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LUNG EPITHELIAL CELLULAR PROLIFERATIVE ACTIVITY ASSOCIATED WITH CONVENTIONAL GOLD MINING IN SUDAN

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ABSTRACT – ***Objective:*** Traditional Sudanese gold mining poses serious health challenges to workers and communities. This issue requires early detection of at-risk populations using affordable, non-invasive sputum cytology. Thus, the present study aimed to evaluate the proliferative activity of lung epithelial cells in Sudanese individuals associated with traditional gold mining.

Materials and Methods: This study is a cross-sectional, descriptive, clinic-based pilot investigation conducted in El-Obeid, North Kordofan State, Sudan. Participants in the study are individuals actively involved in traditional gold mining. The selection of candidates was based on their willingness to participate in the study, independent of demographic characteristics. Lung cytology was performed, and the results were categorized as normal, metaplasia, cytological atypia, and cellular degenerative changes.

Results: Squamous metaplasia was identified in 17 out of 109 individuals (15.6%). Mild cytological atypia was identified in 3.7% of individuals. All four smears with cytological atypia showed squamous metaplasia of the lung. The risk of cytological atypia among individuals with respiratory squamous metaplasia is represented by the Odds Ratio (OR) and the 95% confidence interval (95% CI), which are OR = 28.3 (2.9333 to 273.1792) and $p = 0.0038$.

Conclusions: Pulmonary squamous metaplasia was common and linked with cytological degeneration and inflammation. Mild cytological atypia was infrequent and only linked with respiratory metaplasia. A substantial link exists between inflammatory state, particularly CICI, and respiratory metaplasia (OR = 5.4; $p < 0.003$).

KEYWORDS: Traditional mining, Lung, Squamous metaplasia, Cytological atypia, Sudan.

INTRODUCTION

Conventional gold mining contaminates the environment with mercury and silica dust. Silica dust can induce respiratory complications, and mercury can adversely affect the kidneys and central nervous system. Combined exposure can elicit these effects and synergistic harm[1]. The World Health Organization (WHO) and the International Labour Organization (ILO) are collaborating to produce joint estimates regarding the work-related burden of disease and injury, known as the WHO/ILO Joint Estimates, with

input from numerous individual experts. Research involving human, animal, and mechanistic studies indicates that exposure to dusts and/or fibers, such as silica, asbestos, and coal dust, is a causative factor in pneumoconiosis[2].

Inhaled mineral fibers like asbestos and erionite are harmful due to their crystal-chemical constitution, size, and biodurability. Alveolar macrophages try to ingest and remove mineral fibers from the deep respiratory tract. Some fibers, such as asbestos, are too lengthy for macrophages to ingest, leading to "frustrated phagocytosis," and macrophages continue to leak toxic substances. Three known carcinogenic mineral fibers, crocidolite, chrysotile, and fibrous-asbestiform erionite, are investigated in the upper respiratory tract, deep respiratory tract, and pleural cavity. This grouping of chain silicates (amphiboles), layer silicates (serpentine), and framework silicates (zeolites) includes the most socially and economically significant mineral fibers. The crystal-chemical compositions and qualities of these fibers are used to compare their behavior. From a mineralogical standpoint, especially in light of recent research in this multidisciplinary subject, the known biological and pathological factors that cause cancer are rigorously examined. The causal relationships between lung cancer and malignant mesothelioma are stressed. The literature contains numerous uncertainties, data gaps, and controversies, particularly about the relative potencies of the three mineral fibers for lung cancer and mesothelioma[3].

Cytology is continually applied to the assessment of lung lesions[4]. Cytology is essential for the preliminary assessment and diagnosis of lung cancer in patients. Multiple sampling techniques exist for the acquisition of specimens intended for cytologic assessment of lung tumors. Methods include exfoliative techniques such as induced sputum and thoracentesis; abrasive cytology methods, including bronchoalveolar lavage, bronchial brushing, and bronchial washing; as well as fine needle aspiration (FNA) cytology, which encompasses endobronchial ultrasound-guided, transesophageal, and CT-guided percutaneous/transthoracic approaches[5,6].

Many Sudanese have turned to traditional gold mining to survive the war, which poses serious health and safety dangers[7]. Many miners lack safety training and equipment like helmets, gloves, and protective clothing. Miners often handle mercury and other toxic substances without protection, causing respiratory and neurological difficulties. Traditional gold mining in Sudan needs immediate attention to ensure workers' health and safety[8]. Training, resources, and other economic options must be prioritized to sustain affected communities. Consequently, the current study sought to evaluate the proliferative activity of lung epithelial cells associated with traditional gold mining among Sudanese individuals.

MATERIALS AND METHODS

This study is a cross-sectional, descriptive, clinic-based pilot investigation conducted in El-Obeid, North Kordofan State, Sudan. Participants in the study consist of individuals actively involved in traditional gold mining activities. The selection of candidates was based on their willingness to participate in the study, independent of demographic characteristics.

Criteria for inclusion and exclusion

Applicants were required to possess a minimum of one year of experience in underground gold mining. Candidates with a pre-existing chronic comorbidity, smokers, and cement workers were excluded from consideration for employment in gold mining. Individuals with lung-relevant clinical histories and those who utilized personal protective equipment were excluded.

Sample size and study metrics

All individuals who presented at our healthcare clinics (during the period from January 2025 to January 2026) with respiratory complaints necessitating sputum cytology for diagnostic purposes were included. The research used a focused questionnaire to gather patient data, supplemented by lung cytology to assess changes in the lung epithelium. The variables related to the duration of work and the frequency of intermittent work per year were included only for 75 participants, as they were not considered from the beginning.

Lung cytology was performed, and the results were categorized as normal, metaplasia, cytological atypia, and cellular degenerative changes. Cytology interpretation was performed by a specialized cytopathologist in collaboration with a consultant chest physician.

Statistical analysis

The data were first recorded in a data sheet and then imported into IBM SPSS Statistics for Windows, Version 29 (released 2023; IBM Corp., Armonk, NY, USA). Calculations were performed for percentages, frequencies, and tabulations. Given the relatively small sample size, we applied Fisher's Exact test to calculate the exact probability of observing the data under the null hypothesis, utilizing a hypergeometric distribution. To avoid issues with zero cells during Fisher's exact test, 0.5 was added to all cells in the Table. The odds ratio (OR) is used to measure the association between the exposure and the outcomes. We used the 95% confidence interval (95% CI) to provide a range of values likely to contain the true OR, reflecting the precision of the OR estimate. A *p*-value of less than 0.05 was deemed statistically

significant. Regarding missing variables, we analyzed only the cases with no missing values.

RESULTS

This study investigated 109 traditional mining workers, aged 20 to 62 years, with a mean ± SD of 32 ± 8.984. Most participants were aged 25-29 years, followed by ≤25 years and ≥40 years, representing 37/109 (34%), 23/109 (21%), and 20/109 (18.3%), respectively. Most participants worked in traditional gold mining for 6-11 years, followed by ≥11 and ≤5 years, constituting 42/109 (38.5%), 38/109 (34.9%), and 29/109 (26.6%), in that order, as indicated in Table I and Figure 1.

Cellular degenerative changes were identified in 10/109 (8.3%) individuals; they included 9/10 (90%) pyknosis and 1/10 (10%) karyolysis. The impact of cellular degenerative changes among those with respiratory metaplasia; the OR (95% CI) = 2.6 (0.6007 -11.2708), p = 0.20, z-statistic = 1.279.

Table I. Distribution of the study subjects by duration of gold mining by age.

Age	≤5 years	6-11	≥11	Total
≤25 years	6	15	2	23
25-29	5	17	15	37
30-34	5	5	8	18
35-39	4	2	5	11
≥40	9	3	8	20
Total	29	42	38	109

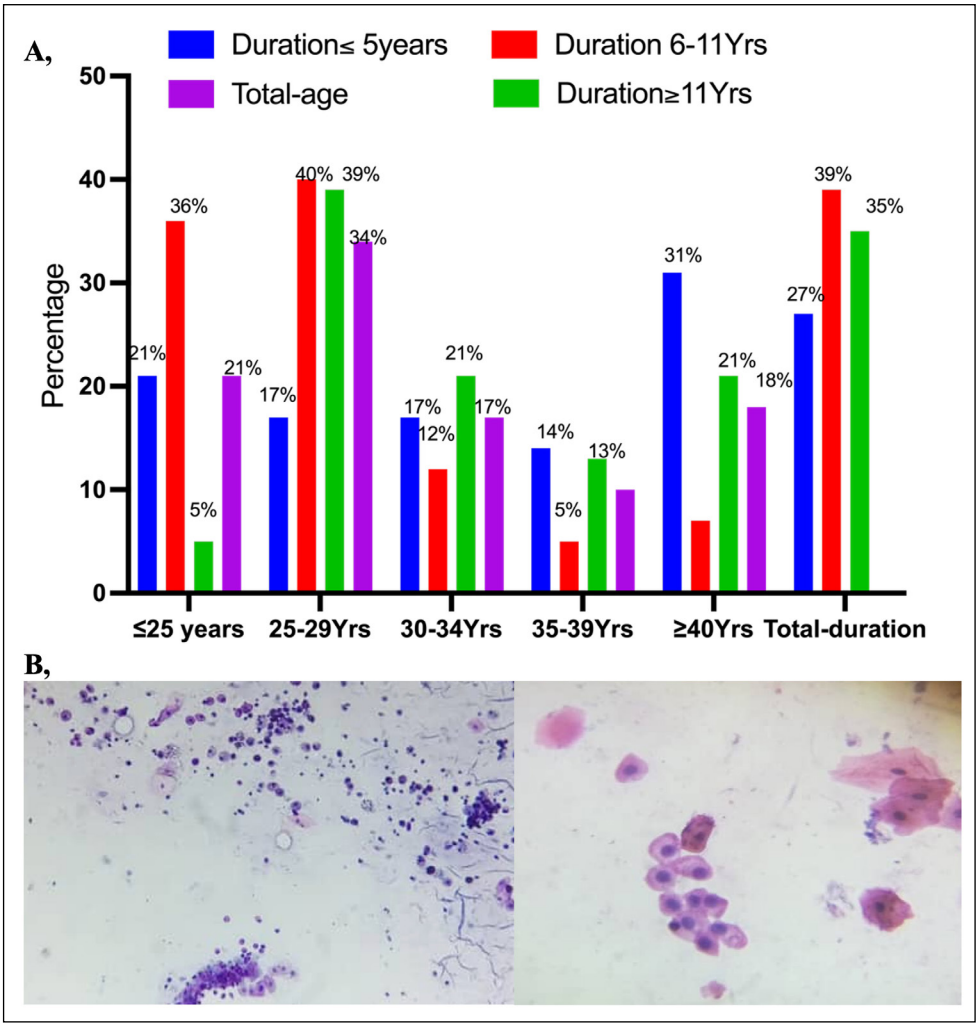

Figure 1. **A**, Description of the study subjects by duration of gold mining by age. Squamous metaplasia was identified in 17 out of 109 individuals (15.6%). Mild cytological atypia was identified in 4/109 (3.7%) individuals. All four smears with cytological atypia showed respiratory metaplasia. **B**, Sputum cytology showing lung squamous metaplasia with mild cytological atypia and pyknosis (400×).

The duration of exposure to mining dust was available for 75 participants, of whom 8/75(10.7%) had metaplasia. Most cases were seen for a duration of ≤5 years, 5/8 (62.5%). The association of respiratory metaplasia with a duration of ≤5 years was OR (95% CI) = 1.3369 (0.4060 - 4.4024), p = 0.633, and z-statistic = 0.478.

The interval of intermittent work in mining per year was available for 75 participants. Most participants have intermittent intervals of ≤5 times/year, 50/75 (66.7%), of whom 5/50 (10%) were found to have respiratory metaplasia. The observed link between respiratory metaplasia and intermittent intervals of ≤5 times per year is represented by an odds ratio (OR) of 0.8148 (95% CI = 0.1783-3.7236), with a p-value of 0.7917 and a z-statistic of 0.264, as indicated in Table II and Figure 2.

Table II. Distribution of the study population by cellular proliferative activity.

Variable	No Metaplasia	Metaplasia	Total
Cytological Atypia			
None	92	13	105
Mild	0	4	4
Total	92	17	109
Degenerative changes			
None	85	14	99
Pyknosis	6	3	9
Karyolysis	1	0	1
Total	91	17	109
Duration of work			
≤5 years	17	5	22
6-10	22	2	24
≥11	28	1	29
Total	67	8	75
Interval of intermittent per year			
≤5 times/year	45	5	50
6-10	18	2	20
≥11	4	1	5
Total	67	8	75

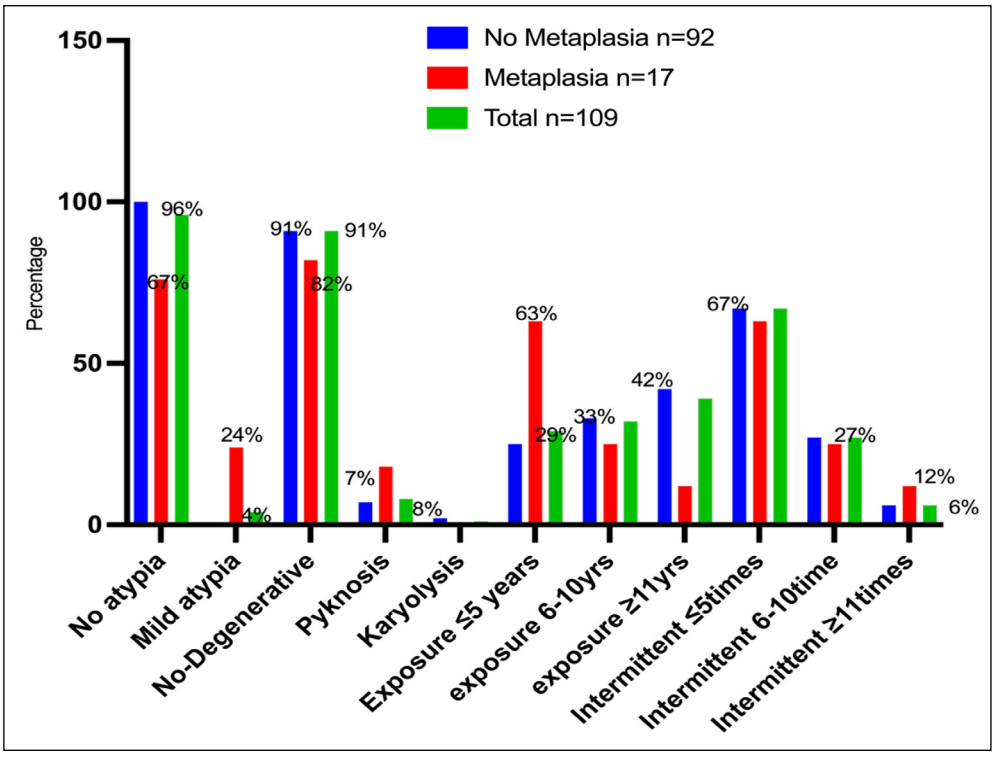

Figure 2. Description of the study population by cellular proliferative activity.

Acute inflammatory cells infiltrating (AICI) were identified in 48/109 (44%) (31 with mild AICI and 17 with moderate AICI). About 10/48 (20.8%) of the cases of metaplasia were found with AICI. The association of AICI with respiratory metaplasia was OR (95% CI) = 2.0301 (0.7095-5.8087), p = 0.1868, z-statistic = 1.32. Three out of 4 cases with cytological atypia were found with AICI. About 5/5 (50%) of those with cellular degenerative changes were found with AICI, as summarized in Table III and Figure 3.

Table III. Distribution of the study population by cellular proliferative activity and acute inflammatory cell infiltrate.

Variable	No-AICI	Mild- AICI	Moderate- AICI	Total
Metaplasia				
No	54	26	12	92
Yes	7	5	5	17
Total	61	31	17	109
Cytological atypia				
No	60	29	16	105
Yes	1	2	1	4
Total	61	31	17	109
Cellular degenerative changes				
No	55	31	12	98
Pyknosis	5	1	4	9
Karyolysis	0	0	1	1
Total	60	32	17	109

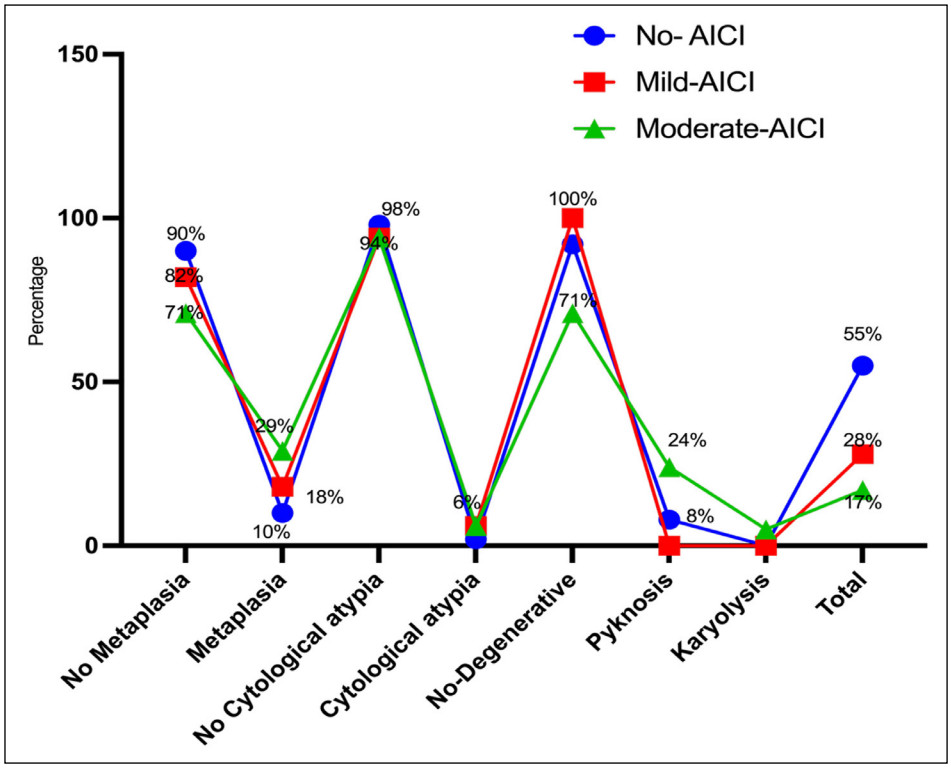

Figure 3. Description of the study population by cellular proliferative activity and acute inflammatory cell infiltrate.

Chronic inflammatory cell infiltration (CICI) was seen in 40/109 (36.7%) study subjects, of whom 12/40 (30%) were found with respiratory metaplasia. The association between CICI and respiratory metaplasia was OR (95% CI) = 5.4857 (1.7652-17.0479), p < 0.003, z-statistic = 2.942. Three out of 4 cases of cytological atypia were found with CICI. About 6/10 (60%) of the cases of cellular degenerative changes were found with CICI. The link between cellular degenerative changes and CICI was OR (95% CI) = 2.8676 (0.7573-10.8584), p = 0.1209, and z-statistic = 1.551, as summarized in Table IV and Figure 4.

Table IV. Distribution of the study population by cellular proliferative activity and CICI.

Variable	None	Mild	Moderate	Total
Metaplasia				
No	64	22	6	92
Yes	5	8	4	17
Total	69	30	10	109
Cytological Atypia				
No	68	28	9	105
Yes	1	2	1	4
Total	69	30	10	109
Cellular degenerative changes				
No	65	26	8	99
Pyknosis	3	4	2	9
Karyolysis	1	0	0	1
Total	69	30	10	109

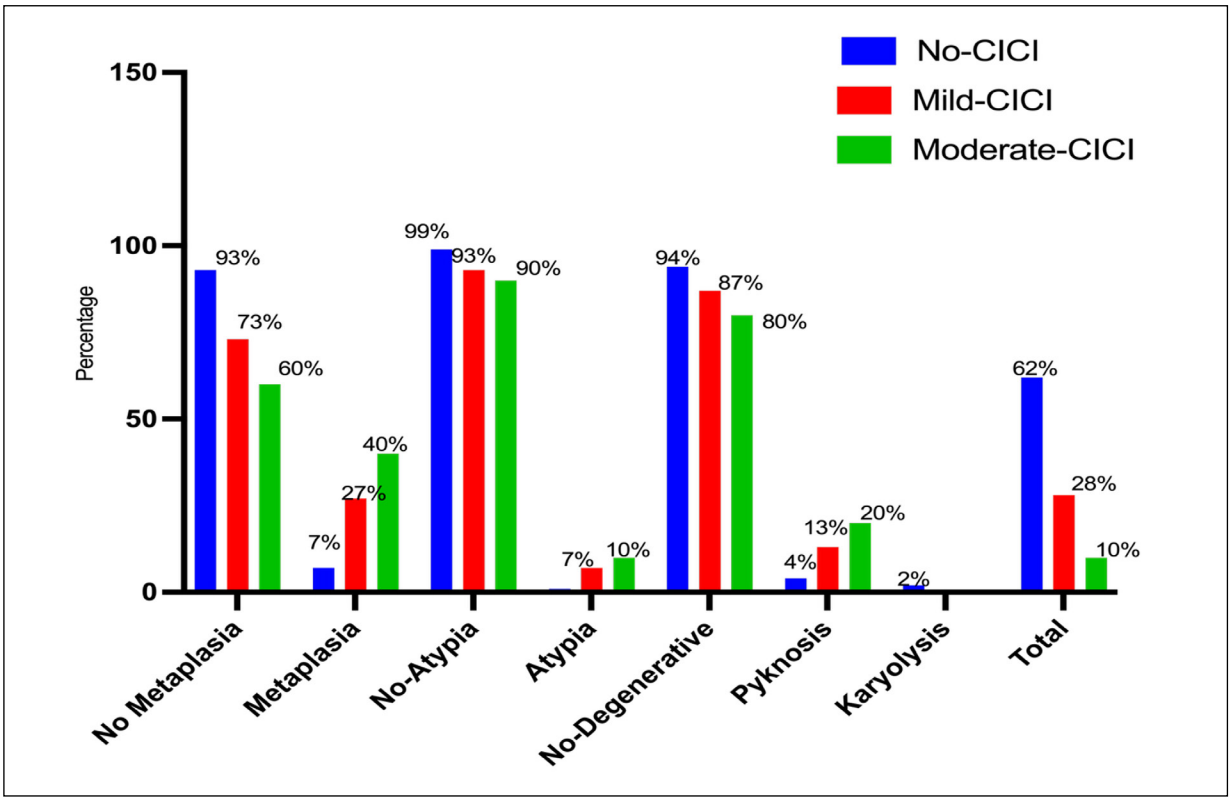

Figure 4. Description of the study population by cellular proliferative activity and chronic inflammatory cell infiltrate.

DISCUSSION

Traditional mining, particularly gold mining, can cause severe lung epithelial alterations because of multiple environmental and occupational exposures. Traditional mining harms the lung epithelium, resulting in both acute and chronic respiratory problems. Intervention strategies are essential for enhancing the working environment and early detecting pulmonary alterations to mitigate health hazards. Therefore, the present study aimed to assess the utility of sputum cytology as a screening approach for early detection of the lung epithelium changes observed due to harmful particles during traditional gold mining without safety measures.

The findings of the present pilot study revealed that over 15% of the participants had respirato-

ry metaplasia. Squamous cell metaplasia (SQM) is a frequent epithelial alteration of the human tracheobronchial mucosa. Initially, during SQM, quiescent basal cells within the pseudostratified epithelium re-enter the cell cycle and become hyperproliferative due to exposure to toxic injury. During the next stage of the process, the epithelium begins to express markers of a squamous phenotype rather than those of the normal pseudostratified epithelium[9]. Chronic lung injury generates metaplasia, which occasionally, but ominously, progresses to squamous dysplasia and squamous lung cancer[10].

The findings of the current investigation showed that all cases with cytological atypia were among the cases of lung SQM. Tissue injury induces a pro-tumorigenic Stress/Tension-Instructive Fibroblast (STIF) state across various organs, which precedes and persists during cancer development. STIF signaling, operating exclusively through fibroblasts rather than epithelial cells, is adequate to trigger embryonic-like plasticity and provoke epithelial pre-cancerous metaplastic lesions. STIFs program pre-malignant phenotypes in neighboring epithelial cells *via* mechanosignaling by activating YAP before tumor emergence. Inhibition of STIFs or mechanosignaling halts or reverses metaplasia and obstructs progression to dysplasia[10].

Respirable crystalline silica (RCS) is associated with the development of lung cancer. However, there is uncertainty around the exposure threshold at which exposure to RCS may pose a clear risk for the development of lung cancer[11]. The International Agency for Research on Cancer (IARC) has recently stated that crystalline silica should be regarded as a potential carcinogen. The IARC bases this statement on a finding that there is limited evidence of carcinogenicity in humans and sufficient evidence for carcinogenicity in animals. Recent laboratory animal experiments demonstrating a carcinogenic response to silica exposure have intensified scientific and regulatory concern for crystalline silica as a respiratory carcinogen. Studies of human populations have been contradictory in demonstrating a causal relationship between crystalline silica exposure and lung cancer[12-14].

In this study, cellular degenerative changes were observed in around 10% of the persons, of whom 90% were observed with pyknosis. Cellular degenerative changes represent the deterioration of cell structure and function, often as a reversible, nonlethal response to injuries like ischemia, toxicity, or chronic disease. Silica, particularly in its crystalline form, is a well-known inducer of severe cellular damage and degenerative changes, most notably in macrophages, which leads to chronic inflammation and fibrosis (silicosis). Exposure to silica, including silica nanoparticles (SiNPs), triggers a cascade of cellular mechanisms resulting in damage, degeneration, and death[15].

Sputum cytology is used in screening programs for individuals exposed to mining dust, primarily to identify early, premalignant cellular changes (atypia) or to detect asbestos bodies in the respiratory tract. It serves as a non-invasive tool, particularly in high-risk populations like miners (e.g., uranium or asbestos miners), to detect centrally located squamous cell lung cancers that may not appear on a chest X-ray[16].

Sputum cytology is a diagnostic method known for its excellent specificity, ranging from 95% to 99%. However, it exhibits low sensitivity, between 40% and 70%. While it is effective at identifying severe atypia, which indicates malignant transformation, its capability to detect and assess squamous metaplasia and mild-to-moderate cytologic atypia requires multiple specimens and expert interpretation[17,18].

Overall, the findings of this study could be utilized to enhance worker protection by instituting regular health checks for traditional miners to assess respiratory health, emphasizing the early identification of metaplasia and other pulmonary disorders. Furthermore, the implementation of cytological screening programs could facilitate the early detection of cellular alterations, particularly among individuals exhibiting symptoms of chronic inflammation. Educational initiatives aimed at increasing awareness of the hazards of dust exposure and the importance of promptly reporting respiratory symptoms should also be promoted. In addition, access to appropriate personal protective equipment (PPE), including high-quality respirators or masks capable of filtering hazardous particles, should be ensured, together with measures to encourage its consistent use.

Limitations

The limitations of this study include: a cross-sectional design that may not prove causality or temporal development from inflammation to metaplasia to atypia. Limited precision due to limited sample size: 4 atypia instances and 10 cellular degeneration cases resulted in wide/unstable CIs and lowered power to detect real connections (e.g., dust duration, intermittent frequency). Limited generalizability due to missing exposure data since duration and intermittent interval were only provided for 75 subjects. Measurement of inflammation relies on cytology, with AICI/CICI inferred from smear results. Potential confounding factors that were not addressed include occupational hygiene and coexisting respiratory disorders.

CONCLUSIONS

In conclusion, among 109 traditional mining workers, respiratory squamous metaplasia was prevalent and associated with cytological degenerative changes and inflammation. Mild cytological atypia was rare and was solely associated with respiratory metaplasia, suggesting advancement along a metaplastic, dysplastic continuum. Cellular degenerative changes were primarily characterized by pyknosis and were seen in conjunction with inflammatory activity.

Most estimates related to the work environment, such as the duration of mining dust exposure and the frequency of intermittent work, reveal no statistically significant association with the risk of metaplasia. In contrast, the inflammatory status, especially CICI, demonstrated a statistically significant correlation with respiratory metaplasia (odds ratio 5.4; $p < 0.003$). Overall, these findings suggest that chronic inflammation is a critical factor associated with respiratory metaplasia in this mining population, while acute inflammation and cellular degeneration may serve as concurrent indicators of cellular stress.

Conflict of Interest
The authors declare no conflict of interest.

Data Availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' Contributions
Amal Khalil Y. Mohamm: conceptualization, writing-original draft, and writing-review and editing. Awad Eljeed Abugooda Alobaid: conceptualization, data collection, and analysis. Eldisugi Hassan M. Humida: writing-review, editing, and critical revision. Ahmed Mirghani O. Ali: conceptualization, writing, review, and data collection. Namarig Alhadi Hamid: conceptualization, writing, review, editing, and administration. Masaged Adam Edrees Mohammed: conceptualization, laboratory investigations, data collection, and approval. Hussain G. Ahmed: conceptualization, editing, and supervision.

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Ethics Approval
The study received ethical approval from the Institutional Research Review Board of the Prof Medical Research Consultancy Center (Prof MRCC) on 5 January 2025 – Approval No. HREC 0021/MRCC.1/25. All study procedures were performed in accordance with the ethical principles outlined in the Declaration of Helsinki and its subsequent amendments.

Informed Consent
The participants' personal information was maintained in strict confidentiality. The data collected from participants were exclusively shared in an anonymous format with the research team. Before enrolment, each participant needed to provide consent for participation in this study.

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