. Our observations are at variance with those of **Rokkas et.al 2007**[24] as we found a direct relationship between H. pylori infection and both types of esophageal cancer.

Conclusion:

Both the hypotheses were proved correct by the present study, that is, a) 'The infection with Helicobacter pylori is a risk factor in esophageal cancer in the North Indian population; where the incidence of esophageal squamous cell carcinoma is higher than esophageal adenocarcinoma'. b) 'The p53 gene polymorphism is a risk factor in esophageal cancer as in many other cancers. Both Helicobacter pylori infection and p53 gene polymorphism were found directly related (risk factor) in esophageal cancer, however, this positive relationship was more marked in cases of esophageal squamous cell carcinoma as compared to esophageal adenocarcinoma.

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