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Original Research Article

Clinico-pathologic profile and pattern of p53 expression of lung adenocarcinoma in non-smokers and smokers in a tertiary cancer centre

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ABSTRACT

Background: Lung cancer is the most common cause of cancer mortality worldwide. Smoking is undoubtedly the major risk factor of lung cancer in both genders. Adenocarcinoma is the most common form of lung cancer in both men and women and the most prevalent subtype in non-smokers. Lung cancer in never-smokers is a distinct entity with sparse studies. We studied the clinico-pathologic profile of lung adenocarcinoma and pattern of p53 expression in smokers and non-smokers.
Methods: A prospective study involving 100 lung adenocarcinoma cases from January 2020 to June 2021 examined p53 expression using immunohistochemistry. Trucut biopsies, fine needle aspiration cytology (FNAC) cell blocks, and pleural effusion were analyzed to identify the predominant morphological subtype of the lung adenocarcinoma.
Results: The most common histological pattern of lung adenocarcinoma was solid, and the presenting symptoms were cough and dyspnoea in both smokers and non-smokers. The incidence of lung adenocarcinoma was higher in non-smokers in the study. p53 expression had a significant correlation with smoking but not with stage of disease or morphological subtype of lung adenocarcinoma.
Conclusions: p53 mutation has a statistical correlation with smoking in adenocarcinomas in our population. Among the adenocarcinoma cases in our study, non-smokers predominate (n=53). Even though our study showed the p53 mutation has no statistical correlation with the stage of the disease or histological subtype in adenocarcinoma, more cases need to be studied to prove this observation.

Keywords: Lung adenocarcinoma, p53 expression, Smokers, Non-smokers

INTRODUCTION

Lung cancer is the most frequently diagnosed major cancer and the most common cause of cancer mortality worldwide.[1] Smoking is undoubtedly the major risk factor for lung cancer in both genders. The incidence of adenocarcinoma has increased significantly in the last two decades, and it is now the most common form of lung cancer in both women and men. The prevalence of lung cancer in non-smokers has been increasing over time, and the World Health Organization (WHO) estimates that 25% of lung cancer worldwide occurs in non-smokers. The most common subtype in never-smokers is adenocarcinoma.[1-3] Lung cancer in never-smokers is a distinct entity, and descriptive studies are still sparse.[4-6] This study aims to describe the characteristics of lung adenocarcinoma in smokers and non-smokers. In the search for causes and biological mechanisms of lung cancer in non-smokers, understanding the histopathological and clinical features may reveal important clues.

Multiple genetic abnormalities leading to the malignant transformation of the bronchial epithelial cells are involved in the development of non-small-cell carcinoma. TP53 mutations in lung cancer in never-smokers are distinct from those in tobacco-associated lung cancer.[7]

In patients with adenocarcinoma, the frequency of TP53 mutations correlated with the amount of tobacco smoked. Furthermore, the prevalence of mutations in TP53 differs for patients with lung cancer who never smoked and those with tobacco-associated lung cancers. To address these issues, it may be necessary to use such promising molecular markers as p53 for stratification in the setting of prospective randomised clinical trials for patients with lung cancer, especially those with adenocarcinoma. Hence, this study intends to describe the pattern of p53 expression in smokers and non-smokers.

METHODS

This was a prospective study including 100 cases of lung adenocarcinoma diagnosed from 01 January 2020 to 30 June 2021, evaluated in Pathology division of Regional Cancer Centre, Trivandrum. It was presented in institutional review board with IRB No:10/2019 and ethical committee with HEC No:40/2019.

Trucut biopsies, cell blocks of FNACs, and pleural effusion of lung adenocarcinoma cases that were positive for TTF1 and negative for p40 were studied for p53 expression by the manual method of immunohistochemistry (IHC). Clinical details were collected using a proforma. Histopathology slides were reviewed to study the predominant morphological subtype of adenocarcinoma. TP53 expression was considered mutant when more than 60% of tumour cells showed strong nuclear staining or a total absence of nuclear staining for p53 (provided p53 was stained by fibroblasts, endothelial cells, or lymphocytes, which acted as internal positive controls). The rest of the cases were considered wild.

Included 100 cases of adenocarcinoma lung diagnosed with small biopsies and cell blocks of FNACs and pleural effusion received in our department. A person was considered a "never smoker if the person declared having smoked fewer than 100 cigarettes during their lifetime. A "lifetime smoker was defined as a person who declared having smoked at least 100 cigarettes during their lifetime. The lung adenocarcinoma cases included were TTF1-positive and p40-negative. Cases where smoking history was not available, adeno-squamous carcinoma, and inadequate biopsies where tissue was insufficient were excluded. The sample size was estimated based on the study by Mogi et al and the minimum sample required for the present study was 96.

All the data were analysed using statistical package for the social sciences (SPSS) 11 software. Continuous variables were represented by the mean and standard deviation. Categorical variables were expressed using frequency and relative proportion. The associations between two categorical variables were assessed using the chi-square test or Fisher's exact test. A significant difference in smoking status and p53 expression was tested using the Chi-square test. A p value of 0.05 was considered to be statistically significant.

RESULTS

In the study group, 53% were non-smokers and 47% were smokers; 63% were males and 37% were females. Females dominated the non-smokers. The majority of people were in the age group of 61–70 years (n=42). The most common symptom among smokers and non-smokers was coughing. Table 1 summarises the distribution of symptoms in smokers and non-smokers.

Table 1: Frequency of initial symptoms in smokers and non-smokers.

Presenting symptoms	Smoker	Non-smoker
Cough	34	33
Dyspnoea	22	29
Haemoptysis	17	8
Chest pain	21	18
Asthenia, weight loss	12	26
Projectile vomiting	4	2
Hoarseness of voice	5	5

The time between the onset of symptoms and the diagnosis of malignancy was 1–5 months in 71% of cases. 79% of the patients had stage 4 disease. The most common location of lung adenocarcinoma was the right upper lobe. 52% of cases presented with both regional lymph nodes and distant metastases. In distant metastasis, the order of involvement was pleura > bones > contralateral lung > supraclavicular lymph node > brain=adrenal > liver > pericardia=axillary lymph node. The predominant histopathological pattern of adenocarcinoma was solid (n=34) (Figure 1). Among them, 20 (58.8%) were smokers, and the rest were non-smokers. 24 (70.6%) were men, and 10 (29.4%) were women. In our analysis, mucinous predominant adenocarcinoma accounted for 18% of cases, with 66.66% of males and 33.3% of females.

About the pattern of P53 expression, 68% showed a wild pattern (Figure 2) and 32% showed mutant expression (Figure 3). Males were the predominant population in both wild and mutant patterns. Among the patients with stage IV disease, 54 (68.4%) cases showed a wild-type pattern of expression, and 25 (31.6%) showed a mutant pattern. p53 expression was correlated with stage at diagnosis, but there was no significant association established between the two (p value=1.00). Furthermore, among cases with a solid pattern, 16 (47.1%) had mutant p53 expression, and 18 (52.9%) showed a wild pattern. while p53 expression was mutant only in 16.6% of cases with mucinous adenocarcinoma. No statistical correlation was established

between p53 expression and the morphological subtype of adenocarcinoma. p53 expression had a significant association with smoking [p value 0.01] and was established using the chi-square test (Table 2).

Table 2: Correlation between p53 status and smoking.

Smoking status	P53		Total	P value
	Wild	Mutant		
Yes	26	21	47	
No	42	11	53	0.010
Total	68	32	100	

Table 3: Age distribution and p53 expression.

Age in years	P53		Total	P value
	Wild	Mutant		
31-40				
n	2	1	3	
%	66.7	33.3	100.0	
41-50				
n	12	3	15	
%	80.0	20.0	100.0	
51-60				
n	18	6	24	
%	75.0	25.0	100.0	
61-70				
n	29	13	42	0.111
%	69.0	31.0	100.0	
71-80				
n	5	9	14	
%	35.7	64.3	100.0	
>81				
n	2	0	2	
%	100.0	0.0	100.0	
Total				
n	68	32	100	
%	68.0	32.0	100.0	

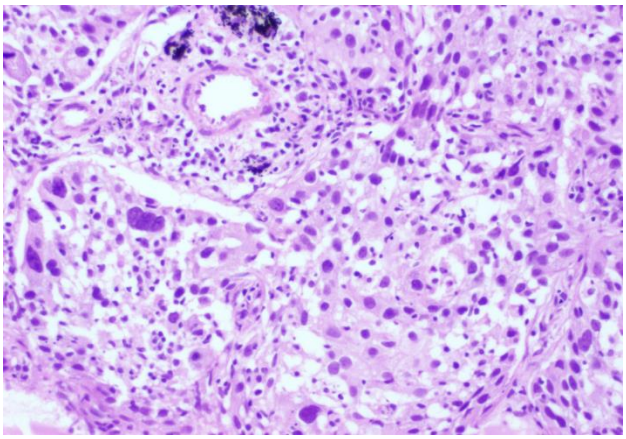

Figure 1: Solid predominant adenocarcinoma (H&E, 400X).

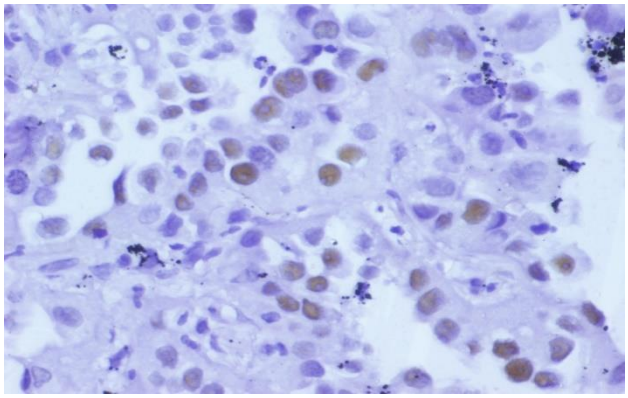

Figure 2: p53 wild pattern (IHC, 400X).

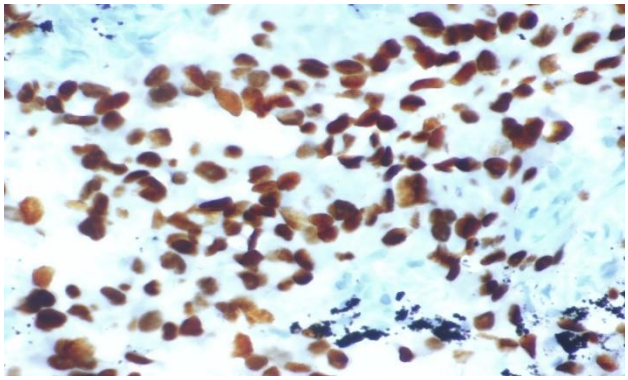

Figure 3: p53 mutant expression (IHC, 400X).

Table 4: Gender distribution and p53 expression.

Gender	P53		Total	P value
	Wild	Mutant		
Male				
n	39	24	63	
%	61.9	38.1	100.0	
Female				
n	29	8	37	0.088
%	78.4	21.6	100.0	
Total				
n	68	32	100	
%	68.0	32.0	100.0	

Table 5: Comparison of p53 expression in smokers and non-smokers in various studies.

Study	p53 expression (%)		P value
	Smokers	Non-smokers	
Lui et al	40	21.2	0.03
Vahakangas et al	67	19	0.016
Chow et al	58	10	0.02
Dosaka-akita et al	45	11	0.01
This study	66	34	0.01

DISCUSSION

Adenocarcinoma is the most common form of lung cancer in women and men, and it is also the most common subtype in non-smokers. Inactivating mutations of the tumour suppressor gene TP53 occur in approximately 45% of lung adenocarcinomas and are commonly smoking-related.[4-6] TP53 gene mutations in lung cancer in never-smokers are distinct from those in tobacco-associated lung cancer. Previous research suggest that the histological subtypes of lung carcinoma differed by gender and age group.[9,10] The younger age group has a higher proclivity for adenocarcinoma.

Among 32 mutant cases of p53, 24 (75%) were men and 8 (25%) were women. Ahrendt et al say p53 mutations have been found more frequently among men than among women.[8] This is due to the fact that men are heavier smokers than women, which is in accordance with our study. Among the 37 females, 21.6% (n=8) had mutant p53 expression, and 78.4% (n=29) had wild p53. According to the American Cancer Society, lung cancer mainly occurs in older people. Most people diagnosed with lung cancer are 65 or older; a very small number are younger than 45. The average age of people diagnosed is about 70. The majority of the diagnosed adenocarcinoma cases belonged to the age group of 61–70 years (n=42). 13 (31%) in this age range had mutant p53, while 29 (69%) had wild p53 expression. But Ahrendt et al found out that p53 mutations have been shown to be associated with a younger age group, which was discordant with our study.[8] The period of time between the onset of symptoms and the diagnosis of malignancy ranged from one to five months, and the majority of patients were found to have stage IV lung cancer, which accounts for the shorter period of time between symptoms and diagnosis. TP53 expression was correlated with stage at diagnosis, but there was no significant association established between the two (p value=1.00). This may be due to our small sample size and the fact that most of our cases were detected at later stages. In contrast to our study, a study by Dosaka-Akita et al showed that abnormal p53 expression was frequently found in both the early and late clinical stages, suggesting that the alteration in the p53 gene is a relatively early genetic event in the development and progression of lung cancer.[11-14] This is in concordance with the hypothesis that the mutation of this gene may play an important role in the development and progression of lung cancer. Long ago, experts established that lung cancer is more common in the upper lobes than the lower lobes, as well as in the right lung versus the left lung, which is similar to our findings. According to Byers et al, the hypothesis that a differential deposition of inhaled particulates is responsible for the predilection of lung cancer for the upper lobes is based on experimental evidence that inhaled particulates deposit more readily in airways supplying the upper lobes, particularly those supplying the right upper lobe.[17,18] Another possibility is that malignancies develop next to scar tissue, which is more common in the higher lobes as a result of infections.

In our study, p53 expression was correlated with the predominant morphological subtype, but there was no significant association (p value=0.303). Consistent with previous studies and the study by Zhang et al, it was found that solid predominant adenocarcinoma patients were more likely to be males and smokers, which was concordant with our study.[20-24] A study by Hung et al showed that lung adenocarcinoma patients with micro-papillary predominant adenocarcinoma and solid predominant adenocarcinoma had worse disease-specific survival.[25] In a study with 440 lung adenocarcinoma patients, Yoshizawa et al showed that the 5-year disease-free survival rate was the lowest in these tumour subtypes, and the tumours in these subgroups recurred more frequently compared with those in other morphological subgroups.[26] Our cases need to be followed up to calculate the disease-free survival rates. A study by Shim et al found that there is a low mutational burden for p53 in invasive mucinous adenocarcinomas (p value=0.007).[27]

P53 gene mutations are less frequent, and the mutation spectra are different in lung cancers of lifelong non-smokers when compared with smokers. Lung cancers in ex-smokers contain a p53 mutation spectrum similar to that in smokers, indicating that tobacco carcinogens cause permanent, identifiable damage in lung tissue that can be found decades after smoking cessation. Multiple strategies have been advocated to restore wild-type p53 function and target the p53 missense mutants frequently found in cancers. This can be utilised to develop new drugs targeted against p53-mutant lung adenocarcinomas in the future. So, detection of the p53 mutation can revolutionise the treatment of lung adenocarcinoma.

The limitations of this study are that IHC overexpression of p53 was used as a surrogate for the TP53 mutation. Splice site mutations can result in p53 wild-type staining and, hence, may be missed. Despite the fact that splicing site mutations are rare in lung cancer, if protein sequencing and immunohistochemistry were combined, the frequency of p53 mutant cases could be higher. Given the fact that the literature suggests the frequency of TP53 mutations is linked to the amount of tobacco smoked in adenocarcinomas, we were unable to link the two since we did not have access to all of the patient's smoking records. Since we didn't follow up with the patients, the significance of p53 mutant histologically bad subtypes of adenocarcinoma in prognosis couldn't be ascertained. Further research has to be done to assess the prognosis of these subtypes in our population.

CONCLUSION

To conclude, the p53 mutation has a statistical correlation with smoking in adenocarcinomas in our population. Among the adenocarcinoma cases in our study, non-smokers predominate (n=53). Even though our study showed the p53 mutation has no statistical correlation with the stage of the disease or histological subtype in

adenocarcinoma, more cases need to be studied to prove this observation.

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