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Original Research Article

Cytological Patterns in Sputum and Buccal Smears among Chronic Asthmatic Patients in Shendi Town, Sudan

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Abstract: ***Background:*** Asthma is a common chronic respiratory disorder that induces cytological alterations in the airways and oral mucosa. Persistent inflammation and long-term inhalation therapy may contribute to epithelial hyperplasia, metaplasia, and atypia. ***Objective:*** To assess cytological changes in sputum and buccal smears among chronic asthmatic patients in Shendi, Sudan. ***Methods:*** A descriptive cross-sectional case-control study was conducted on 60 chronic asthmatic patients and 60 matched healthy controls. Sputum and buccal smears were collected, stained using the Papanicolaou technique, and examined microscopically. Data were analyzed using SPSS, and statistical significance was set at $p < 0.05$. ***Results:*** Nuclear atypia, inflammation, infection, cytoplasmic vacuolization, and prenuclear halos were significantly more frequent in sputum samples from asthmatic patients compared with controls ($p < 0.001$). Buccal smears showed increased inflammation and infection among patients, particularly those on long-term inhaled therapy, while nuclear atypia was not statistically significant. Poor housing conditions and positive family history were strongly associated with asthma prevalence. ***Conclusion:*** Chronic asthma is linked to marked cytological alterations in sputum and, to a lesser extent, buccal mucosa. These findings highlight the role of cytological screening as a simple and cost-effective tool for evaluating cellular changes in asthmatic patients.

Keywords: *Asthma, Cytology, Sputum, Buccal Mucosa, Nuclear Atypia, Shendi, Sudan*.

1. INTRODUCTION

Asthma is a long-term condition affecting both children and adults. It is characterized by narrowing of the airways in the lungs due to inflammation and tightening of the surrounding smooth muscles, leading to symptoms such as cough, wheezing, shortness of breath, and chest tightness. Based on the frequency and severity of symptoms, asthma is classified into four categories: mild intermittent, mild persistent, moderate persistent, and severe persistent [1]. Several clinical types of asthma have been described, including well-controlled and poorly controlled asthma, adult-onset asthma, allergic and non-allergic asthma, occupational and work-exacerbated asthma, nocturnal asthma, seasonal asthma, viral asthma, thunderstorm asthma, refractory or difficult-to-treat asthma, silent asthma, and chronic asthma as part of chronic obstructive pulmonary disease (COPD) overlap. Diagnosing asthma is preferably done through spirometry, assessment of allergic status, radiological examination, and evaluation of eosinophilic airway inflammation [2]. Management depends on its type and severity, and involves the use of short-acting relievers, long-acting bronchodilators, corticosteroids, and steroid-sparing agents. In addition to diagnosis and treatment, cytological examination of sputum plays an important role in detecting both malignant and non-malignant lung conditions such as pneumonia, tuberculosis, and other inflammatory diseases [3]. A variety of structural changes in the epithelium and airways, collectively called “airway remodeling,” are

often seen in asthma. These changes include epithelial hypertrophy, metaplasia, hyperplasia, and cell death, and have been linked to disease severity [4–8]. Chronic airway inflammation has been proposed as a potential factor connecting asthma with lung cancer development. Persistent inflammatory responses in the lungs may promote genetic alterations or interact with genetic predispositions such as polymorphisms, aiding carcinogenesis. Bronchial fibroblasts play a key role in this process. Experimental studies show that lung cancer cells exposed to signals from fibroblasts of asthmatic patients display greater motility compared to those exposed to fibroblasts from non-asthmatics. While these lab findings may not fully replicate in vivo conditions, they shed light on possible biological mechanisms underlying the link between asthma and lung cancer [9]. Epidemiological evidence supports this connection. A 2017 meta-analysis demonstrated that people with asthma are 44% more likely to develop lung cancer, with the association consistent across different ethnic groups, genders, and smoking statuses [10]. Likewise, a large 2019 cohort study found that asthma was linked to a 25% increased risk of lung cancer, though no connection was seen with breast or prostate cancers [11]. Another UK population-based study involving 1.2 million people reported that never-smokers with asthma severe enough to require treatment were 32% more likely to develop lung cancer [12]. Furthermore, a systematic study by the International Lung Cancer Consortium revealed that asthma was most strongly associated with squamous cell carcinoma (69% increase) and small cell carcinoma (71% increase), with a weaker link to adenocarcinoma (9% increase) [13]. Overall, these findings suggest that asthma is not only a chronic inflammatory airway disease but also a possible risk factor for lung cancer, likely driven by ongoing inflammation, airway remodeling, and fibroblast-related cellular interactions.

2. MATERIALS AND METHODS

2.1. Study Design

This was a descriptive cross-sectional study.

2.2. Study Area

The study was conducted in Shendi town, Sudan. Sputum and buccal samples were collected from patients with chronic asthma and transferred to the Histopathology and Cytology Laboratory at Shendi University for processing and examination.

2.3. Study Duration

The study was carried out over four months, from September to December 2021.

2.4. Study Population

The study population consisted of two groups: patients diagnosed with chronic asthma (case group) and apparently healthy individuals (control group).

2.5. Inclusion and Exclusion Criteria

All participants in the case group were confirmed chronic asthmatic patients, while the control group consisted of healthy individuals. Both groups were matched according to selected sociodemographic characteristics, except for the presence of asthma in the case group. Patients with pulmonary comorbidities such as tuberculosis, interstitial lung diseases, lung cancer, or other pulmonary infections were excluded from the study.

2.6. Study Samples

Both sputum and buccal smears were obtained from each participant to detect cytomorphological changes.

2.7. Sample Size

A total of 240 samples were collected, consisting of 120 sputum samples (60 cases and 60 controls) and 120 buccal samples (60 cases and 60 controls).

2.8. Data Collection Tool

Data were collected using structured questionnaire sheets to record sociodemographic information and sample details. All information was compiled into a master sheet for analysis.

2.9. Study Variables and Detection Methods

Cytomorphological changes were assessed using the Papanicolaou staining method. Analytical and sociodemographic data were processed using statistical methods.

2.10. Sample Collection and Processing

Fresh early morning sputum specimens were obtained by deep coughing and collected in sterile, disposable sputum containers. Each container was properly labeled and sealed to ensure sample integrity. The collected specimens were preserved in 50% ethanol and transported to the laboratory. In the laboratory, smears were prepared from the sputum and fixed in 95% ethanol for at least 15 minutes. Following fixation, the slides were stained using the Papanicolaou staining method. For buccal samples, each participant was instructed to rinse their mouth thoroughly with water to minimize contamination. The buccal mucosa was then gently scraped using a sterile disposable spatula. The collected material was immediately smeared onto pre-labeled, frosted-end microscopic glass slides before drying occurred. Each slide was fixed in 95% ethanol for at least 15 minutes and subsequently stained using the Papanicolaou staining method.

2.11 Papanicolaou Staining Technique

Each fixed smear was rehydrated sequentially in 90% ethanol, 70% ethanol, and distilled water for 2 minutes each. After rehydration, the slides were stained with Harris's hematoxylin for 5 minutes, followed by differentiation in 1% acid alcohol. The smears were then

blued under running tap water for 10 minutes and subsequently rinsed in 95% ethanol. Staining with Orange G6 was performed for 3 minutes, after which the slides were washed in 95% ethanol. Eosin Azure 50 was then applied for 2 minutes. The slides were dehydrated in absolute ethanol, cleared in xylene, and mounted in Distrene Plasticizer Xylene (DPX). Finally, the smears were examined under a light microscope by the researchers and independently confirmed by experienced cytologists [14].

2.12 Interpretation of Results

Cellular changes were identified based on established cytological criteria. Malignancy was indicated by the presence of primary features such as irregular chromatin patterns, chromatin strands of unequal size and shape, and condensation of large chromatin clumps along the nuclear border with an empty central area. Nuclear atypia was identified by features including hyperchromasia, increased chromatin content, an elevated nuclear-to-cytoplasmic ratio, multinucleation, irregular nuclear borders, abnormal mitotic figures, and the presence of enlarged nuclei with prominent nucleoli. Chronic inflammation was determined by the presence of chronic inflammatory cells, while keratotic changes were identified by parakeratosis and hyperkeratosis. Acute inflammatory changes were indicated by neutrophilia. Evidence of infection was determined by the identification of specific infectious agents or the presence of perinuclear halos [15].

2.14. Quality Control

Sterile disposable tools were used to collect all samples. Buccal smears were obtained directly and immediately fixed in 95% ethanol to avoid air-drying artifacts and to preserve nuclear details. Sputum samples were collected in sterile containers, preserved in 50% ethanol, and subsequently processed in the laboratory for smearing. All staining solutions were filtered and checked before use, and coplin jars and dishes were thoroughly cleaned before and after each procedure, then tightly sealed during work to prevent evaporation and contamination. Further precautions were taken to avoid contamination during mounting and coverslipping. The adequacy of sputum samples was confirmed by the presence of numerous pulmonary macrophages.

2.15. Data analysis and Presentation

Data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 2021. Means, frequencies, and percentages were calculated and results were presented in tables and figures. A p-value < 0.05 was considered statistically significant.

3. RESULTS

A total of 120 sputum and 120 buccal samples were examined, equally divided between 60 chronic asthmatic patients and 60 apparently healthy controls. The cytological findings revealed distinct alterations between the two groups. In sputum samples, the majority of asthmatic patients demonstrated significant cellular changes compared with controls. Nuclear atypia was observed in almost 41% of cases, while only 6% of controls showed this feature. Similarly, signs of inflammation and bacterial infection were markedly higher among cases compared to controls. In addition, cytoplasmic vacuolization and prenuclear halo were exclusively or predominantly observed in the asthmatic group. These findings are summarized in Table 1. Regarding buccal smears, cytological alterations were less prominent compared with sputum. Cellular changes were more frequent among patients with long disease duration and those using long-acting inhaled medications. However, nuclear atypia was not statistically significant between groups, suggesting that oral epithelial changes may be less directly influenced by asthma itself. In contrast, inflammation and infection were more common among asthmatic patients, particularly those on long-term therapy. Details of these findings are presented in Table 2. Environmental and hereditary factors were also evaluated. Housing conditions showed a clear association with asthma severity, as patients living in poorer conditions were more frequently affected (Table 3). Moreover, family history played a substantial role, with nearly three-quarters of asthmatic patients reporting a positive family history (Table 4).

Table 1: Cytological alterations in sputum samples among asthmatic patients and controls

Category	Variable	Case	Control	Total	P-value
Cellular changes	Present (Frequency)	51	3	54	0.000
	Present (Percentage)	42.5%	2.5%	45.0%	0.000
	Absent (Frequency)	9	57	66	0.000
	Absent (Percentage)	7.5%	47.5%	55%	0.000
Nuclear atypia	Present (Frequency)	49	7	56	0.000
	Present (Percentage)	40.8%	5.8%	46.6%	0.000
Inflammation	Present (Frequency)	31	28	59	0.000
Infection	Present (Frequency)	50	13	63	0.000
Cytoplasmic vacuolization	Present (Frequency)	11	0	11	0.000
Prenuclear halo	Present (Frequency)	17	1	18	0.000

Table 2: Cytological alterations in buccal samples among asthmatic patients

Category	Observation	Frequency (Long acting)	Frequency (Short acting)	Total	P-value
Cellular changes	Childhood onset	12	6	18	0.000
	>10 years duration	24	7	31	0.000
	<10 years duration	2	9	11	0.000
Nuclear atypia	Present (long-acting drugs)	37	1	38	0.741
Inflammation	Present	30	18	48	0.008
Infection	Present	31	13	44	0.002
Cytoplasmic vacuolization	Present	8	1	9	0.134
Prenuclear halo	Present	17	0	17	0.143

Table 3: Housing condition among asthmatic patients

Condition	Frequency	Percentage
Good	12	20%
Moderate	22	33%
Poor	26	47%
Total	60	100%

Table 4: Family history of asthma

Family history	Frequency	Percentage
Yes	43	72%
No	17	28%
Total	60	100%

4. DISCUSSION

This descriptive cross-sectional study was conducted from September to December 2021 in Shendi town, aiming to detect cytological changes in sputum and buccal smears among chronic asthmatic patients. A total of 120 samples were collected, including 60 from chronic asthmatic patients and 60 from apparently healthy individuals as controls, matched for sociodemographic characteristics. The age distribution showed that approximately two-thirds of asthmatic patients were between 41 and 60 years, which aligns with the fact that asthma commonly affects middle-aged and older adults. Regarding gender distribution, two-thirds of the patients were female, a finding supported by the American study of Ruchi Shah (2018), which reported that women have a higher prevalence of asthma than men, particularly due to hormonal influences at different life stages [16]. Occupational distribution revealed that the most common occupation among patients was employee, which may be related to local housing, working conditions, and family history. Cytological examination of sputum revealed important findings. Hemoptysis was absent in all patients, consistent with the understanding that hemoptysis is usually associated with tuberculosis and aspergillosis rather than asthma [17]. In contrast, purulent sputum was present in more than half of the patients, suggesting ongoing inflammation or bacterial infection. This observation agrees with Fedoseev *et al*., [18], who demonstrated that sputum cytograms in patients show various phenotypes, including eosinophilic, neutrophilic, epithelial, macrophagic, and mixed-cell types. A strong association was found between chronic asthma and cytological changes in sputum ($p < 0.05$). Long-term asthma and continuous medication use may reduce immunity and predispose patients to such changes. Nuclear atypia was also significantly associated with asthma ($p < 0.05$), supporting the findings of Ruchi Shah [16], who reported sexual dimorphism in asthma across different hormonal phases of life. Inflammation was common in asthmatic patients and strongly associated with the disease ($p < 0.05$), aligning with Gibson *et al*., [19], who described airway inflammation as a central pathological feature characterized by eosinophilic infiltration, epithelial damage, and mucus plugging. Infection in sputum was also significantly associated with asthma ($p < 0.05$), consistent with Saetta and Turato [20], who reported that asthma involves infiltration of CD4 T-lymphocytes, eosinophils, and mast cells. Additionally, cytoplasmic vacuolization and prenuclear halos were significantly linked to asthma ($p < 0.05$), reflecting nonspecific infection and inflammation [21]. Similar associations were observed in buccal smears. A strong relationship was identified between asthma and cytological changes in buccal cells ($p < 0.05$), likely due to prolonged use of inhaled medications. This finding matches the Indian study by Mohammed Ismail [22], which concluded that long-term inhalational therapy in asthma patients induces epithelial changes in oral mucosa. However, nuclear atypia in buccal cells did not show any significant association with asthma ($p > 0.05$), suggesting that asthma itself may not directly affect nuclear morphology in the oral cavity. Conversely, buccal inflammation was significantly linked to asthma and its medications ($p < 0.05$), in line with Mohammed Ismail [22]. Infection in buccal smears was also associated with asthma ($p < 0.05$), in agreement with Godara [23], who reported that inhaled drugs can predispose patients to oral health

complications such as xerostomia, candidiasis, dental caries, and mucosal ulcerations. Additionally, cytoplasmic vacuolization and prenuclear halos were strongly associated with buccal smears of asthmatic patients ($p < 0.05$), likely reflecting inflammation related to the disease and its treatment. Environmental and familial factors were also evident. Poor housing conditions were associated with a higher frequency of asthma, whereas improved housing was linked to a reduced incidence. This supports previous studies that identified poor housing—such as dampness, mold, pest infestation, and overcrowding—as risk factors for asthma [24]. Moreover, family history was strongly associated with asthma in this study, as nearly three-quarters of patients reported a positive family history. Literature confirms that family history is a major determinant of asthma risk and can be used to identify individuals at high risk [25]. Overall, the findings of this study demonstrate that chronic asthma is strongly associated with cytological changes in both sputum and buccal samples. These changes are influenced not only by the disease itself but also by long-term use of inhaled medications, environmental conditions, and genetic predisposition.

Limitations

This study is limited by its relatively small sample size and restriction to a single geographic area, which may affect the generalizability of the findings. In addition, only conventional cytological staining was used without molecular confirmation. Finally, the cross-sectional design prevents establishing causal relationships or long-term effects of asthma and inhaled medications on cellular changes.

5. CONCLUSION

Chronic asthma is associated with significant cytological alterations in sputum, including nuclear atypia, inflammation, infection, and cytoplasmic changes. Buccal smears showed fewer alterations, mainly related to long-term inhalation therapy. Cytology thus offers a simple and cost-effective tool for monitoring cellular changes in asthmatic patients.

Recommendations

Further studies are recommended with larger sample sizes and diverse populations to confirm and extend these findings. Future research should exclude confounding variables such as smoking and coexisting pulmonary diseases, while employing advanced molecular and immunohistochemical methods for more precise detection of cytological changes. Longitudinal studies are also encouraged to evaluate the long-term effects of asthma and inhaled therapies on cellular morphology in both sputum and buccal mucosa.

Ethical Considerations

The study was approved by the Department of Histopathology and Cytology, Faculty of Medical Laboratory Sciences, and the Faculty of Higher Studies and Scientific Research at Shendi University. Verbal informed consent was obtained from all participants after explaining the study objectives and benefits, with assurances of confidentiality.

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REFERENCES

1. World Health Organization. Asthma [Internet]. Geneva: WHO; 2021 [cited 2025 Sep 10]. Available from: https://www.who.int/news-room/fact-sheets/detail/asthma.
2. Ralston N, Penman B, Strachan S. Davidson's Principles and Practice of Medicine. 21st ed. Edinburgh: Churchill Livingstone; 2010. p. 662-5.
3. Healthwise Staff. Asthma [Internet]. Michigan Medicine, University of Michigan; 2020 [cited 2025 Sep 10]. Available from: https://www.uofmhealth.org.
4. Elias, Jack A., *et al.,* "Airway remodeling in asthma." The Journal of clinical investigation 104.8 (1999): 1001-1006.
5. Laitinen, Lauri A., *et al.,* "Damage of the airway epithelium and bronchial reactivity in patients with asthma." American Review of Respiratory Disease 131.4 (1985): 599-606.
6. Jeffery, P. K., *et al.,* "Bronchial biopsies in asthma." Am Rev Respir Dis 140 (1989): 1745-1753.
7. Barbato, Angelo, *et al.,* "Epithelial damage and angiogenesis in the airways of children with asthma." American journal of respiratory and critical care medicine 174.9 (2006): 975-981.
8. Holgate, Stephen T. "Epithelium dysfunction in asthma." Journal of Allergy and Clinical Immunology 120.6 (2007): 1233-1244.
9. Ryszawy, Damian, *et al.,* "Invasive bronchial fibroblasts derived from asthmatic patients activate lung cancer A549 cells in vitro." Oncology letters 16.5 (2018): 6582-6588.
10. Qu, Yan-Liang, *et al.,* "Asthma and the risk of lung cancer: a meta-analysis." Oncotarget 8.7 (2017): 11614.
11. Kantor, Elizabeth D., *et al.,* "Allergies and asthma in relation to cancer risk." Cancer Epidemiology, Biomarkers & Prevention 28.8 (2019): 1395-1403.
12. Pirie, Kirstin, *et al.,* "Lung cancer in never smokers in the UK Million Women Study." International journal of cancer 139.2 (2016): 347-354.

13. Rosenberger, Albert, *et al.,* "Asthma and lung cancer risk: a systematic investigation by the International Lung Cancer Consortium." Carcinogenesis 33.3 (2012): 587-597.
14. Bancroft JD, Gamble M, editors. Theory and practice of histological techniques. Elsevier health sciences; 2008.
15. Nga ME. Practical Diagnostic Approaches in Non-Gynaecologic Cytology. Springer Singapore; 2021.
16. Shah, Ruchi, and Dawn C. Newcomb. "Sex bias in asthma prevalence and pathogenesis." Frontiers in immunology 9 (2018): 2997.
17. Irfan M, Gnananandha D, Munavvar M. Unusual cause of hemoptysis in a patient with asthma. Eur Respir J. 2009;18:114.
18. Fedoseev, G. B., *et al.,* "Cytological phenotypes of spontaneous sputum in the assessment of the presence and type of bronchopulmonary inflammation in patients with asthma and chronic obstructive pulmonary disease." J Lung Pulm Respir Res 2.1 (2015): 10-5.
19. Gibson, P. G., *et al.,* "Cellular characteristics of sputum from patients with asthma and chronic bronchitis." Thorax 44.9 (1989): 693-699.
20. Dey, Surajit, *et al.,* "Pathogenesis, clinical features of asthma COPD overlap, and therapeutic modalities." American Journal of Physiology-Lung Cellular and Molecular Physiology 322.1 (2022): L64-L83.
21. Edmund S, Barbara S. Diagnostic Cytology: Principles and Clinical Correlates. 3rd ed. Philadelphia: Saunders; 2009. p. 67.
22. Mohammed IB, Harikrishnan P, Muthusamy R, *et al.,* Effect of inhalation therapy on buccal mucosal cells in asthmatic patients. Rambam Maimonides Med J. 2020;11:1-3.
23. Godara, Navneet, Ramya Godara, and Megha Khullar. "Impact of inhalation therapy on oral health." Lung India 28.4 (2011): 272-275.
24. Helms, Veronica E., Brian A. King, and Peter J. Ashley. "Cigarette smoking and adverse health outcomes among adults receiving federal housing assistance." Preventive medicine 99 (2017): 171-177.
25. Liu, Tiebin, *et al.,* "The association between family history of asthma and the prevalence of asthma among US adults: National Health and Nutrition Examination Survey, 1999–2004." Genetics in Medicine 11.5 (2009): 323-328.