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Original Article

Cytological assessment of sputum smear and total blood count of workers in wood and timber industries

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ABSTRACT

Background and aim: Inhalation of airborne dust at the wood/timber workplace puts the worker at risk of occupational disease. This study was aimed at comparing selected staining methods for the histological assessment of sputum smear from wood and timber workers.

Methodology: Fifty timber workers, 25 males and 25 females of eighteen (18) years and above from Abakaliki timber industries were recruited for this study. Four civil servants who do not live close to the wood industries were used as control. Blood and sputum samples were collected from the subjects. Giemsa stain and Papanicolaou stain were used for the histological analysis.

Results: Out of 50 participants, 26 of them had normal range of PCV value, 7 have abnormally high range of the PCV and 17 of the workers had abnormally low range of PCV. Forty-four workers had normal range of neutrophils, 6 of the workers had abnormal level of neutrophils of which 3 were within higher range and 3 of lower range. Normal lymphocytes level was seen in 15 participants while abnormal level was seen in 35 subjects, of which twenty (20) are high and fifteen (15) low. Eosinophils level of 18 participants were found to fall within the normal range while 32 were seen to be abnormally low. There are observable histological changes in the test groups when compared with the control group in both staining techniques. Giemsa stain tends to render the cell nuclei thinner and more spread-out than the Papanicolaou stain.

Conclusion: The findings suggest that exposure to wood dust especially for a prolonged period of time is highly detrimental to human health.

Keywords:
Sputum; Wood workers; Wood dust; Cytological analysis

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INTRODUCTION

Inhalation of airborne dust at the wood/timber workplace puts the worker at risk of occupational disease. In developed and developing countries, overexposure to dust causes diseases, temporary and permanent disabilities and even deaths. Airborne microflora from secondary infection of the wood with moulds is also implicated as part of the respiratory hazard (Agu *et al*., 2016). Wood or timber industries are mechanical wood industries which produce sawn wood in dusty processes. Wood and timber workers are exposed to a variety of hazards notable among which is wood dust – a combustible organic vegetable dust which is a cause of particulate air pollution (Agu *et al*., 2016). The association between occupational exposure to wood dust in sawmill workers and other (Mohan *et al*., 2013) woodworkers and impaired lung function, chronic bronchitis, occupational asthma, external allergic alveolitis, nasal cancer, increased risk of lung cancer in both developed and developing countries have been shown (Mohan *et al*., 2013; Vallieres *et al*., 2015).

Mucus is the fluid secreted by the airways (also known as bronchial and windpipes) and lungs. In the setting of an infection or a longstanding health condition, the term of phlegm is also used. The mixture of saliva and mucus specifically coughed up from the respiratory tract, often either following an infection or an irritation of the mucosa, is precisely called sputum. Sputum product of the respiratory tract is the result of interaction between mucocilliary apparatus and the immune system of host and between the animate and inanimate invaders from the environment. It is the most frequently examined specimen from the respiratory tract (Bibbo and Wilbur 2018). Sputum is composed predominantly of mucoid substances as well as variable numbers of inflammatory and epithelial cells and is the most common specimen for pulmonary cytology (Matee *et al*., 2018; Shema *et*

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al., 2020).

Variation in the number of macrophages, neutrophils and epithelial cells can yield significant insight into underlying pathologic process. The sputum specimens stained by Papanicolaou technique and other special stains can provide a rapid preliminary diagnosis which will enable the clinician to decide on the therapy before definitive diagnosis is provided by more sensitive methods which include culture and polymerase chain reaction (PCR) (Matee *et al*., 2018; Shema *et al*., 2020). Sputum histology is rapid, accurate and minimally invasive method which can be used to diagnose opportunistic infections and cellular changes in the pulmonary system prior to confirmation by microbiological techniques (Shema *et al*., 2020).

The Giemsa stain was adapted to histology due to its unique staining of chromatin, nuclear membranes, and cytoplasmic elements. The color of the various cellular components is influenced by pretreatment of the specimen material. Clinical histological material like sputum smears from fine needle aspiration biopsies (FNAB), rinses, and touch preps are also used as starting material for the Giemsa stain (Weinstein, 2014).

The understanding of the mechanisms of diseases and their correct diagnosis has been made possible by analysis of body fluids such as sputum in several areas of clinical study. In the past, sputum analysis was considered not to be reliable or reproducible enough to assist in the understanding of the mechanisms of respiratory diseases. More recently, significant advances related to the processing of sputum samples using Giemsa stain have allowed the method of sputum examination to become feasible, reproducible, valid, and responsive to interventions. Several researchers have used this method to study the various aspects of airway inflammation in asthma. The use of sputum examination has been further extended to chronic obstructive pulmonary disease (COPD), cystic fibrosis, chronic cough, idiopathic pulmonary fibrosis, and other respiratory diseases.

Pulmonary infections affecting wood and timber workers have been associated with high rate of mortality in previous years. This study will improve the histological diagnosis based on non-culture technique, which takes minimal time, more sensitive, and cost effective requiring essentially the microscope, which is more affordable to the average laboratory.

The aim of this study is to histologically assess the sputum smear and total blood count from wood/timber workers. The result of the study would inform assessment of health risk exposures of dust from timber industry in the life of the workers. This could also possibly serve as evidence-based study that may be useful in health, environment and safety policy formulation towards occupation safety and environmental protection.

MATERIALS AND METHODS

Study Design

The study was designed to cytologically analyze sputum and blood sample from humans to evaluate the effect of exposure to wood dust on the respiratory system of workers from timber industries. Ethical approval was gotten from the Research and Ethics Committee of David Umahi Federal University of Health Sciences with the code DUFUHS/1704/02/001.

Study Area

The study area was solely based in the timber industries in Abakaliki metropolis where there are timber workers exposed to the timber/wood dust. Fifty (50) subjects who have worked in the industry for more than ten years were selected for the research work while 4 civil servants who do not live near the timber industry were selected as control.

Sputum collection

Fifty workers, 18 years and above from timber industries in Abakaliki metropolis were recruited. They were recruited using questionnaire to ascertain if they have previously worked in any other industry and to document their medical history so as to help us confirm that they do not have a previous medical condition. Their sex, age, and nationality were documented. The consent of the subjects was obtained before collection of their sputum. The sputum was collected first thing in the morning. First, the mouth was rinsed with water to remove debris, deep breathing exercise was done to help loosen mucus. A sterile sputum collection container was opened and cough forcefully to expel sputum into the container. A sufficient amount was collected (usually a teaspoon or more). The container lid was closed tightly and labelled. The specimen was then stored in a refrigerator until transport to the laboratory.

Blood Collection

The timber workers were told to relax and expose one arm, and tourniquet was placed tight around their cubital fossa at the upper limb. The median cubital vein that appears was cleaned with alcohol pad. Needle (with syringe) was inserted through the skin to access the vein. Blood was seen coming through the hollow needle into a syringe, tourniquet was removed, gentle pressure was held on the venipuncture site, drew 5ml of blood and dispose into EDTA container.

Haematological Analysis

The Automated Hematological Analyzer (2800 Haematology Auto-Analyzer) (Ode *et al*., 2017) was used to analyze blood samples.

Cytological Procedure

Sputum Collection

Early morning sputum was collected from each subject by coughing into sterilized container that was given to the individuals. Each individual was asked to rinse out their mouth with water or a saline solution. This was to help to remove food particles from their mouth after which the subject was asked to breathe in deeply and cough out sputum into the sterilized container.

Preparation of sputum collected

A drop of fresh sputum sample was placed on a grease free slide using pasture pipette. Then a smear was made on a slide and fixed immediately in 95% ethanol for 30 minutes after which it was arranged in a staining rack and stained with Papanicolaou stain (Demay, 1996).

Cytological Techniques for Sputum Analysis

The sputum smear was treated with 95% alcohol for 1 minute, 70% alcohol for 1 minute and rinsed with distilled water for 3 minutes. It was primarily stained in Harris haematoxylin for 3 minutes and rinsed in water for 1 minute. Afterwards, it was treated with 1% acid alcohol for 3 seconds, rinsed in water and blue in tap water for 2 minutes. This was followed by treatment with 70% alcohol for 2 minutes, 95% alcohol for 2 minutes, and counterstained in O. G 6 solution for 2minutes. The sample again was treated with 95% alcohol for 2 minutes, and further counterstained again in E.A 50 solution for 3 minutes, with 95% alcohol for 2 minutes, with absolute alcohol for 1min, and then with xylene for 2minutes and mount as desired (Gill, 1976).

Microscopic Study

The slides collected were viewed through a microscope and photomicrographs of the cells in the sputum were taken and interpreted.

Preparation of stain

Pap Stain

Solutions required include Harris Haematoxylin without acetic acid**,** 20ml of 0.5% orange G6 in 95% alcohol, 30mg of Phosphotungstic acid**,** 90ml of 0.5% light green SF yellowish in 95% alcohol, 20ml of 0.5% Bismark brown in 95% alcohol, 90ml of 0.5% eosin Y in 95% alcohol, 0.4g of Phosphotungstic acid, 2 drops of saturated aqueous lithium carstine were mixed together.

Giemsa Stain

Giemsa (dry powder) was prepared by adding 1.2g of dried powder of Giemsa stain to 100ml of methanol, it was mixed and the powder was dissolved, 50ml of glycerin was added, the solution was stored in brown bottle after filtering and was ready to be used before 1 ml stock solution was diluted with 9ml phosphate buffer.

Leishman Stain

Combine 30ml of Leishman solution with 150ml of distilled water and with 20ml of pH 6.8 buffer solution.

Staining Method/Staining procedure

Pap Stain

The smear of the sputum was fixed while still wet in 95% alcohol for 30 minutes and rinsed in water. The smear was stained in Harris hematoxylin for 5 minutes and rinsed in water after which it was differentiated in 1% acid alcohol for 15 seconds and blued in tap water for 5 minutes. It was then scot in tap water for a minute and rinsed in 70% alcohol, 95% alcohol. It was further stained in OG6 for 2 minutes and rinsed in 95% alcohol for 3 changes after which it was again stained in EA36 for 2 minutes, rinsed in 95% alcohol for 3 changes, rinsed in absolute alcohol for 2 changes and finally clear the smear in xylene and mount in DPX (Gill, 1976).

Giemsa Stain

As described by Mokobi, 2022, the sputum smear was air dried, fix in 95% methanol for 30 minutes. It was diluted in 1ml of Giemsa stain with 9ml phosphate buffer of pH 6.8 and poured on the smear for 10min after which it was differentiated in phosphate buffer of pH 6.8 briefly and air dried.

Leishman Stain

According to the procedure described by Muhibi et al., 2019, prepared sputum smear was allowed to dry, Leishman solution was dropped on the fixed blood smear and allowed to react for a minute. Buffered water was added, gently stirred and allowed to react for 5mins. The smear was rinsed using the pH 6.8 phosphate buffer solution and finally dried the preparation.

RESULTS

Hematological result from timber workers.

The table below showed the normal and abnormal (high and low) hematological parameters collected from timber workers. The normal percentage range of PCV in males is between 38.3% and 48.6% while that of females is between 35.5% and 44.9%. It was therefore observed that out of 50 participants, 26 of them had normal range of PCV value while 7 have abnormally high range of the PCV and 17 of the workers had abnormally low range of PCV. In the neutrophil, the normal range in a healthy adult is between 2,500 and 7,000 neutrophils per microliter of blood. Forty-four workers had normal range of neutrophils while 6 of the workers had abnormal level of neutrophils of which 3 were within higher range and 3 were of lower range. For the lymphocytes, the normal range of lymphocytes for both males and females is between 1,000 and 4,800 lymphocytes per microliter of blood in adults. It was seen in the result that normal lymphocytes level was seen in 15 participants while abnormal level was seen in 35 subjects, of which twenty (20) are high and fifteen (15) low. The normal range of monocyte count for healthy adults is between 2% and 8% of white blood cells or 200 to 800 monocytes per microliter of blood. The observed abnormal monocyte level was found in zero participants. A normal range for eosinophils in both males and females is typically between 30 and 350 cells per microliter of blood. The observed eosinophils level of 18 participants were found to fall within the normal range while 32 were seen to be abnormally low.

Table 1. Hematological result of sputum from timber/wood workers

Parameters	Normal Range	Abnormal Range	
		High level	Low level
PCV	26	7	17
Neutrophils	44	3	3
Lymphocytes	15	20	15
Monocytes	50	0	0
Eosinophils	18	0	32

Photomicrographs of Sputum samples

The photomicrographs of sputum samples stained with Giemsa stain reveal the cytoplasm appearing irregular and in clusters, some has the cells clumped together and has presence of granular debris. Some cytoplasm appears feathery and ill-defined. The samples stained with Papanicolaou stain reveal granuloma formation with multinucleated giant cells across the samples.

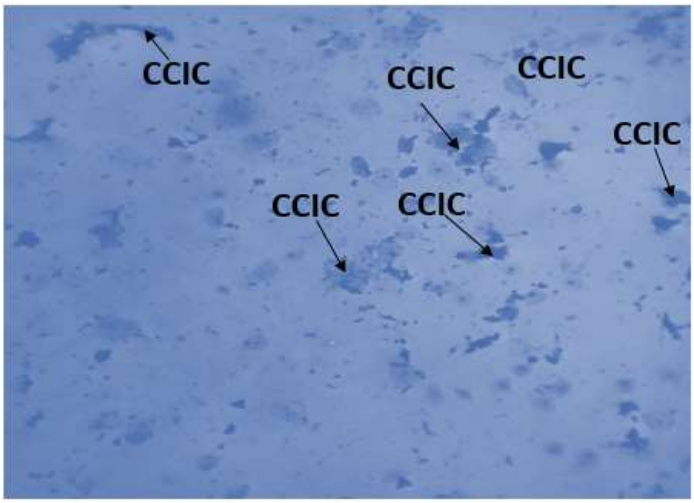

Plate 1: Photomicrograph of control sample shows cohesive clusters of irregular cytoplasm (CCIC) (Giemsa Stain; 100x).

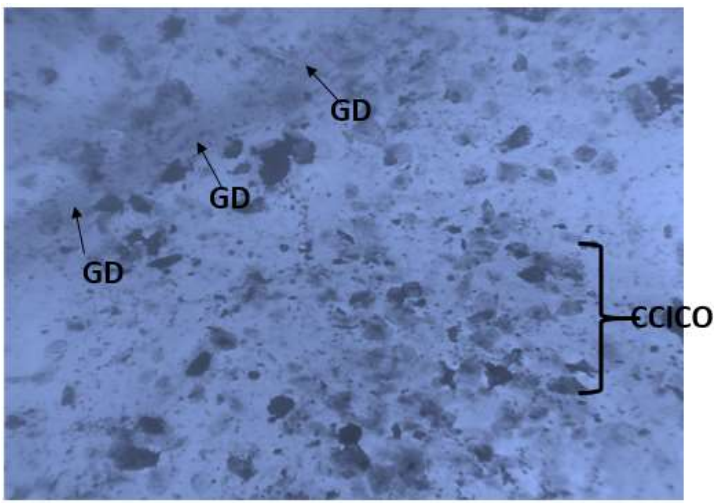

Plate 2: Photomicrograph of wood sputum sample shows clumping of cells with irregular cytoplasmic outline (CCICO) and granular debris (GD) represented with small black arrows (Giemsa Stain; 100x).

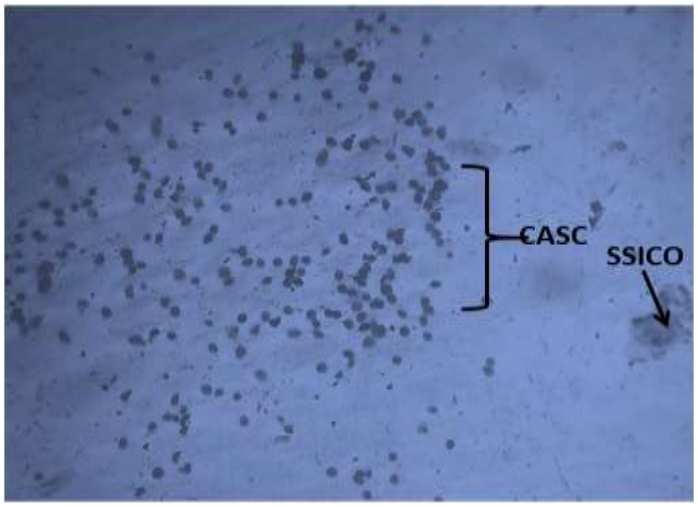

Plate 3: Photomicrograph of wood sputum sample shows cellular aggregates with scanty cytoplasm (CASC) and single squamous with irregular cytoplasmic outlines (SSICO) (Giemsa Stain; 100x).

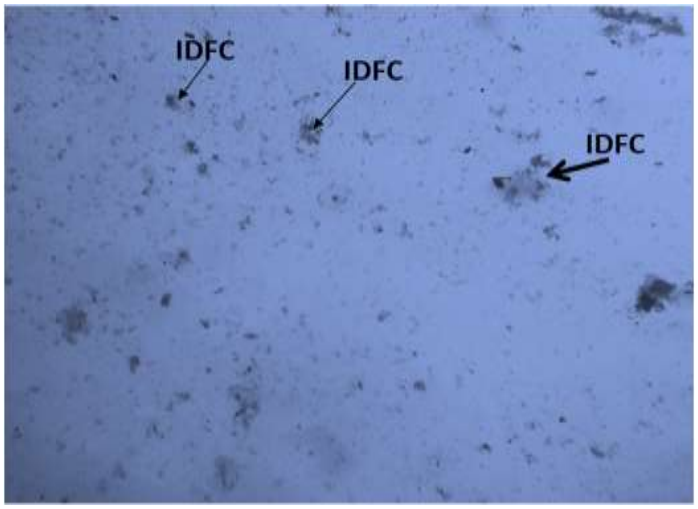

Plate 4: Photomicrograph of wood sputum sample shows ill-defined, feathery cytoplasm (IDFC) (Giemsa Stain; 100x).

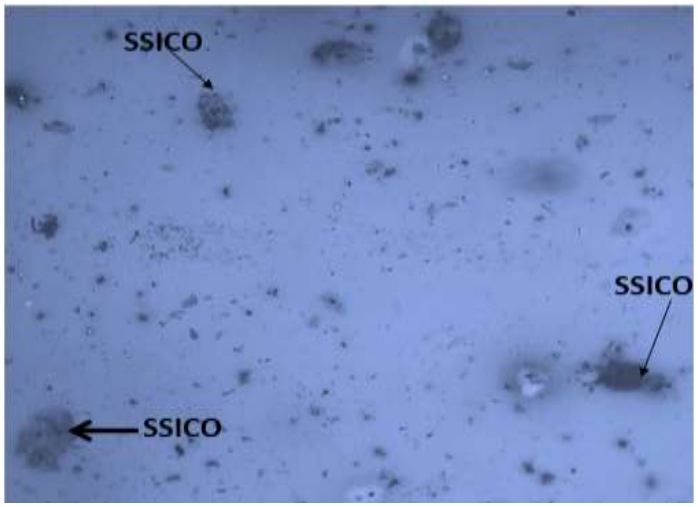

Plate 5: Photomicrograph of wood sputum sample shows single squamous with irregular cytoplasmic outline (SSICO) (Giemsa Stain; 100x).

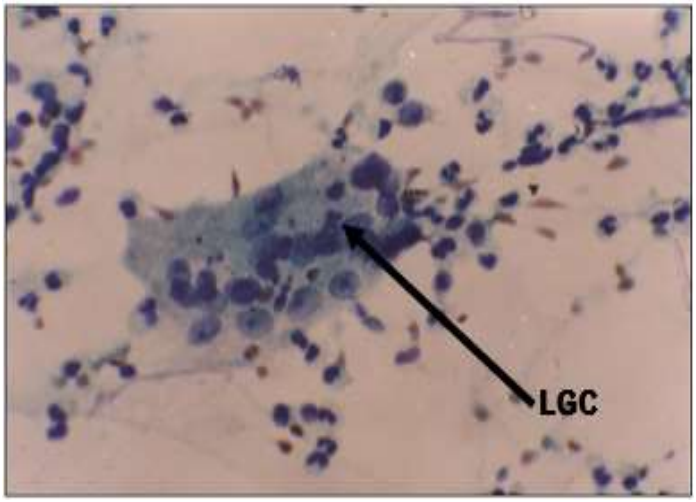

Plate 6: Photomicrograph of control sample showing Langhan's giant cell (LGC) (Papanicolaou stain: 400x).

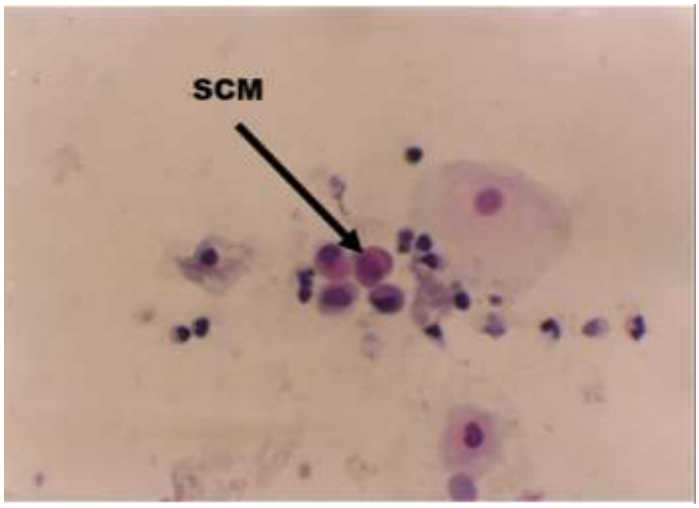

Plate 7: Photomicrograph of wood sputum sample showing squamous cell metaplasia (SCM) with atypia (Papanicolaou stain: 400x).

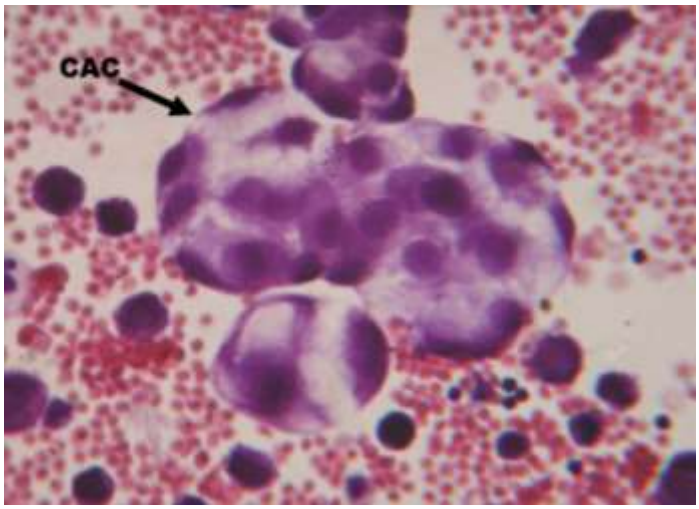

Plate 8: Photomicrograph of wood sputum sample showing cells of adenocarcinoma (CAC) (Papanicolaou stain: 400x).

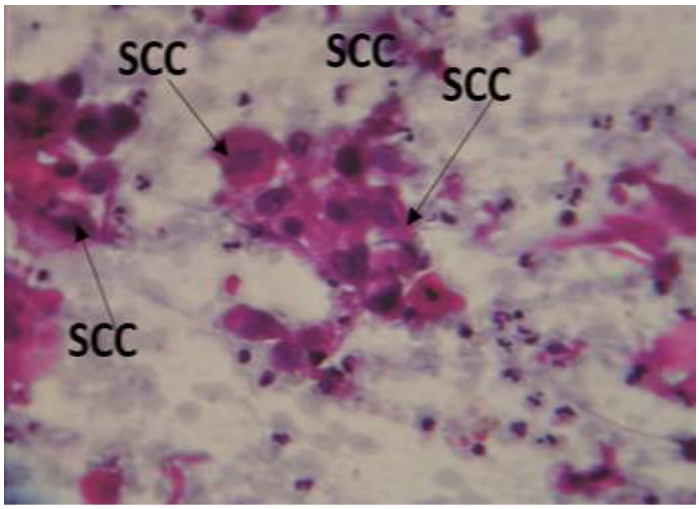

Plate 9: Photomicrograph of wood sputum sample showing squamous cell carcinoma (SCC) (Papanicolaou stain: 400x).

DISCUSSION

The findings in this study revealed that chronic occupational exposure to timber dust for years may have hematological effect. Twenty-six (26) timber workers had normal range of PCV value while 44 workers had normal range of neutrophils, normal lymphocyte and eosinophil levels was seen in 15 and 18 participants respectively while non was observed in monocytes. This study is not in agreement with a study conducted by Farheen *et al*. (2017) who reported that packed cell volume was significantly lower after work shift but MCV and MCH were unaffected. Similarly, there exist a contrast with the findings of Ashwini *et al*. (2016), Jude *et al*. (2001) and Mojiminiyi *et al*. (2008) wherein they found significant lowering in the red cell count, packed cell volume and hemoglobin. This may be due to effect of components of dust on hemopoietic system. Naik *et al*. (2012) reported no change in Hb levels. A case-control study done in India reported significantly reduced Hb, PCV and MCV whereas MCHC increased significantly (Mandal and Suva, 2014). Okonkwo *et al*. (2015) reported no change in Hb and PCV. The obstruction of the airways experienced by the timber workers as a result of exposure to dust will lead to more erythropoietin production and consequently more production of red cells (Uzoma *et al*., 2021).

There was no significant variation in the total leukocyte count probably owing to adaptation of the immune response to chronic exposure to grain dust. Similar adaptation has been noted in workers exposed to cement dust and rice dust (Patil *et al*., 2015).

Plate 1 shows cohesive clusters of irregular cytoplasm (CCIC). Also plate 2 shows clumping of cells with irregular cytoplasmic outline and granular debris while plate 3 shows cellular aggregates with scanty cytoplasm and single squamous with irregular cytoplasmic outlines. Sputum sample of plate 4 showed ill-defined, feathery cytoplasm. Plate 5 shows single squamous with irregular cytoplasmic outline. The prominent aggregation of cells common to all the samples may be an indication of triggered response to infection as a result of dust particles. The sputum sample also showed Langhan's giant cell. Granuloma formation with

multinucleated giant cells is seen in numerous diseases. A granuloma is a focus of chronic inflammation consisting of a microscopic aggregation of macrophages surrounded by a collar of lymphocytes and plasma cells. Some of the giant cells exhibited arrangement of the nuclei at both poles as can be seen in plate 6. Nuclei were uniform and appeared normal and vesicular. The giant cells were not associated with hemorrhagic foci, which were fairly observed. The histopathology was suggestive of a chronic granulomatous disease. Granulomatous inflammation is a distinctive pattern of chronic inflammatory reaction characterized by focal accumulation of activated macrophages, which often develop an epithelioid appearance.

In Papanicolaou (H and E) sections, epithelioid cells have a pale purple cytoplasm with indistinct cell boundaries and appear to be merging with one another. These cells can fuse to form giant cells with diameter of 40-50 µm. These giant cells have a large mass of cytoplasm with 20 or more nuclei arranged haphazardly or in a horseshoe pattern peripherally-the Langhans type (Kumar *et al*., 2013). These giant cells can be formed by cell fusion and nuclear division without cytoplasmic separation. In Langhans giant cells, the nuclei are either arranged in a horseshoe pattern at the periphery or clustered at the two poles of the giant cell (Kumar *et al*., 2013).

Moreover, it was observed in this research that wood sputum sample showed squamous cell metaplasia with atypia. Cytologically, metaplastic cells have variable amounts of basophilic cytoplasm with well-defined borders, variable nuclear/cytoplasm (N/C) ratios, round centrally placed nuclei with smooth nuclear membranes and occasional nucleoli (Idowu and Powers, 2010). Metaplastic squamous cells may occasionally be significantly atypical and be mistaken for malignancy.

Photomicrograph of wood sputum sample in this study showed cells of adenocarcinoma. Adenocarcinoma usually arises in a peripheral location and may thus be sampled by CT-guided percutaneous/transthoracic FNA (Idowu and Powers, 2010). There are several variants of adenocarcinoma, including the more common acinar, papillary, mixed acinar-papillary and solid. Bronchioloalveolar carcinoma (BAC), defined by convention as lacking stromal, vascular or capsular invasion, is often included in this category. Typical cytomorphological features of adenocarcinoma include cellular clusters with depth of focus; however, there may also be individual cells or acinar (glandular) arrangements (Idowu and Powers, 2010). Depth of focus is one of the major cytological features, because adenocarcinoma is often present as three-dimensional clusters of large vacuolated cells. These cells are columnar, cuboidal or polygonal with variable cell size and variable quantity of fine vacuolated cytoplasm. The nucleus is variably sized round to oval nuclei, often eccentric with high nuclear/cytoplasm (N/C) ratio, finely granular chromatin. Prominent central cherry red nucleoli are variably identified. On a cautionary note, BAC may have a large number of bland neoplastic cells (which may resemble alveolar macrophages/bronchial cells) that may suggest a reactive or reparative process. The presence of papillary fronds with fibrovascular septa and/or psammoma bodies should suggest the diagnosis of BAC (Idowu and Powers, 2010).

Conclusion: This study revealed that exposure to wood dust is highly detrimental to human health. It is therefore of paramount importance that workers in wood and timber industries apply occupational safety precautionary measures during work hours. These measures may include but not limited to wearing safety protective apron, facial/nose protective masks, hand gloves and helmets.

Conflict of interest: None declared.

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