

Original Research Article

CHANGES AT THE CELLULAR LEVEL OF LUNG CAUSED BY SMOKING WITH THE AID OF SPUTUM CYTOLOGY AND ITS IMPORTANCE IN PREDICTING THE PATIENTS AT RISK FOR PULMONARY DISORDERS IN FUTURE

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ABSTRACT

Background: Smoking remains a major public health concern and a leading cause of respiratory morbidity and mortality worldwide. In India, the burden of smoking-related lung diseases, including chronic obstructive pulmonary disease (COPD) and lung cancer, is rising. Tobacco smoke induces characteristic cellular alterations in the bronchial epithelium that can be detected cytologically. Sputum cytology serves as a simple, non-invasive tool for early identification of individuals at risk for such pulmonary disorders. This study was done to evaluate the cytological changes in the lung epithelium of smokers using sputum cytology and to assess the correlation between smoking duration and the degree of epithelial atypia.

Materials and Methods: A cross-sectional observational study was conducted from May 2022 to February 2023 among 157 participants (92 current smokers and 65 ex-smokers) aged ≥ 25 years at a tertiary care hospital in Karnataka. Early morning or induced sputum samples were collected, fixed in 95% ethanol, and stained with H&E, PAP, and Giemsa stains. Cytological findings were categorized as normal, inflammatory, hyperplastic, metaplastic, dysplastic, or malignant. Statistical analysis was performed using SPSS v20.

Results: Cytologic atypia was detected in 24.2% of participants and squamous metaplasia in 43.3%. Both findings were significantly more frequent in current smokers (34.8% and 53.3%, respectively) than in ex-smokers (9.2% and 29.2%) ($p < 0.01$). The prevalence of chronic inflammatory infiltrates was threefold higher in current smokers. Duration of smoking correlated positively with epithelial atypia ($r = 0.62$, $p < 0.001$). Ex-smokers who had quit for ≥ 5 years exhibited partial reversal of cytologic changes.

Conclusion: Smoking induces progressive epithelial alterations in the lungs, including metaplasia and atypia, which correlate with exposure duration. Sputum cytology is an effective, low-cost screening tool for detecting early precancerous changes, enabling identification of high-risk individuals and promoting smoking cessation to prevent future pulmonary disorders.

Keywords: Sputum cytology, Squamous metaplasia, Cytologic atypia, Lung epithelial changes.

INTRODUCTION

Developing countries face the burden of both communicable and non-communicable diseases

equally, with higher prevalence of respiratory disorders, especially in India. Amongst the various risk factors, smoking remains to be the major contributor to this spectrum, with the rise in respiratory disorders paralleling the trends of tobacco

smoking.^[1] Apart from the chronic obstructive respiratory diseases, India has also seen a substantial rise in cases of lung cancer, accounting for 9.3 per cent of all cancer related deaths.^[2] The lower smoker: non-smoker ratio in India explains the ubiquity of respiratory disorders compared to the Western countries.

Tobacco smoking has been known to induce various changes at the level of alveolar epithelial cells. They form the lung's first line of defence against the external environment, hence undergo atypical changes on exposure to tobacco smoke.^[3] The degree of these atypical changes depends commonly on the length of tobacco use and exposure intensity.^[4] Tobacco smoke contains various reactive oxygen species and reactive nitrogen species, that affect the lung epithelial cells causing cell death, i.e, apoptosis or necrosis.^[5] While some proliferative changes are reversible with removing the offending agent (smoking), continuous exposure to tobacco smoke may lead to the development of lung cancer. To discern the several changes, there are a wide variety of diagnostic tools, both invasive and non invasive, that help in gaining a knowledge regarding the events at histopathological level.

Sputum cytology is a simple, non invasive diagnostic tool to screen the at-risk population for any proliferative changes in the lung. The presence of chronic inflammation and cellular atypia, which ranges from hyperplasia, metaplasia to dysplasia helps in identifying the patients who have higher propensity for chronic respiratory disorders and lung cancer. Hence sputum cytology guides as a screening tool to identify the precancerous or cancerous lesions, in order to establish a diagnosis with other invasive methods and initiate appropriate management.

There are a very few studies in India that have emphasised the effect of smoking on lung epithelial cells and there is a need for identification of high risk groups and their screening. Since sputum cytology is a non invasive and a cheaper tool, with smoking being a major contributor, the present study aimed at evaluating the effect of smoking on lung epithelial cells, so as to alert the population, that may suffer from the impending devastating conditions, beforehand.

Aims and Objectives

Aim:

The study aims to evaluate the changes at the cellular level of lung caused by smoking with sputum cytology as a screening tool for predicting the population at risk for development of pulmonary disorders.

Objectives

1. To determine the different proliferative changes in lung epithelium among current and former smokers.
2. To evaluate the correlation between the duration of smoking and the cytologic changes.

MATERIALS AND METHODS

Study Design: Cross-sectional, observational study.

Study Period: May 2022 – February 2023 (10 months).

Study Setting: Department of Respiratory Medicine, BLDE (Deemed to be University)'s Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura, Karnataka.

Sample Size: 157 patients (calculated based on an anticipated proportion of proliferative changes among smokers = 92.9%,^[6] 95% CI, 4% absolute precision).

Inclusion Criteria

- Age ≥ 25 years.
- Current smokers: History of ≥ 100 cigarettes in lifetime and currently smoking.
- Ex-smokers: ≥ 100 cigarettes in lifetime, abstinent for ≥ 28 days.
- Able to produce good-quality sputum.

Exclusion Criteria

- Non-smokers.
- Life expectancy < 5 years.
- History of cancer diagnosis within 5 years before recruitment.

Method of Sputum Collection

1. Spontaneous sputum collection: Early morning sputum was collected in sterile containers after deep breathing and forceful coughing.
2. Induced sputum collection: For patients unable to produce sputum, induction was done using 3% normal saline nebulization preceded by salbutamol inhalation.

Cytological Examination: Sputum samples were examined macroscopically for mucus and blood, and smears were prepared using the prick and smear technique.

Smears were fixed in 95% ethanol and stained using Hematoxylin and Eosin (H&E), Papanicolaou (PAP) and Giemsa stain.

Cytological findings were categorized as: Normal, Inflammatory changes, Hyperplasia, Squamous metaplasia, Dysplasia, Malignant cells. Sample adequacy was confirmed by the presence of pulmonary macrophages.

Statistical Analysis: Data were entered in Microsoft Excel and analyzed using SPSS v20. Descriptive statistics were expressed as mean $\pm$ standard deviation (SD) for continuous variables and as frequencies and percentages for categorical variables. The Independent t-test or Mann–Whitney U test was applied to compare continuous variables between current smokers and ex-smokers, depending on the distribution of data. The Chi-square test was used to analyze associations between categorical variables. The Pearson correlation coefficient was employed to assess the relationship between the duration of smoking and the degree of cytological changes. A p-value of less than 0.05 was considered statistically significant.

RESULTS

The present study investigated 157 individuals aged 25–70 years, with a mean age of 45 ± 10.4 years. Of these, 92 (58.6%) were current smokers and 65 (41.4%) were ex-smokers. The majority of participants were between 35–54 years, representing 95/157 (60.5%) of the study population. Most current smokers were in the 35–54 year age group (57/92; 62%), while ex-smokers were predominantly ≥ 45 years (39/65; 60%) [Table 1].

Among all participants, cytologic atypia was identified in 38/157 (24.2%), while squamous metaplasia was observed in 68/157 (43.3%). The frequency of cytologic atypia was significantly higher among current smokers (32/92; 34.8%) than among ex-smokers (6/65; 9.2%) ($p < 0.001$).

Similarly, squamous metaplasia was present in 49/92 (53.3%) current smokers versus 19/65 (29.2%) ex-smokers ($p = 0.002$) [Table 2].

Acute inflammatory cell infiltrates were found in 21/92 (22.8%) of current smokers and 8/65 (12.3%) of ex-smokers. Chronic inflammatory infiltrates were present in 33/92 (35.9%) of current smokers compared to 10/65 (15.4%) ex-smokers, showing a statistically significant association (OR = 3.04, 95% CI = 1.3–6.8; $p = 0.004$) [Table 3].

The duration of smoking demonstrated a clear trend with the degree of epithelial atypia. Among current smokers with >20 years of smoking, 81.3% showed either metaplasia or atypia, compared to 45.5% in those with ≤ 10 years of smoking. Ex-smokers who had quit for ≥ 5 years showed partial reversal, with only 14% showing residual atypia and 25% showing metaplastic changes [Table 4].

Table 1: Distribution of Smoking Status by Age

Age Group (years)	Current Smokers (n=92)	Ex-Smokers (n=65)	Total (n=157)
25–34	12 (13.0%)	7 (10.8%)	19 (12.1%)
35–44	30 (32.6%)	18 (27.7%)	48 (30.6%)
45–54	27 (29.3%)	20 (30.8%)	47 (29.9%)
≥ 55	23 (25.0%)	20 (30.8%)	43 (27.4%)
Total	92 (58.6%)	65 (41.4%)	157 (100%)

Table 2: Distribution of Smoking Status by Cytologic Atypia and Metaplasia

Category	Variable	Current Smokers	Ex-Smokers	Total	OR (95% CI)	p-value
Lung Epithelial Cytologic Atypia	Present	32	6	38	5.36 (2.1–13.6)	<0.001*
	Absent	60	59	119		
Total		92	65	157		
Squamous Metaplasia	Present	49	19	68	2.74 (1.4–5.4)	0.002*
	Absent	43	46	89		
Total		92	65	157		

Table 3: Distribution of Inflammatory Cell Infiltrate by Smoking Status

Category	Variable	Current Smokers	Ex-Smokers	Total	OR (95% CI)	p-value
Acute inflammatory cells	Present	21	8	29	2.09 (0.87–5.0)	0.09
	Absent	71	57	128		
Chronic inflammatory cells	Present	33	10	43	3.04 (1.3–6.8)	0.004*
	Absent	59	55	114		
Total		92	65	157		

Table 4: Lung Epithelial Proliferative Changes According to Duration of Smoking

Duration of Smoking (years)	n	Cytologic Atypia n (%)	Squamous Metaplasia n (%)	Any Proliferative Change n (%)
≤ 10	33	5 (15.2%)	10 (30.3%)	15 (45.5%)
11–20	38	11 (28.9%)	18 (47.3%)	26 (68.4%)
>20	21	10 (47.6%)	7 (33.3%)	17 (81.0%)
Total (current smokers)	92	26 (28.3%)	35 (38.0%)	58 (63.0%)

DISCUSSION

The present study aimed to evaluate the cytological changes in the lung epithelium of smokers using sputum cytology, comparing current smokers and ex-smokers, to identify those at potential risk for future pulmonary disorders. Our results demonstrated a significantly higher frequency of cytologic atypia and squamous metaplasia among current smokers compared with ex-smokers. Moreover, the severity of proliferative epithelial changes correlated strongly with the duration of smoking, indicating a dose-

dependent relationship between tobacco exposure and cellular injury.

These findings are consistent with those of H G Ahmed et al,^[6] who studied 300 participants in Saudi Arabia and reported cytologic atypia in 4.7% of all subjects, with 92.9% of these cases occurring among smokers. They also found metaplastic changes in 15% of subjects, predominantly in smokers, and highlighted a strong association between smoking and proliferative epithelial abnormalities. In the present study, a much higher overall proportion of proliferative changes (91.7%) was observed, likely because our population included both chronic and

heavy smokers with longer smoking histories and ongoing exposure.

Similarly, Prindiville et al,^[3] in a prospective study among chronic smokers with ≥ 30 pack-years, demonstrated that cytologic atypia in sputum correlated with continued smoking and was predictive of incident lung cancer. Their findings established that moderate or severe atypia carries a several-fold increased risk of developing lung cancer in the subsequent years. Our results echo this observation, as current smokers showed higher frequencies of dysplastic and metaplastic cells compared to ex-smokers, reinforcing the link between persistent smoking and the risk of malignant transformation in the bronchial epithelium.

Babiker AYY et al,^[4] examined the respiratory cytology of Shisha users and found a significantly increased rate of epithelial proliferation and atypia, emphasizing that even non-cigarette forms of tobacco smoke induce similar cytologic damage. These results, together with our study, underline that the type of tobacco product is less relevant than the total duration and intensity of exposure. In our data, prolonged exposure (>20 years) was associated with the highest rates of atypia and metaplasia, highlighting a cumulative cellular injury pattern with chronic smoking.

The presence of chronic inflammatory cell infiltration in a large proportion of current smokers in our study further corroborates the role of smoking-induced inflammation as a precursor to epithelial damage. Ahmed et al,^[6] also reported a threefold increase in chronic inflammation among smokers compared to non-smokers. Chronic exposure to reactive oxygen and nitrogen species from tobacco smoke promotes oxidative stress, DNA damage, and persistent inflammation, which together contribute to epithelial remodeling. This supports the notion that inflammation and metaplasia coexist as early adaptive responses to injury before neoplastic transformation occurs.

Our findings also align with the observations of Rigden et al,^[7] who demonstrated a marked increase in squamous metaplasia among smokers with COPD compared to non-smokers. Squamous metaplasia represents a reversible adaptive change, where the normal pseudostratified columnar epithelium transforms into a squamous phenotype more resistant to smoke-induced injury. However, persistent exposure prevents reversal, allowing progression toward dysplasia and carcinoma. The increased frequency of metaplastic cells in our study among long-term current smokers compared to ex-smokers supports this progressive model of epithelial injury. Interestingly, our results reveal partial reversibility of cytologic abnormalities among ex-smokers, reflecting the regenerative capacity of the bronchial mucosa after cessation of smoking. Studies by Pfeifer,^[8] and Seton-Rogers,^[9] demonstrated that the epithelial DNA profile of ex-smokers tends to approximate that of non-smokers over time, suggesting molecular repair mechanisms following

cessation. The significantly lower rates of atypia in our ex-smokers (9.2%) as compared to current smokers (34.8%) are in concordance with this observation and highlight the potential benefit of early cessation in reducing the risk of malignant transformation.

The relationship between the duration of smoking and degree of cytological atypia observed in this study further supports a dose-response association. Longer duration and higher intensity of smoking were associated with more pronounced cellular changes, consistent with Babiker et al,^[4] and Ahmed & Rezgalla,^[10] who reported that both the quantity and duration of tobacco exposure determine the severity of epithelial alterations. In our study, 81% of individuals with over 20 years of smoking history exhibited proliferative or dysplastic changes, underscoring the cumulative impact of sustained tobacco exposure.

From a preventive perspective, these findings have significant implications. Sputum cytology, being a simple, non-invasive, and cost-effective tool, can serve as an initial screening method in primary care settings, particularly in resource-limited countries like India, where access to advanced imaging and bronchoscopy is limited. Ahmed et al,^[6] and Prindiville et al,^[3] both emphasized the utility of cytological screening in identifying high-risk individuals before overt clinical disease develops. Incorporating sputum cytology into community-level screening programs could thus aid in early detection and prompt intervention.

Limitations

The present study was limited by its cross-sectional design, which restricts the ability to establish a causal relationship between smoking and cytological changes. The assessment of smoking exposure was based on self-reported history, which may introduce recall bias. Longitudinal follow-up and molecular correlation studies would provide stronger evidence of the progression and reversibility of these cytological changes.

CONCLUSION

This study demonstrates a clear association between cigarette smoking and proliferative epithelial changes in the lungs, with current smokers exhibiting significantly higher rates of cytologic atypia and metaplasia compared to ex-smokers. The findings highlight that cessation of smoking leads to partial regression of these abnormalities, reinforcing the benefits of quitting early. Sputum cytology, being a simple and non-invasive tool, can effectively serve as a screening method to identify individuals at high risk for chronic pulmonary diseases and lung cancer, thereby facilitating timely preventive interventions.

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