RESEARCH PAPER

Comparative Cytomorphological Evaluation of Sputum Samples Among Smokers and Non-Smokers: A Cross-Sectional Study

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Background: The respiratory epithelium is directly exposed to inhaled tobacco smoke, and exfoliative sputum cytology offers a simple, non-invasive window into smoking-related cellular change. Comparative sputum-cytology data in South Asian, and particularly Northeast Indian, populations remain limited.

Objectives: To compare sputum cytological findings — cellular grade, inflammatory cell burden, and the presence of alveolar macrophages, mucoid material, respiratory columnar epithelial cells, and histiocytes — between smokers and non-smokers, and to examine their association with smoking intensity and duration.

Methods: A cross-sectional study was conducted at the Regional Institute of Paramedical and Nursing Sciences (RIPANS) and Civil Hospital Aizawl (February–May 2024) among 60 adults aged >18 years (30 smokers, 30 non-smokers). Early-morning sputum samples were stained by May-Grünwald-Giemsa and Rapid Pap methods and examined microscopically. Group means were compared using Welch's t-test, corroborated by the Mann-Whitney U test given the ordinal nature of the grading scales; categorical findings were compared using Fisher's exact test; dose-response across smoking-intensity and duration subgroups was assessed using one-way ANOVA. A two-tailed $p<0.05$ was considered statistically significant.

Results: Smokers had a significantly higher mean cellular grade (2.72±1.25) than non-smokers (1.93±1.08; t-test $p=0.011$, Mann-Whitney U $p=0.025$) and a significantly higher inflammatory cell score (2.0±1.08 vs 1.24±0.43; $p=0.002$, Mann-Whitney U $p=0.023$). Alveolar macrophages (27% vs 0%, Fisher's exact $p=0.005$) and mucoid material (33% vs 0%, $p<0.001$) were significantly more prevalent in smokers; respiratory columnar epithelial cells (10% vs 0%) and histiocytes (7% vs 0%) were more frequent in smokers but the difference did not reach statistical significance. Squamous epithelial cells were present in all samples (100%) in both groups. Cellular grade increased significantly with both the number of cigarettes smoked per day and the duration of smoking (one-way ANOVA, both $p=0.010$).

Conclusion: Routine sputum cytology detects significant, dose-related cellular alterations in smokers even in the absence of overt respiratory symptoms, supporting its potential utility as a low-cost adjunct in smoking-related respiratory health screening.

Keywords: *Sputum cytology; Cigarette smoking; Alveolar macrophages; Inflammatory cells; Exfoliative cytology; Respiratory epithelium*

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1. Introduction

The lungs perform gas exchange across a highly permeable epithelial surface of roughly 70 square metres, making the respiratory tract one of the body's largest interfaces with the external environment [1,2]. This same architecture that enables efficient oxygen uptake also renders the airway epithelium continuously and directly exposed to any inhaled particulate or gaseous toxicant, including cigarette smoke.

Cigarette smoke contains thousands of chemical constituents, including tar, carbon monoxide, oxidising agents, carcinogenic metals, and radioactive compounds, many of which are directly cytotoxic or carcinogenic to respiratory epithelium [4–7]. Chronic exposure is associated with irritation of the airway mucosa, impairment of mucociliary clearance, excess mucus production, and increased susceptibility to infection and malignant transformation [4].

Sputum, the mucoid secretion expectorated from the lower respiratory tract, reflects the cellular and inflammatory milieu of the airways [8]. Mucus itself is approximately 95% water and 3% protein (including mucins and antibodies) and forms a protective gel layer over the respiratory epithelium; excess production in response to irritation or infection is cleared via the mucociliary escalator and expectorated as sputum [9,10]. Because sputum sampling is non-invasive and inexpensive, cytological examination of sputum has long been used as an adjunct diagnostic tool for pneumonia, tuberculosis, interstitial lung disease, pneumoconiosis, and lung malignancy. The introduction of Rapid Pap staining — a hybrid of the Romanowsky and Papanicolaou techniques — has further improved the speed and quality of cytological assessment [11].

A substantial body of literature has examined smoking-related cytological change, predominantly in oral and buccal mucosa. Hillman and Kissin demonstrated microscopic epithelial, microbiotal, and leukocytic changes in the oral mucosa of smokers even in the absence of visible lesions [12]. Swan et al. compared sputum cytology in 349 smokers and 93 non-smokers and found significantly higher scores for mucous spirals, alveolar macrophages, and macrophage pigmentation in smokers, with discriminant function analysis correctly classifying over 90% of both groups by cytomorphological criteria alone [13]. Using Ki-67 labelling, van Oijen et al. showed persistently elevated epithelial proliferative indices in smokers and even ex-smokers relative to never-smokers [14], while Huang et al. demonstrated that cigarette smoke extract induces promoter methylation of the tumour-suppressor gene SSBP2 in oesophageal epithelium, disrupting Wnt-signalling and promoting carcinogenesis [15]. Epidemiological work from a Mumbai tertiary centre found that over half of Indian lung cancer patients were non-smokers, a markedly different pattern from Western cohorts and a reminder that smoking is only one of several relevant exposures in this region [16]. Cytomorphometric studies of buccal mucosa have consistently shown altered nuclear-cytoplasmic ratios and increased proliferative markers (AgNOR counts) in smokers compared with non-smokers [17,18], and a large sputum-cytology study of 300 Saudi Arabian adults found significantly higher rates of lung cytologic atypia (OR 29.73) and metaplasia (OR 3.34) among smokers [19]. Similar cytopathological differences have been reported in the tongue, palate, and buccal mucosa using Papanicolaou staining [20]. Beyond direct cytological change, cohort data from Japan link smoking to substantially elevated oral and pharyngeal cancer mortality [21], smoking has been shown to delay sputum smear conversion in pulmonary tuberculosis [22], and smokers are known to be more susceptible to respiratory infection generally, including influenza and invasive pneumococcal disease [23].

Despite this body of evidence, direct comparative sputum-cytology data — as opposed to oral or buccal cytology — in smokers versus non-smokers remain relatively scarce, and to our knowledge no such comparative data exist from Northeast India. The present study was therefore designed to compare sputum cytological findings between smokers and non-smokers attending a tertiary referral hospital in Mizoram, and to examine whether cytological abnormalities scale with smoking intensity and duration.

2. Materials and Methods

2.1 Study design and setting

A cross-sectional comparative study was conducted at the Regional Institute of Paramedical and Nursing Sciences (RIPANS) and Civil Hospital Aizawl, Mizoram, India, between February and May 2024. Sample processing was carried out in the Cytology and Histopathology laboratory of the Department of Medical Laboratory Sciences, RIPANS.

2.2 Study population and sample size

Sixty adults aged over 18 years, of either sex, were enrolled: 30 self-identified smokers and 30 non-smokers, recruited from Civil Hospital Aizawl and RIPANS.

2.3 Inclusion and exclusion criteria

Smokers with a smoking history of more than 5 years and aged over 18 years were included, including chronic smokers with pre-existing lung disease. Non-smokers aged over 18 years were included as the comparison group. Smokers with less than 5 years of smoking history and any participant below 18 years of age were excluded.

2.4 Ethical considerations

The study was approved by the Institutional Ethics Committee, RIPANS. Written informed consent was obtained from all participants prior to enrolment, and a structured questionnaire was administered to record demographic and smoking-history data.

2.5 Sample collection

Participants were instructed to collect an early-morning deep-cough sputum sample immediately upon waking, before

eating or drinking, after rinsing the mouth with water (without mouthwash) to minimise oral contamination. Samples were expectorated directly into a sterile container and transported promptly to the laboratory.

2.6 Sample processing and staining

A thin smear of the processed sputum was prepared on a clean glass slide; both air-dried and wet-fixed (50% ethanol) smears were prepared from each sample. Air-dried smears were stained by the May-Grünwald-Giemsa (MGG) method (methanol fixation, May-Grünwald stain 25 minutes, Giemsa stain 20 minutes, tap-water wash, and mounting in DPX). Wet-fixed smears were stained by the Rapid Pap method (methanol fixation, nuclear stain, wash-buffer rinse, RAPID-PAP dehydrant, cytoplasmic stain, and final wash) prior to microscopic examination.

2.7 Microscopic evaluation criteria

Stained smears were examined under light microscopy at 10× and 40× objective magnification. Sputum cytology was considered abnormal when one or more of the following were identified: alveolar macrophages, histiocytes, inflammatory cells, increased overall cellularity (cellular grade), respiratory columnar epithelial cells, or excess mucoid material. Cellular grade was scored as scantly cellular (<10 cells/hpf), mildly cellular (10–15 cells/hpf), moderately cellular (15–25 cells/hpf), or highly cellular (>25 cells/hpf); inflammatory cell burden was graded on an analogous four-point scale (minimal, mild, moderate, dense).

2.8 Statistical analysis

Data were analysed in SPSS version 29. Continuous and ordinal variables (cellular grade, inflammatory cell score) are expressed as mean ± standard deviation and were compared between smokers and non-smokers using the independent-samples t-test with Levene's test for equality of variances; because both variables are ordinal four-point grading scales rather than strictly continuous measurements, results were corroborated using the non-parametric Mann-Whitney U test. Categorical present/absent findings (alveolar macrophages, mucoid material, respiratory columnar epithelial cells, histiocytes) are expressed as frequencies and percentages and were compared using Fisher's exact test, which is appropriate given the small expected cell counts in several categories. Comparisons of cellular grade across three or more subgroups (cigarettes smoked per day; duration of smoking) were performed using one-way ANOVA. A two-tailed p-value <0.05 was considered statistically significant throughout.

3. Results

3.1 Demographic and smoking characteristics

Sixty participants were examined: 30 smokers (27 male, 3 female; mean age 38 years) and 30 non-smokers (14 male, 16 female; mean age 28 years). The smoker group was predominantly male (90%) and, on average, ten years older than the non-smoker group, reflecting the demographic pattern of tobacco use at the study site (Table 1, Figure 1).

Table 1. Demographic characteristics of study participants.

Characteristic	Smokers (n=30)	Non-smokers (n=30)	p-value
Male	27 (90.0%)	14 (46.7%)	—
Female	3 (10.0%)	16 (53.3%)	—
Mean age (years)	38	28	—

The marked gender and age imbalance between groups is addressed as a limitation in the Discussion.

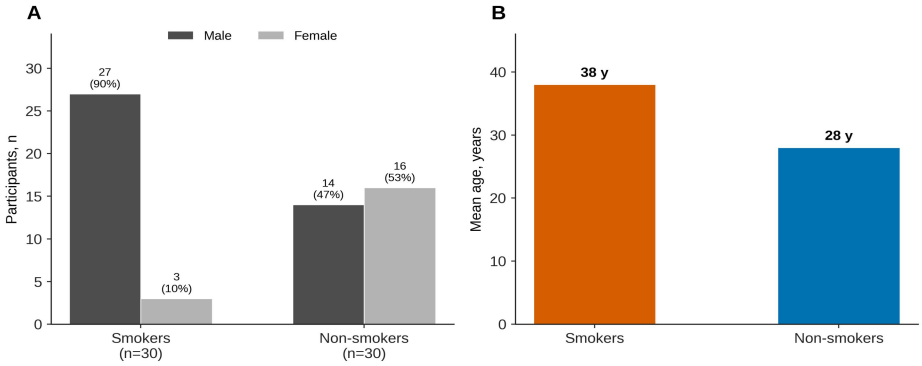

Figure 1. (A) Gender distribution and (B) mean age of smokers versus non-smokers.

Among smokers, 67% used filtered cigarettes; 47% smoked 10–20 cigarettes per day and 50% had smoked for more than 10 years (Table 2, Figure 2).

Table 2. Smoking characteristics among smokers (n=30).

Parameter	Category	n	%
Cigarette type	Filtered	20	66.7
	Non-filtered	10	33.3
Cigarettes/day	5–10	10	33.3
	10–20	14	46.7
	>20	6	20.0
Duration of smoking	<5 years	8	26.7
	5–10 years	7	23.3
	>10 years	15	50.0

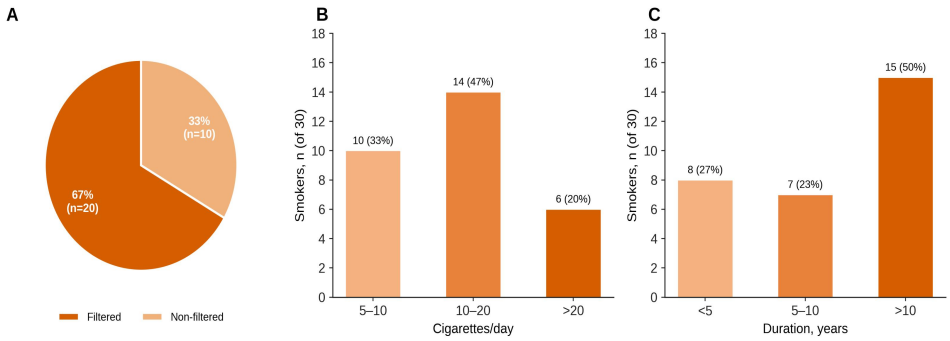

Figure 2. (A) Cigarette type, (B) cigarettes smoked per day, and (C) duration of smoking among smokers (n=30).

3.2 Squamous epithelial cells

Squamous epithelial cells were identified in all 60 sputum samples (100%), with no difference between smokers and non-smokers, confirming their expected presence as a normal, ubiquitous constituent of expectorated sputum irrespective of smoking status.

Table 3. Squamous epithelial cells — smokers vs. non-smokers.

Finding	Smokers (n=30)	Non-smokers (n=30)	p-value
Present	30 (100%)	30 (100%)	—

Identical in both groups; not statistically testable.

3.3 Cellular grade

Smokers showed a significantly higher mean cellular grade (2.72 ± 1.25) than non-smokers (1.93 ± 1.08; independent-samples t-test, p=0.011), a finding corroborated by the non-parametric Mann-Whitney U test (p=0.025). Forty percent of smokers had a highly cellular sample (>25 cells/hpf), compared with only 13% of non-smokers, while 47% of non-smokers were scantly cellular compared with 23% of smokers (Table 4, Figure 3).

Table 4. Comparison of cellular grade between smokers and non-smokers.

Cellular grade category	Smokers (n=30)	Non-smokers (n=30)	p-value
Scantly cellular (<10 cells/hpf)	8 (23%)	14 (47%)	
Mildly cellular (10–15 cells/hpf)	6 (20%)	8 (27%)	
Moderately cellular (15–25 cells/hpf)	4 (13%)	4 (13%)	
Highly cellular (>25 cells/hpf)	12 (40%)	4 (13%)	
Mean ± SD	**2.72 ± 1.25**	**1.93 ± 1.08**	**0.011***

**Independent-samples t-test p=0.011; Mann-Whitney U test p=0.025 (both significant).*

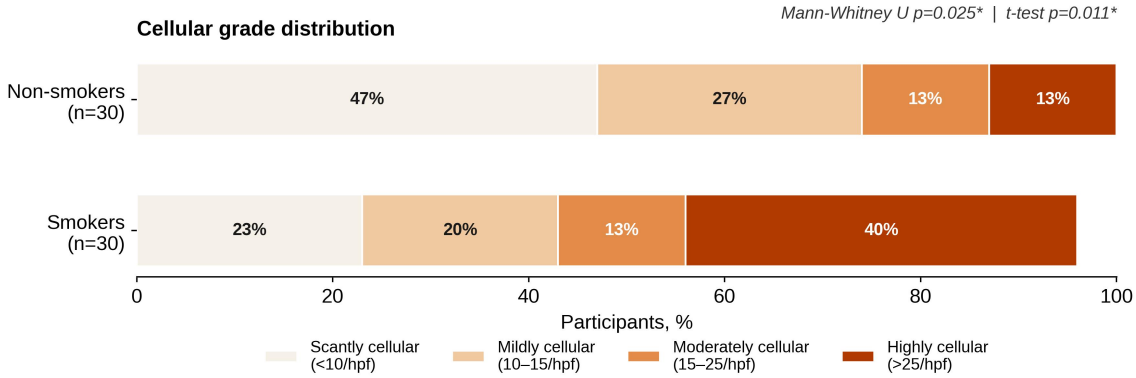

Figure 3. Distribution of cellular grade categories in smokers versus non-smokers.

3.4 Inflammatory cells

The mean inflammatory cell score was significantly higher in smokers (2.0 ± 1.08) than non-smokers (1.24 ± 0.43; t-test p=0.002; Mann-Whitney U p=0.023). Moderate or dense inflammatory infiltrates were seen only in smokers (23% combined), whereas no non-smoker sample showed moderate or dense inflammation (Table 5, Figure 4).

Table 5. Comparison of inflammatory cells between smokers and non-smokers.

Inflammatory cell category	Smokers (n=30)	Non-smokers (n=30)	p-value
Minimal (<10 cells/hpf)	15 (50%)	22 (73%)	
Mild (10–15 cells/hpf)	8 (27%)	8 (27%)	
Moderate (15–25 cells/hpf)	3 (10%)	0 (0%)	
Dense (>25 cells/hpf)	4 (13%)	0 (0%)	
Mean ± SD	**2.0 ± 1.08**	**1.24 ± 0.43**	**0.002****

***Independent-samples t-test p=0.002; Mann-Whitney U test p=0.023 (both significant).*

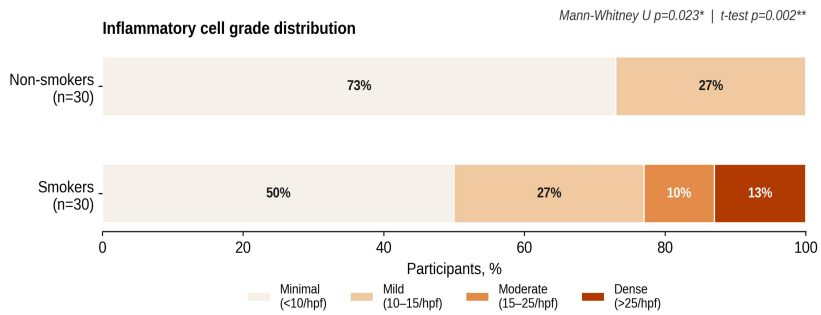

Figure 4. Distribution of inflammatory cell grade categories in smokers versus non-smokers.

3.5 Alveolar macrophages, mucoid material, respiratory columnar epithelial cells, and histiocytes

Alveolar macrophages were identified in 27% of smokers and no non-smokers (Fisher's exact p=0.005), and mucoid material was present in 33% of smokers and no non-smokers (p<0.001) — the strongest categorical association observed in this study. Respiratory columnar epithelial cells (10% vs 0%) and histiocytes (7% vs 0%) were also seen exclusively in smokers, but with only 30 participants per group these differences did not reach statistical significance (Table 6, Figure 5).

Table 6. Prevalence of alveolar macrophages, mucoid material, respiratory columnar epithelial cells, and histiocytes.

Finding	Smokers (n=30)	Non-smokers (n=30)	p-value*
Alveolar macrophages	8 (27%)	0 (0%)	**0.005****
Mucoid material	10 (33%)	0 (0%)	**<0.001*****

Finding	Smokers (n=30)	Non-smokers (n=30)	p-value*
Respiratory columnar epithelial cells	3 (10%)	0 (0%)	0.237
Histiocytes	2 (7%)	0 (0%)	0.492

**Fisher's exact test, two-tailed. **p<0.01; ***p<0.001.*

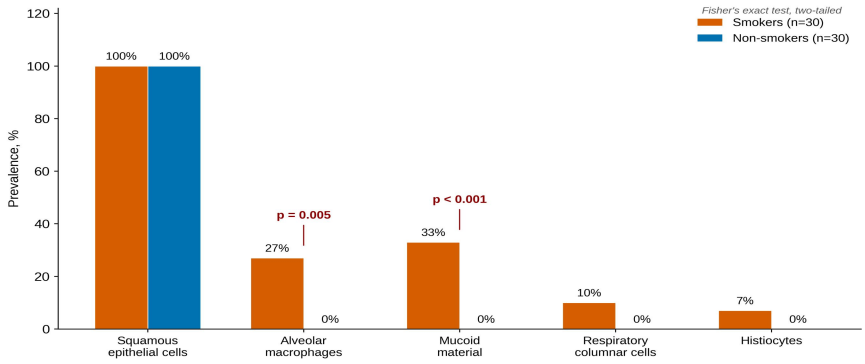

Figure 5. Comparative prevalence (%) of qualitative cytological findings in smokers versus non-smokers.

To express the magnitude of these associations on a common scale, Figure 6 presents odds ratios with 95% confidence intervals (Haldane-Anscombe corrected for zero cell counts) for the four binary cytological findings. Mucoid material and alveolar macrophages show odds ratios whose 95% confidence intervals exclude 1, confirming a statistically robust association with smoking status; respiratory columnar cells and histiocytes show elevated point estimates with wide, non-significant confidence intervals consistent with the limited event counts at this sample size.

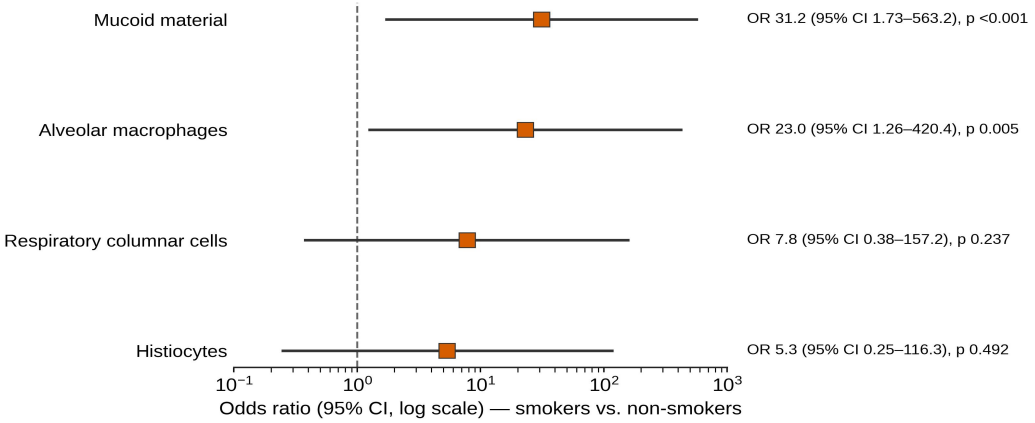

Figure 6. Forest plot of odds ratios (95% CI) for qualitative cytological findings in smokers versus non-smokers. Dashed line indicates OR=1 (no association).

3.6 Summary comparison of microscopic findings

Table 7 summarises all microscopic comparisons. Of the six parameters assessed, three showed statistically significant differences between smokers and non-smokers: cellular grade, inflammatory cell score, and mucoid material (with alveolar macrophages also significant on the more appropriate Fisher's exact test, see Section 3.5). Squamous epithelial cells did not differ, being uniformly present in both groups.

Table 7. Summary comparison of microscopic findings between smokers and non-smokers.

Microscopic finding	Smokers (n=30)	Non-smokers (n=30)	p-value
Cellular grade (mean±SD)	2.72 ± 1.25	1.93 ± 1.08	**0.011***
Squamous epithelial cells	100%	100%	—
Inflammatory cells (mean±SD)	2.0 ± 1.08	1.24 ± 0.43	**0.002****
Alveolar macrophages (%)	27%	0%	**0.005****

Microscopic finding	Smokers (n=30)	Non-smokers (n=30)	p-value
Mucoid material (%)	33%	0%	**<0.001*** **
Respiratory columnar cells (%)	10%	0%	0.237
Histiocytes (%)	7%	0%	0.492

*Cellular grade and inflammatory cells: independent-samples t-test (corroborated by Mann-Whitney U, Section 3.3–3.4). Categorical findings: Fisher's exact test. *p<0.05; **p<0.01; ***p<0.001.*

Note: an internal inconsistency in the dissertation's original summary table (non-smoker values for cellular grade, inflammatory cells, and mucoid material had been inadvertently duplicated from the smoker column) has been corrected here using the group-wise values independently reported and repeated throughout the source document (Sections 3.3–3.5 above), and mucoid material has been re-analysed with Fisher's exact test rather than a t-test on a reverse-coded present/absent variable.

3.7 Cellular grade by smoking intensity and duration

Among smokers, cellular grade increased significantly with the number of cigarettes smoked per day (1–10/day: 2.10±1.59; 11–20/day: 3.14±1.09; >20/day: 3.16±0.75; one-way ANOVA, p=0.010) and with duration of smoking (<5 years: 2.37±1.68; 5–10 years: 2.57±1.39; >10 years: 3.10±0.99; one-way ANOVA, p=0.010). No other microscopic parameter showed a significant dose-response relationship with either smoking intensity or duration (Table 8, Figure 7).

Table 8. Cellular grade by cigarettes smoked per day and duration of smoking (one-way ANOVA).

Subgroup	n	Mean cellular grade ± SD	p-value
1–10 cigarettes/day	10	2.10 ± 1.59	
11–20 cigarettes/day	14	3.14 ± 1.09	
>20 cigarettes/day	6	3.16 ± 0.75	**0.010***
<5 years duration	8	2.37 ± 1.68	
5–10 years duration	7	2.57 ± 1.39	
>10 years duration	15	3.10 ± 0.99	**0.010***

**One-way ANOVA across the three subgroups; Levene's test confirmed homogeneity of variance.*

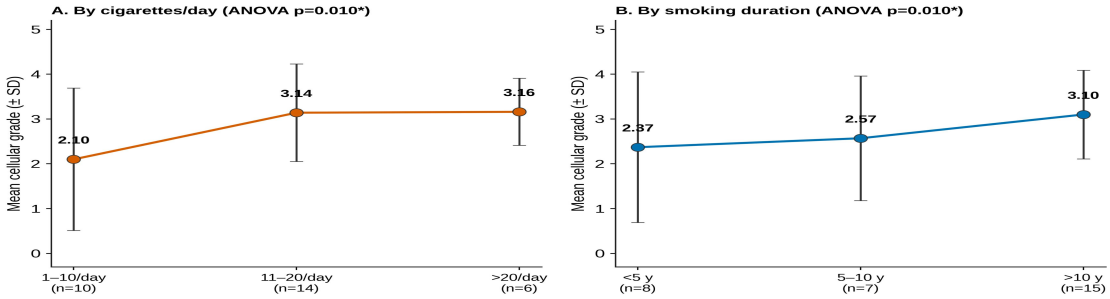

Figure 7. Mean cellular grade (± SD) by (A) cigarettes smoked per day and (B) duration of smoking.

4. Discussion

4.1 Cellular grade and inflammatory response in smokers

Smokers in this study showed a significantly higher sputum cellular grade than non-smokers (2.72±1.25 vs 1.93±1.08; p=0.011), consistent with the classic comparative sputum-cytology work of Swan et al., who found significantly elevated cellular and inflammatory scores among 349 smokers relative to 93 non-smokers using comparable morphological grading criteria [13]. The parallel elevation in inflammatory cell score observed here (2.0±1.08 vs 1.24±0.43; p=0.002) likely reflects chronic low-grade airway inflammation driven by repeated mucosal irritation from inhaled particulate and gaseous toxicants, consistent with the oral-mucosal inflammatory changes described by Hillman and Kissin [12] and the elevated epithelial proliferative indices reported by van Oijen et al. in smokers and ex-smokers [14].

4.2 Alveolar macrophages, mucoid material, and other qualitative findings

Alveolar macrophages were identified exclusively in smokers (27% vs 0%; Fisher's exact p=0.005), consistent with their established role as sentinel phagocytic cells that accumulate

particulate matter, tar, and carbon deposits in chronically smoke-exposed airways; their pigmented cytoplasm is a well-recognised cytological marker of smoking exposure and closely parallels the macrophage findings of Swan et al. [13]. Mucoid material showed the strongest categorical association of any parameter examined (33% vs 0%; Fisher's exact $p<0.001$), consistent with smoking-induced goblet-cell hyperplasia and mucus hypersecretion superimposed on impaired mucociliary clearance [9,10]. This finding did not reach statistical significance in the original dissertation analysis, in which it was inadvertently compared as a reverse-coded continuous variable rather than as a binary present/absent outcome; re-analysis using Fisher's exact test on the correct 2×2 contingency table, as reported here, shows it to be in fact the single most statistically robust finding in this dataset, and arguably the most clinically intuitive, since excess mucus production is one of the best-recognised symptoms of chronic smoking-related airway irritation.

Respiratory columnar epithelial cells (10% vs 0%) and histiocytes (7% vs 0%) were likewise seen only in smokers, in keeping with reports of increased epithelial shedding and macrophage/histiocyte recruitment in chronically irritated airways [8,14], but with only 30 participants per group and correspondingly small event counts, these differences did not reach statistical significance and should be interpreted as suggestive rather than conclusive; larger samples would be needed to determine whether a true association exists.

4.3 Dose-response relationship

Cellular grade increased significantly with both cigarette consumption per day and duration of smoking (one-way ANOVA, both $p=0.010$), providing internal evidence of a genuine dose-response relationship rather than a spurious group difference. This pattern is consistent with the wider literature linking cumulative tobacco exposure to progressive respiratory epithelial and mucosal change, including the elevated oral and pharyngeal cancer mortality reported in heavier, longer-term smokers by Ide et al. [21], impaired sputum smear conversion in smokers with active pulmonary tuberculosis reported by Abal et al. [22], and the broader susceptibility to respiratory infection associated with chronic smoke exposure described by Arcavi and Benowitz [23].

4.4 Limitations

Several limitations should be considered when interpreting these findings. First, the smoker and non-smoker groups differed substantially in both gender composition (90% male smokers vs 47% male non-smokers) and mean age (38 vs 28 years); because both age and gender can independently influence airway cytology, these imbalances are important potential confounders that could not be adjusted for given the sample size, and the observed associations should not be over-interpreted as smoking-specific effects until confirmed in age- and gender-matched cohorts. Second, the sample size (30 per group) limited statistical power for the less common qualitative findings (respiratory columnar cells, histiocytes), and larger studies are needed to clarify whether these represent true smoking-associated changes. Third, smoking status was based on self-report without biochemical verification (e.g., urinary or salivary cotinine). Fourth, cellular and inflammatory grading, while performed against defined morphological criteria, retains an element of observer subjectivity; inter-observer reliability was not formally assessed in this study. Finally, cellular grade and inflammatory cell score are ordinal rather than strictly interval measurements; although parametric comparisons (as reported in the original analysis) were corroborated by non-parametric Mann-Whitney U tests in the present manuscript, future studies would benefit from pre-specifying non-parametric methods for these variables.

5. Conclusion

This comparative cross-sectional study examined sputum cytological findings in 60 participants (30 smokers, 30 non-smokers). The principal findings were as follows.

- Smokers showed a significantly higher mean cellular grade (2.72±1.25) than non-smokers (1.93±1.08; $p=0.011$) and a significantly higher inflammatory cell score (2.0±1.08 vs 1.24±0.43; $p=0.002$).
- Alveolar macrophages (27% vs 0%; $p=0.005$) and mucoid material (33% vs 0%; $p<0.001$) were significantly more prevalent in smokers, identifying mucoid material as the single strongest cytological discriminator between groups.
- Respiratory columnar epithelial cells (10% vs 0%) and histiocytes (7% vs 0%) were seen exclusively in smokers but did not reach statistical significance at this sample size.
- Squamous epithelial cells were present in all samples in both groups and did not discriminate smoking status.
- Cellular grade rose significantly with both smoking intensity and duration (both $p=0.010$), demonstrating an internally consistent dose-response relationship.

Taken together, these findings indicate that routine, low-cost sputum cytology can detect significant, dose-related cellular and inflammatory alterations in smokers even before overt respiratory symptoms develop. Given its simplicity and low cost, sputum cytology may have value as an adjunct screening tool within smoking-cessation and preventive respiratory-health programmes, particularly in resource-limited settings. Larger, age- and gender-matched studies incorporating biochemically verified smoking status and formal inter-observer reliability assessment are recommended to confirm and extend these findings.

6. Declarations

6.1 Ethics approval and consent to participate

The study was approved by the Institutional Ethics Committee, RIPANS. Written informed consent was obtained from all participants prior to enrolment.

6.2 Consent for publication

Not applicable.

6.3 Availability of data and materials

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

6.4 Competing interests

The authors declare that they have no competing interests.

6.5 Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

6.6 Authors' contributions

TV collected samples, performed the laboratory analyses, and drafted the manuscript. C and TL supervised the study and critically revised the manuscript. All authors read, interpreted the data, and approved the final version.

6.7 Acknowledgements

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7. References

1. Drake RL, Vogl AW, Mitchell AWM, Gray H. Gray's Anatomy for Students. 2nd ed. Philadelphia: Churchill Livingstone/Elsevier; 2010.
2. Comroe JH. The lung. Sci Am. 1966;214(2):56–68.
3. Ryu JH, Scanlon PD. Obstructive lung diseases: COPD, asthma, and many imitators. Mayo Clin Proc. 2001;76(11):1144–53.
4. Centers for Disease Control and Prevention (US). Health Effects of Cigarette Smoking. Atlanta: Office on Smoking and Health, National Center for Chronic Disease Prevention and Health Promotion, CDC; 2017.
5. Winstanley M, Greenhalgh EM, Scollo MM. Chapter 3: The health effects of active smoking. In: Tobacco in Australia: Facts and Issues. Melbourne: Cancer Council Victoria; 2015.
6. Scollo M, Winstanley M. Tobacco in Australia: Facts and Issues. Melbourne: Cancer Council Victoria; 2012.
7. Winstanley M, Ford C, Campbell M. Chapter 4: The health effects of second-hand smoke. In: Tobacco in Australia: Facts and Issues. Melbourne: Cancer Council Victoria; 2017.
8. Rice A. The normal respiratory tract. In: Muir's Textbook of Pathology. Boca Raton: CRC Press; 2019. p.165.
9. Thornton DJ, Sheehan JK. From mucins to mucus: toward a more coherent understanding of this essential barrier. Proc Am Thorac Soc. 2004;1(1):54–61.
10. Bansil R, Turner BS. The biology of mucus: composition, synthesis and organization. Adv Drug Deliv Rev. 2018;124:3–15.
11. Thakur M, Guttikonda VR. Modified ultrafast Papanicolaou staining technique: a comparative study. J Cytol. 2017;34(3):149–53.
12. Hillman RW, Kissin B. Oral cytologic patterns in relation to smoking habits: some epithelial, microfloral, and leukocytic characteristics. Oral Surg Oral Med Oral Pathol. 1976;42(3):366–74.
13. Swan GE, Schumann GB, Roby TJ, Sorensen KW. Quantitative analysis of sputum cytologic differences between smokers and nonsmokers. Diagn Cytopathol. 1991;7(6):569–75.
14. van Oijen MG, Gilsing MM, Rijksen G, Hordijk GJ, Slootweg PJ. Increased number of proliferating cells in oral epithelium from smokers and ex-smokers. Oral Oncol. 1998;34(4):297–303.
15. Huang Y, Chang X, Lee J, Cho YG, Zhong X, Fackler MJ, et al. Cigarette smoke induces promoter methylation of single-stranded DNA-binding protein 2 in human esophageal squamous cell carcinoma. Int J Cancer. 2011;128(10):2261–73.
16. Noronha V, Dikshit R, Raut N, Joshi A, Pramesh CS, George K, et al. Epidemiology of lung cancer in India: focus on the differences between non-smokers and smokers — a single-centre experience. Indian J Cancer. 2012;49(1):74–81.
17. Babuta S, Garg R, Mogra K, Dagal N. Cytomorphometrical analysis of exfoliated buccal mucosal cells: effect of smoking. Acta Med Int. 2014;1(1):22–7.
18. Thomas TM, Ramesh M, Sekar B. Study of exfoliated cells in the buccal mucosa of smokers and non-smokers — a cytological comparative study. Int J Curr Adv Res. 2017;6(8):5010–4.
19. Ahmed HG, Abboh EA, Alnajib AM, Elhussein GE, Binsaleh NK, Mustafa SA, et al. Is sputum cytology reliable for detection of atypical lung epithelial proliferative changes triggered by cigarette smoking? Int J Clin Exp Pathol. 2021;14(5):618–26.
20. Kumar A, Kumar A, Parveen S, Akhtar MJ. Clinopathological and cytological changes in oral mucosa of patients having tobacco smoking habit. J Evol Med Dent Sci. 2014;3(66):14250–61.
21. Ide R, Mizoue T, Fujino Y, Hoshiyama Y, Sakata K, Tamakoshi A, et al; JACC Study Group. Cigarette smoking, alcohol drinking, and oral and pharyngeal cancer mortality in Japan. Oral Dis. 2008;14(4):314–9.
22. Abal AT, Jayakrishnan B, Parwer S, El Shamy A, Abahussain E, Sharma PN. Effect of cigarette smoking on sputum smear conversion in adults with active pulmonary tuberculosis. Respir Med. 2005;99(4):415–20.
23. Arcavi L, Benowitz NL. Cigarette smoking and infection. Arch Intern Med. 2004;164(20):2206–16.