

## Original Article

# Is sputum cytology reliable for detection of atypical lung epithelial proliferative changes triggered by cigarette smoking?

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**Abstract:** Background: In recent years, Saudi Arabia has witnessed major tobacco smoking-related disease, such as cardiovascular disease and cancer, particularly among the younger population. Methodology: The present study aimed at evaluating the effect of cigarette smoke on lung epithelial cells. Results: This was a cross-sectional case-control study involving 300 apparently healthy volunteers living in Ha'il, Northern Saudi Arabia. Cigarette smokers (N = 100) were used as cases, and non-smokers (N = 200) were used as controls. A sputum specimen was obtained from each participant, employing all necessary safety precautions and sample adequacy measures. Results: Among 300 study subjects, cytologic atypia was identified in 14/300 (4.7%). Among the 14 cases with atypical cytologic changes, 13/14 (92.9%) were in smokers and 1/14 (7.1%) was in a non-smoker. The risk of lung cytologic atypia associated with cigarette smoking, was OR (95% CI) = 29.73 (3.82-230.87), P = 0.0001. Out of 300 study subjects, metaplasia was identified in 45/300 (15%). Among 45 cases with metaplastic changes, 26/45 (57.8%) were in the smokers and 19/45 (42.2%) were in non-smokers. The risk of lung epithelial metaplasia associated with cigarette smoking was OR (95% CI) = 3.34 (1.74-6.41), P = 0.0003. Conclusion: Cigarette smoking is a significant risk for developing lung epithelial atypia, lung metaplasia, and inflammatory cell infiltrate (especially chronic inflammation). Sputum cytology is a simple, non-invasive method that can be used in screening at-risk populations for early detection of lung proliferative changes associated with tobacco smoking.

**Keywords:** Tobacco smoking, lung, cytology, sputum, atypia, metaplasia

## Introduction

About 85% of cases of lung cancer are attributed to tobacco smoking worldwide. This is in addition to the cancer cases resulting from passive smoking due to second-hand smoke exposure. However, lung cancer risk due to smoking is reversible, and can be reduced by smoking cessation, if patients quit early in life. The global map of lung cancer in different geographic regions correlates with tobacco smoking consumption proportion [1].

In recent years, Saudi Arabia has witnessed major tobacco smoking-related disease, particularly among the younger population [2]. A

recent study from Northern Saudi Arabia had shown that the prevalence of current smoking among male secondary school students is 40.8%. The most commonly smoked tobacco form is cigarettes, representing 67.3%, followed by Shisha, constituting 22.4%. Other forms of smoked tobacco such as Hashish were reported (2.1%) to be used, in adolescents. In a Saudi Arabian study, about 39.8% of adolescents reported daily smoking, with 29.6% smoking less than five cigarettes daily [3].

It is well established that lung epithelial cells' exposure to tobacco smoke causes epithelial cells to undergo atypical changes [4]. The degree of these atypical changes depends on

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**Table 1.** Distribution of smoking status by age

| Age        | Non-smokers | Smokers | Total |
|------------|-------------|---------|-------|
| ≤ 25 years | 61          | 4       | 65    |
| 26-35      | 98          | 44      | 142   |
| 36-45      | 22          | 28      | 50    |
| ≥ 46       | 19          | 24      | 43    |
| Total      | 200         | 100     | 300   |

most occasions on the length of tobacco use and exposure intensity [5]. Although there is increasing use of cigarette smoking in most of Saudi Arabia, no study investigated tobacco smoking's effect on the lung epithelium by cytologic methods. Lung epithelial cellular proliferative activity represents a model for detecting early signs of lung cancer. Therefore, the present study aimed at evaluating the effect of cigarette smoke on lung epithelial cells.

### Materials and methods

In this cross-sectional case-control study, 300 apparently healthy volunteers were selected by simple random method regardless of their demographic characteristics. All study subjects were males living in the city of Ha'il, Northern Saudi Arabia. As smoking is considered a social stigma among females (no response), all study subjects were males. Cigarette smokers (N = 100) were used as cases, and non-smokers (N = 200) were used as controls. A sputum specimen was obtained from each participant (employing all necessary safety precautions and sample adequacy measures). Cytologic material was carefully selected from the coloured sputum contents and was evenly smeared on a cleaned frosted-end glass-slide. The coloured contents most probably contained abnormal cytologic changes because of inflammatory reactions. Each smear was immediately fixed in 95% ethyl alcohol for 15 minutes. The smears were then transferred to the histopathology laboratory at the College of Medicine, University of Ha'il, Saudi Arabia, for staining and diagnosis. All smears were stained using the Papanicolaou (Pap) method, adopting a procedure described by Ahmed and Rezgalla [7].

### Cytologic assessment

All control measures were employed before the screening of the stained smears. Ten smears with known lung cancer were included as an internal positive control. All cytologic samples

(cases, controls, and internal positive controls) were re-indexed so that the examiner did not know whether the sample belonged to a case or a control. Two independent cytologists examined the samples. Conflicting positive samples were discussed and a joint decision was employed.

Cytologic atypia was defined as increased nuclear-cytoplasmic ratio associated with nuclear enlargement, chromatin clumping with uneven distribution, and condensation at the periphery of the cellular nuclei, bi- or multinucleation, irregular nuclear borders, and hyperchromasia. The decision of atypia was made when three or more of the features were present.

According to the features included, squamous metaplasia was defined as: a spherical group of cells with enlarged hyperchromatic nuclei mimicking squamous epithelial cells.

Acute inflammatory cells infiltrate was noted when the following features were present: polymorphonuclear leukocytes, evidence of necrosis, and eosinophilia.

Chronic inflammatory cell infiltrate was noted when there were: alveolar macrophages, lymphocytes, monocytes, or giant cells.

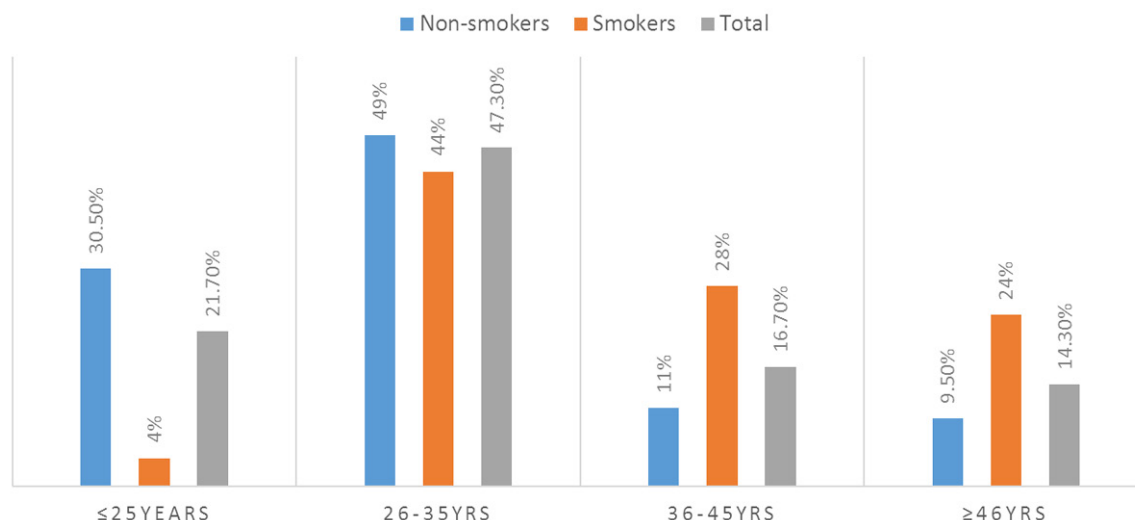
### Statistical analysis

Obtained data were entered into computer software, Statistical Package for Social Sciences (SPSS version 16; SPSS Inc., Chicago, IL). Chi-square test was employed for statistical significance (P < 0.05 was considered significant). Cross-tabulations and odds ratios applying 95% confidence interval were used.

### Results

The present study investigated 300 healthy individuals aged 21-63 years, with a mean age of 34 years. The majority of the participants were 26-35 years old, followed by ≤ 25 years, and 36-45 years, representing 142/300 (47.3%), 65/300 (21.7%), and 50/300 (16.7%), respectively. Most of the non-smokers were in the age group 26-35 years, followed by ≤ 25 years constituting 98/200 (49%), and 61/200 (30.5%), in that order. Most smokers were in the age range 26-35 years, followed by 36-45 years, representing 44/100 (44%) and 28/100 (28%), as indicated in **Table 1; Figure 1**.

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**Figure 1.** Description of smoking status by age.

**Table 2.** Distribution of smoking status by cytologic atypia and metaplasia

| Category                         | Variable | Non-smokers | Smokers | Total | OR (95% CI)                        | z statistic |
|----------------------------------|----------|-------------|---------|-------|------------------------------------|-------------|
| Lung Epithelial Cytologic Atypia |          |             |         |       | 29.73 (3.82-230.87),<br>P = 0.0001 | 3.244       |
|                                  | Yes      | 1           | 13      | 14    |                                    |             |
|                                  | No       | 199         | 87      | 286   |                                    |             |
|                                  | Total    | 200         | 100     | 300   |                                    |             |
| Lung Epithelial Metaplasia       |          |             |         |       | 3.34 (1.74-6.41), P =<br>0.0003    | 3.640       |
|                                  | Present  | 19          | 26      | 45    |                                    |             |
|                                  | Absent   | 181         | 74      | 255   |                                    |             |
|                                  | Total    | 200         | 100     | 300   |                                    |             |

Out of the 300 study subjects, cytologic atypia was identified in 14/300 (4.7%) and was absent in 286/300 (95.3%). Out of the 14 cases with atypical cytologic changes, 13/14 (92.9%) were in smokers and 1/14 (7.1%) were in non-smokers. The risk of lung cytologic atypia associated with cigarette smoking, had an the odds ratio (OR), and 95% confidence interval (95% CI) = 29.73 (3.82-230.87), P = 0.0001, as indicated in **Table 2; Figure 2; Image 2**.

Out of the 300 study subjects, metaplasia was identified in 45/300 (15%) and negative in 255/300 (85%). Out of the 45 cases with squamous metaplasia, 26/45 (57.8%) were in smokers and 19/45 (42.2%) were in non-smokers. The risk of lung epithelial metaplasia associated with cigarette smoking was OR (95% CI) = 3.34 (1.74-6.41), P = 0.0003 as indicated in **Table 2; Figure 2; Image 1**.

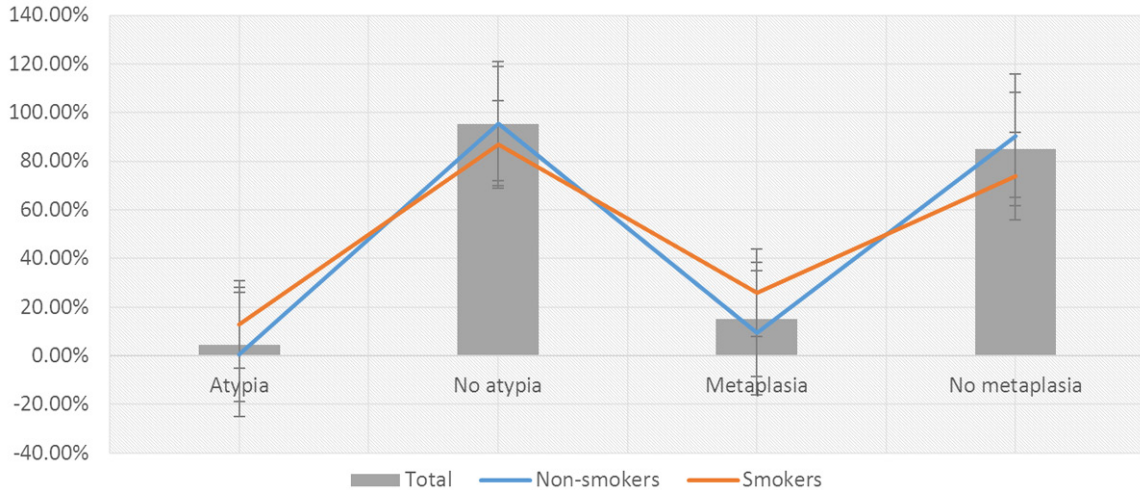
**Table 3; Figure 3**, show the lung epithelial proliferative changes according to the duration of

cigarette smoking. Most of the cases with cytologic atypia were seen in the duration ≤ 5 years, followed by 11-15, 16-20, and ≥ 21 years, representing 6/13 (46%), 3/13 (23%), 2/13 (15%), and 2/13 (15%), respectively. Most cases of metaplasia were associated with a duration of 6-10 years, followed by 11-15 and ≥ 21 years, constituting 9/26 (35%), 8/26 (31%), and 7/26 (27%) respectively.

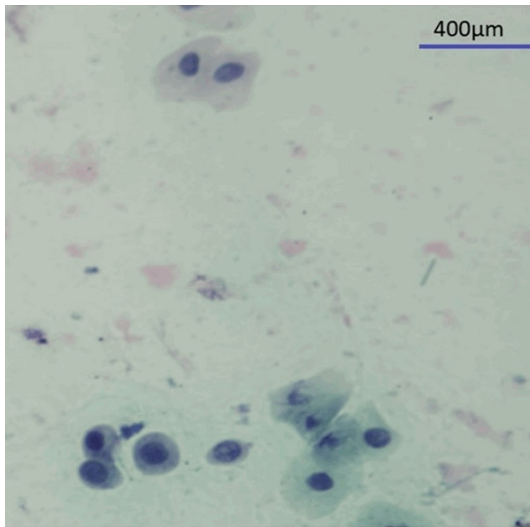
Acute inflammatory cells were identified in 22/200 (11%) of non-smokers and 19/100 (19%) of smokers. The risk of acute inflammatory changes associated with cigarette smoking had an OR (95% CI) = 1.89 (0.97-3.7), P = 0.06.

Chronic inflammatory cells were identified in 25/200 (12.5%) of non-smokers and 33/100 (33%) of smokers. The risk of chronic inflammatory changes associated with cigarette smoking had an OR (95% CI) = 3.34 (1.9-6.22), P = 0.0001, as indicated in **Table 4; Figure 4; Image 3**.

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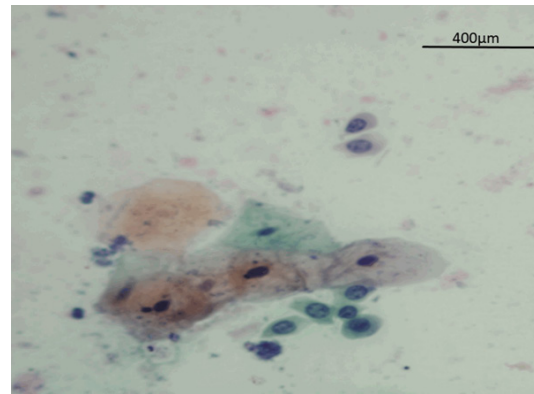
**Figure 2.** Description of smoking status by cytologic atypia and metaplasia.



**Image 1.** Papanicolaou staining (magnification 400), sputum smear obtained from cigarette smoker showing squamous metaplasia.

Acute inflammatory cell infiltrate was predominantly seen at the duration of smoking  $\leq 5$  years followed by 6-10 and  $\geq 21$  years, constituting 8/19 (42%), 6/19 (32%), and 3/19 (16%), in that order. Chronic inflammatory cell infiltrate was predominantly seen at the duration of smoking  $\leq 5$  years followed by 11-15 and both 6-10 &  $\geq 21$  years, constituting 9/33 (27%), 9/33 (27%), and 7/33 (21%), respectively, as indicated in **Table 5; Figure 5**.

About 6/13 (46%) of the atypical cytologic cases were seen in age groups 26-35 & 46+ years. Most of the cases of metaplasia were



**Image 2.** Papanicolaou staining (magnification 400), sputum smear obtained from cigarette smoker showing features of cytologic atypia (Chromatin clumps, hyperchromatic nuclei, apparent nucleoli, and condensation of chromatin at the periphery of the nuclei).

seen in the age range 26-35 years, followed by both age groups 36-45 & 46+ years, representing 13/26 (50%), and 6/26 (23%), respectively, as indicated in **Table 6; Figure 6**.

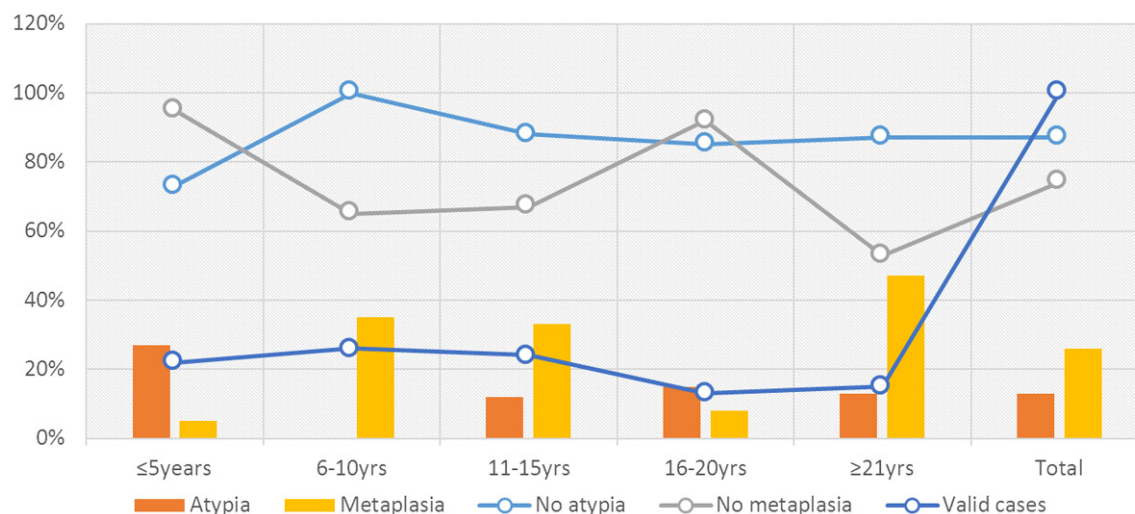
### Discussion

Tobacco smoking is one of the major preventable global causes of cancer, particularly lung cancer. Early detection of precancerous tobacco-related lung lesions can enhance the overall management of patients. Sputum cytology is a simple, reliable, non-invasive method with representative sensitivity and specificity measures, to screen at-risk populations. As the cig-

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**Table 3.** Lung epithelial proliferative changes according to duration of cigarette smoking

| Variable                                  | ≤ 5 years | 6-10 | 11-15 | 16-20 | ≥ 21 | Total |
|---|-----------|------|-------|-------|------|-------|
| <i>Lung Epithelial Cytological atypia</i> |           |      |       |       |      |       |
| No  | 16        | 26   | 21    | 11    | 13   | 87    |
| Yes                                       | 6         | 0    | 3     | 2     | 2    | 13    |
| Total                                     | 22        | 26   | 24    | 13    | 15   | 100   |
| <i>Lung Epithelial Metaplasia</i>         |           |      |       |       |      |       |
| No  | 21        | 17   | 16    | 12    | 8    | 74    |
| Yes                                       | 1         | 9    | 8     | 1     | 7    | 26    |
| Total                                     | 22        | 26   | 24    | 13    | 15   | 100   |



**Figure 3.** Lung epithelial proliferative changes according to duration of cigarette smoking by group.

**Table 4.** Distribution of smoking status by inflammatory cell infiltrate

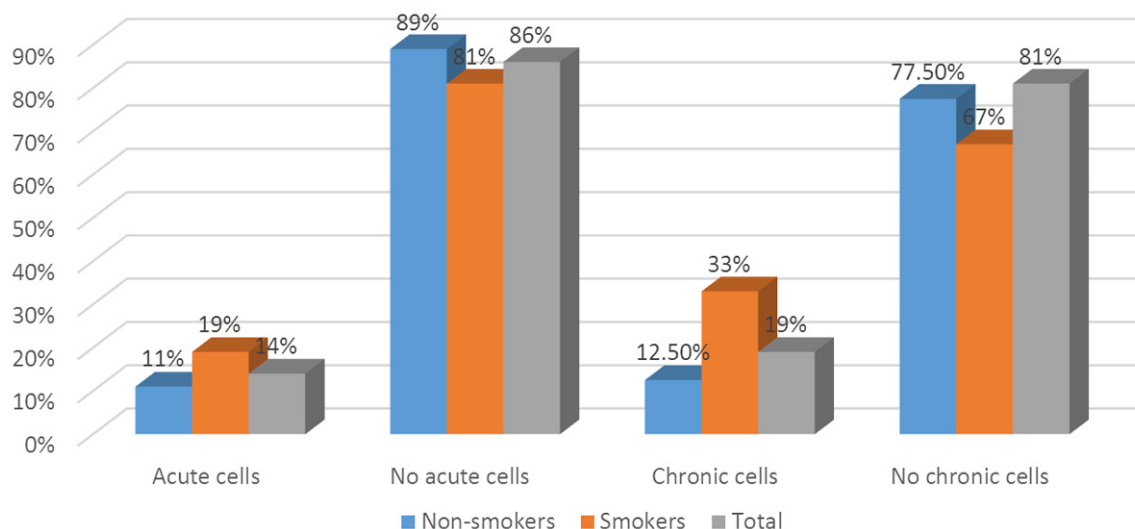
| Category                          | Variable | Non-smokers | Smokers | Total | OR (95% CI)      | z statistic |
|-----------------------------------|----------|-------------|---------|-------|------------------|-------------|
| <i>Acute inflammatory cells</i>   |          |             |         |       | 1.89 (0.97-3.7), | 1.881       |
|                                   | Present  | 22          | 19      | 41    | P = 0.06         |             |
|                                   | Absent   | 178         | 81      | 259   |                  |             |
|                                   | Total    | 200         | 100     | 300   |                  |             |
| <i>Chronic inflammatory cells</i> |          |             |         |       | 3.34 (1.9-6.22), | 4.104       |
|                                   | Present  | 25          | 33      | 58    | P = 0.0001       |             |
|                                   | Absent   | 175         | 67      | 242   |                  |             |
|                                   | Total    | 200         | 100     | 300   |                  |             |

arettte smoking habit is tremendously increasing in Saudi Arabia [7, 8], our current investigation was to predict the early precancerous changes (cytologic atypia) in a series of Saudi smokers.

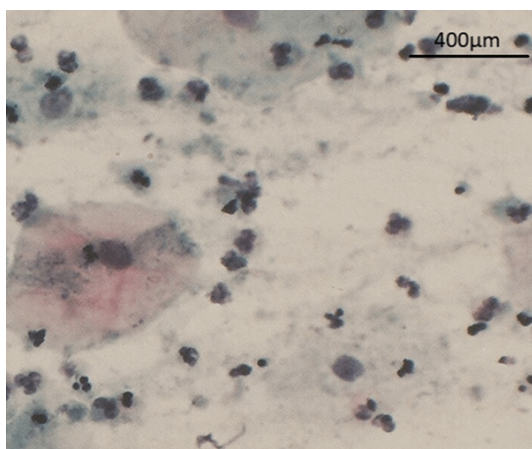
In the present study, the risk of lung cytologic atypia associated with cigarette smoking had an OR (95% CI) = 29.73 (3.82-230.87), P =

0.0001. This confirms the harmful effects of cigarette smoking among the Saudi population. The complex effects of cigarette smoking on lung epithelium are well-documented. Cigarette smoking can initiate several mutations in lung tissues [9]. It was reported that normal cells of smokers usually harbour mutations similar to those associated with a lung cancer profile.

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**Figure 4.** Description of the smoking status by inflammatory cell infiltrate.



**Image 3.** Papanicolaou staining (magnification 400), sputum smear obtained from cigarette smoker showing inflammatory cell infiltrate.

On the other hand, normal cells of ex-smokers possessed profiles very close to the typical cell profile [10]. With the wide application of highly sophisticated diagnostic methods (such as molecular diagnosis), there is a lack of cancer-related reports employing sputum cytology [11]. The current investigation may inspire researchers and healthcare providers to use sputum cytology as a screening method to detect precancerous conditions and cancer.

The present study shows a risk of lung epithelial metaplasia associated with cigarette smoking; OR (95% CI) = 3.34 (1.74-6.41),  $P = 0.0003$ . Squamous metaplasia results from transforming the delicate pseudostratified co-

lumnar lung epithelium into squamous cells due to exposure to the harmful effects of tobacco smoke components [12, 13]. Besides exposure to smoking, respiratory squamous metaplasia can be caused by other factors such as lung chronic inflammatory diseases [14]. Although lung squamous metaplasia is a reversible change, with the persistence of exposure, it can progress to squamous cell carcinoma [15].

The current findings showed that the risks of both cytologic atypia and squamous metaplasia were cumulative with the increase of duration of exposure to tobacco smoke. However, such findings were previously reported [16].

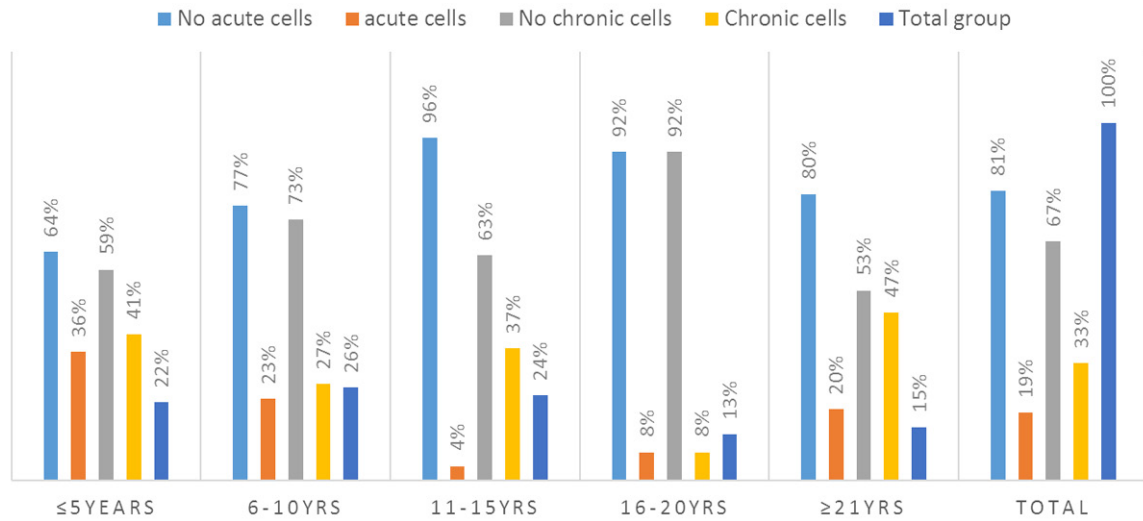
Inflammatory cell infiltrates were experienced in the present study. Acute inflammatory cell infiltrate showed relatively elevated significance ( $P < 0.06$ ), whereas a chronic inflammatory cell infiltrate was significantly associated with tobacco smoking ( $P = 0.0001$ ). Previous studies have reported on the positive role of cigarette smoking in causing inflammation in the lung [17]. Cigarette smoking leads to an inflammatory cell infiltrate, mainly chronic inflammatory cells (e.g. infiltration of macrophage in the lung mucosa), resulting in the unrestrained secretion of inflammatory mediators promoting a continuous inflammatory response [18-20].

Although the present study suggests a limited, simple, and reliable non-invasive method, it

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**Table 5.** Inflammatory cell infiltrate by the duration of cigarette smoking

| Variable                                    | ≤ 5 years | 6-10 | 11-15 | 16-20 | ≥ 21 | Total |
|---|-----------|------|-------|-------|------|-------|
| <i>Acute inflammatory cell infiltrate</i>   |           |      |       |       |      |       |
| No  | 14        | 20   | 23    | 12    | 12   | 81    |
| Yes   | 8         | 6    | 1     | 1     | 3    | 19    |
| Total                                       | 22        | 26   | 24    | 13    | 15   | 100   |
| <i>Chronic inflammatory cell infiltrate</i> |           |      |       |       |      |       |
| No  | 13        | 19   | 15    | 12    | 8    | 67    |
| Yes   | 9         | 7    | 9     | 1     | 7    | 33    |
| Total                                       | 22        | 26   | 24    | 13    | 15   | 100   |



**Figure 5.** Description of inflammatory cell infiltrate according to duration of cigarette smoking by group.

**Table 6.** Cytologic proliferative changes by age

| Variable          | ≤ 25 years | 26-35 | 36-45 | 46+ | Total |
|-------------------|------------|-------|-------|-----|-------|
| <i>Atypia</i>     |            |       |       |     |       |
| Yes               | 0          | 6     | 1     | 6   | 13    |
| No                | 4          | 38    | 27    | 18  | 87    |
| Total             | 4          | 44    | 28    | 24  | 100   |
| <i>Metaplasia</i> |            |       |       |     |       |
| Yes               | 1          | 13    | 6     | 6   | 26    |
| No                | 3          | 31    | 22    | 18  | 74    |
| Total             | 4          | 44    | 28    | 24  | 100   |

has some limitations, including its cross-sectional sampling setting and calibration with available highly sophisticated methods (e.g., polymerase chain reaction (PCR)).

In conclusion, cigarette smoking is a significant risk for developing lung epithelial atypia, lung metaplasia, and inflammatory cell infiltrate (especially chronic inflammation). Sputum cytology is a simple, non-invasive method that

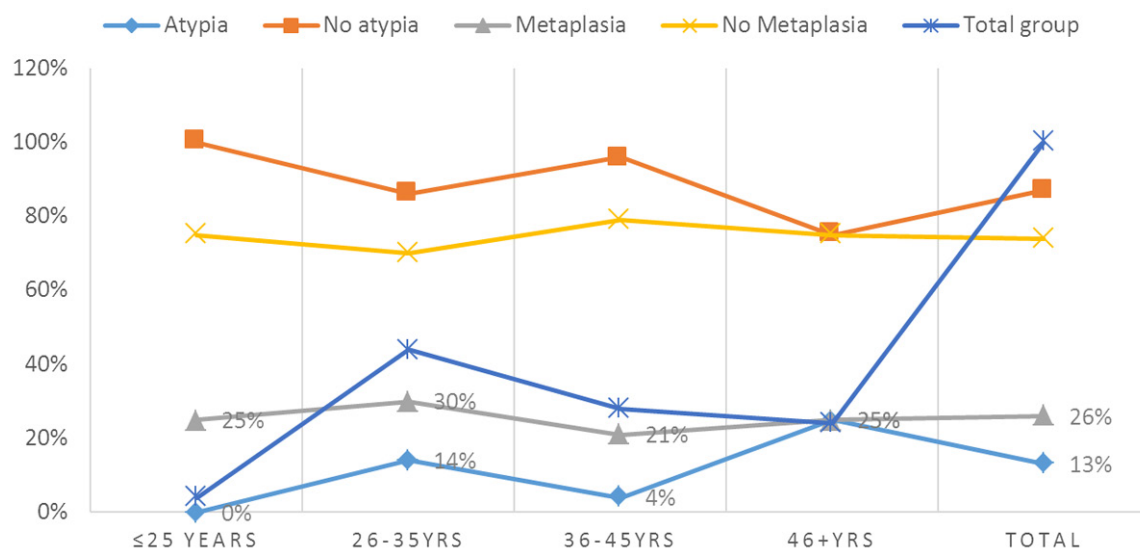
can be used in screening at-risk populations for early detection of early lung proliferative changes associated with smoking.

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Written & Oral informed consent was obtained from all individual participants included in the

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**Figure 6.** Cytologic proliferative changes within age groups.

study. Additional informed consent was obtained from all individual participants for whom identifying information is included in this manuscript.

### Disclosure of conflict of interest

None.

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