ORIGINAL ARTICLE

Cytological and Molecular Assessment of Sputum Specimens of the Individuals Suspected to Have Tuberculosis in Wua Settlement in Nasarawa State, Nigeria

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ABSTRACT

Background: Approximately 2 million individuals worldwide die of lung tumors each year, accounting for 25% of all cancer-related deaths. Nigeria has the sixth highest rate of tuberculosis worldwide and the highest incidence in Africa. ***Objective:*** This study aimed to evaluate cytological and molecular parameters of sputum specimens collected from individuals suspected to have tuberculosis in Wua Settlement in Nasarawa State, Nigeria. ***Methods:*** The cross-sectional study was carried out from June to October of 2024. A structured questionnaire was used to collect demographic characteristics, knowledge and awareness, and behavioural factors of the respondents. One hundred and eleven subjects participated in this research. Sputum specimens were collected from each participant using sterile sputum containers. Papanicolaou's stain, GeneXpert, and direct Gram of the sputum were conducted. ***Results:*** Of the 111 participants, 94(84.7%) were farmers by occupation. About risk factors, 22(19.8%) reported being smokers, with smoking duration ranging between 1 and 20 years, while 50(45%) admitted to consuming alcohol. 93(88.3%) participants had normal sputum smears, 17(15.5%) showed signs of inflammation, and 1(0.9%) had a premalignant condition. No case of MTB was detected. Other findings included Gram-positive cocci arranged in chains in 86(77.5%) participants, in clusters in 17(15.5%), Gram-positive diplococci in 2(1.8%), and Gram-negative bacilli in 6(5.4%). ***Conclusion:*** Cytological and molecular assessment techniques played a vital role in sputum analysed for determination of the bacterial infections' distribution and percentage distribution of cellular abnormalities in the study participants.

Keywords: Pulmonary tuberculosis, sputum test, cytopathologic technique, GeneXpert

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INTRODUCTION

Respiratory cytology has been used extensively in the diagnosis of both cancerous and non-neoplastic conditions over decades. Sputum cytology is a component of exfoliative cytology, which is based on the examination of cells spontaneously shed from the lining of an organ into the cavity. It is an easy, precise, reliable, economical, and non-invasive diagnostic cytopathologic technique for screening of respiratory tract disorders, including pre-invasive and invasive cancers.[1] About 2 million fatalities worldwide are attributed to lung tumours each year, accounting for 25% of all cancer-related deaths.[2] According to the 2020 WHO Global Cancer Observatory projections, lung and airway cancer is 14th most common in Nigeria with 1789 cases newly diagnosed and 1643 deaths yearly (which is approximately 2.1% of all cancer-associated deaths). These are the most frequently cited current lung cancer incidence and mortality statistics in Nigeria.[3]

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Squamous cell carcinoma (SCC), the second most prevalent neoplasm, is typically aggressive and is among the top causes of cancer-related deaths.[4] This variant of lung cancer is known as non-small cell lung cancer (NSCLC). Squamous metaplasia (SM), grade I–III dysplasia, carcinoma in situ (CIS), and basal cell hyperplasia (BCH) are the morphological alterations in the bronchial epithelium that give rise to lung SCC.

When goblet cells are absent and ciliated cells and three or more layers of basal cells are seen, BCH is diagnosed. SM is characterized by the squamous epithelium replacing the cylindrical ciliated epithelium. There are three categories of dysplasia: mild (I), moderate (II), and severe (III).[5] Smoking has a higher risk of LSCC than any other form of NSCLC. Age, family history, exposure to indirect smoke, metal and mineral fragments, or asbestos is other risk factors for SCC.[6] Evidence showed that SCC accounts for about 30% of all lung cancer cases, 40% of cases in males, and 25% in women.[7] Approximately 18% of LSCC individuals survive for five years.[5] Even though immunotherapy has improved the prognosis of some of these malignancies, LSCC still has a poor overall prognosis, and no precise biomarkers are available.[8,9]

Respiratory tract infections (RTIs) are the most common infections in humans, with lower respiratory tract infections (LRTIs) making up about 90% of these cases.[10] Acute respiratory infections (ARIs), which are the cause of both upper respiratory tract infections (URIs) and lower respiratory tract infections (LRIs), are a leading etiology of mortality for children under five years old, especially in nations with limited resources where the disease burden is two to five times higher than in developed nations.[11] The incidence of lower respiratory tract infections has historically varied greatly throughout the world, especially among the poor and young.[12] 4.4% of deaths across all age groups are caused by lower respiratory tract infections (LRTIs), making them a major cause of morbidity and mortality globally.[12,13] Following the introduction of conjugate vaccines against *Haemophilus influenzae* type b, pertussis, and pneumococcal infections, reductions in death rates from LRTIs have been observed on a global scale. Conversely, in low- and middle-income nations, LRTI continues to be a major cause of death for both young children and the elderly.[12,13] Lower respiratory tract infections were the sixth largest cause of mortality for all ages and the most common cause of death for children under five years old in 2016, accounting for about 2.38 million fatalities.[12] Individuals with immunocompromising ailments or those from families with irregular or inadequate access to clean cooking fuel, vaccinations, proper diet, and hygiene are frequently at the highest risk of developing or dying from lower respiratory infections.[14] The World Health Organization (WHO) reported that the most epidemics of infectious diseases now occur in Africa, where there are significant risks of transmission.[15] Additionally, risk factors like human-animal interfaces—caused by residential closeness to livestock – are on the rise in emerging regions, both rural and urban. There have been requests for enhanced ARI surveillance as a result of these shifting epidemiological patterns, particularly in areas with a high risk of transmission.[2]

In Nigeria, several risk factors linked to ARI in children, such as gender, age, congestion, nutritional status, and socioeconomic position, are extremely common, also there is currently little research on ARIs. Due to these wide-ranging risk factors and scarce resources, community-based initiatives for surveillance and interventions are required.[11] Distinguishing TB from other LRTIs, like bacterial pneumonia, is a significant clinical problem in developing nations. If TB fails to distinguish from other LRTIs, the patients may experience worse health outcomes, which could culminate in an elevated death rate.[16] In developing nations, tuberculosis is a highly dreaded disease.[10] *Mycobacterium tuberculosis* (*M. tuberculosis*) is the bacterium that causes tuberculosis (TB), a potentially lethal bacterial infection. The lungs are the main organs affected by this highly infectious droplet infection. But it may involve several organ systems. Although TB can be efficiently treated, it is still a major public health concern and an important cause of morbidity and mortality globally, particularly in low-income countries. Over 1.7 billion people have been reported to be infected with MTB.[17] The overall prevalence of tuberculosis has decreased. Research showed that Nigeria has the sixth-highest TB burden worldwide and the highest in Africa,[18,19] with an estimated 108,000 Nigerians contracted tuberculosis in 2020, and 23,000 individuals died from the infection.[18] Research showed that 13.25% of the patients had *M. tuberculosis* in the North

Central Nigeria investigated through GeneXpert as 2800 sputum specimens were collected from probable pulmonary TB patients presenting at secondary health care centres. The highest occurrence was 39.08% among patients aged 30-39, with male respondents accounting for 13.88% and female respondents for 12.56%. Additionally, HIV co-infection was present in 20.6% of PTB patients. The incidence of tuberculosis was 14.2% and 12.8% among smokers and non-smokers, respectively, and 13.24% and 13.26% among alcoholic and non-alcoholic individuals. The study also highlighted the significance of education in relation to PTB occurrence, revealing that respondents with secondary and primary education had the highest occurrence (13.6%), followed closely by those with higher education (13.4%), while those with no formal education (12.4%) had the lowest frequency.[20]

METHODS

The cross-sectional study was carried out in Dalhatu Araf Specialist Hospital Lafia, Nasarawa State of North Central Nigeria, from June to October of 2024. The study comprised individual suspected of having *Mycobacterium tuberculosis* in Wua settlement, Nasarawa State in Nigeria. A survey was administered and the demographic, knowledge and awareness, and behavioural factors were obtained. Random sampling techniques were used for this study. The sample size was calculated using Taro Yamane's formula to be 111. The residents of Wua village who are above five years and are not on TB drugs were used for this study. The individuals who are not resident of Wua village and those on TB medication were excluded from study. The residents of the settlement who are below five years were excluded from the study. Also, the individuals who could not produce sputum were excluded from the study. A structured questionnaire sectioned into, Demographic information (age, gender, occupation, formal education level and marital status), Knowledge and awareness, behavioural factors and clinical conditions were used to collect the data of the subjects who participated in the research. A labelled Clean, sterile leak proof screw capped wide mouth container were used. The subjects were instructed to rinse their mouth with water and produce 2-5 ml of deeply cough sputum from lower respiratory tract in a sterile screw-cap container.

The samples collected were stained with Papanicolaou (PAP) stain to assess the cytological changes and other infections (Aspergillus) as described by Sathawane et al.[21] Direct sputum Gram stain was carried out on the samples as described by Paray et al.[22] Using the GeneXpert assay machine (Cepheid, Sunnyvale, CA, USA), *M. tuberculosis* was detected from sputum specimens. The subject's sputum specimens were digested in sample reagent in a 2:1 ratio and waited to settle at room temperature. Two millilitres (2 ml) of the mixture were pipetted into the GeneXpert cartridge and loaded in the GeneXpert machine for two hours. The outcome was regarded as either "MTB detected" or "MTB not detected".[23]

Data collected and the result obtained were analysed using the Statistical Package for the Social Sciences (SPSS) software version 27.0 for Windows. Descriptive statistics and a Chi-square test were conducted. P value <0.05 was considered as statistically significant

RESULTS

Table 1 shows the demographic characteristics of the 111 respondents. 57(51.4%) of the respondents were male, while 54(48.6%) were female. Regarding age distribution, the largest group of respondents 36 (32.4%) fell within the 41-50 age range, followed by 31-40 years at 28(25.2%), 11-20 years at 18(16.2%), and 21-30 years at 17 (15.3%). The smallest age group was those 51 years and older, comprising 12(10.8%) of the respondents. Occupationally, the majority were farmers, accounting for 94(84.7%), while students made up 16(14.4%). Only one respondent 1(0.9%) was a civil servant, and there were no teachers 0(0.0%). In terms of marital status, the majority were married, constituting 80(72.1%), while 31(27.9%) were single. There were no widows or widowers among the respondents. Table 2 shows the knowledge and awareness of tuberculosis (TB) among the 111 respondents indicate that only 19(17.1%) knew what TB is, while the majority, 92(82.9%), were unaware of the disease. Regarding family history, 108 (97.3%) of the respondents reported no family history of TB, while only 3(2.7%) had a family history of the disease. When asked about the mode of transmission of TB, 56(50.5%) incorrectly believed it was through the consumption of unpasteurized milk, while 8(7.2%) correctly

identified coughing as a transmission method. Another 3(2.7%) thought sneezing was a transmission route, and 44(39.6%) did not know the mode of transmission. Only 2(1.8%) of respondents had treated Mycobacterium tuberculosis (MTB), while 109(98.2%) had never done so. Table 3 shows the behavioral and clinical data of the respondents. 22(19.8%) were smokers, while the majority, 89(80.2%), did not smoke. Among the smokers, 8(7.2%) had smoked for 1-5 years, 6(5.4%) for 6-10 years, 3(2.7%) for 11-15 years, and 5(4.5%) for 16-20 years, with 89(80.2%) unable to recall their smoking duration. In terms of alcohol consumption, 50(45.0%) reported drinking alcohol, while 61(55.0%) did not. Of those who consumed alcohol, 8(7.2%) had done so for 1-5 years, 20(18.0%) for 6-10 years, 4(3.6%) for 11-15 years, and 21(18.9%) for 16-20 years, with 58(52.3%) unable to recall the duration. Regarding clinical conditions, 75(67.6%) of respondents reported having a productive cough, and 36(32.4%) experienced a cough without blood. There were no cases of profuse night sweating, coughing with blood, or weight loss among the respondents. Additionally, none of the respondents had underlying conditions such as HIV/AIDS, diabetes mellitus, or kidney disease, with all 111 respondents (100%) indicating the absence of any underlying health conditions. Table 4 presents the prevalence of microorganisms found in the sputum specimens of the respondents. None of the participants was identified with *Mycobacterium tuberculosis.* Most of the isolates were Gram-positive cocci in chains, identified in 86(77.5%) specimens. Gram-positive cocci in clusters were observed in 17(15.3%) specimens. Gram-positive diplococci were found in 2(1.8%) specimens. Lastly, Gram-negative bacilli were isolated in 6(5.4%) specimens. Table 5 shows the percentage of abnormalities observed in the sputum smears of the respondents. 1(0.9%) Premalignant changes were identified in respondent. There was no case of malignant abnormalities. Inflammation was detected in 17(15.3%) respondents. Most of the sputum smears, 93(83.8%) show no abnormalities. Table 6 shows the relationship between gender, occupation, smoking status, and the presence of inflammation among the respondents. For gender, among the males, 9(8.1%) sputum smears of the respondents showed inflammation, and 48(43.2%) sputum smears did not show inflammation, while among the females' respondents, 8(7.2%) sputum smears were inflamed and 46(41.4%) were not, with (X^2=0.020; P>0.05), which indicate no significant association. Regarding occupation, inflammation was observed in sputum smears of 14(12.6%) farmers compared to 80(72.1%) sputum smears that did not show inflammation. Inflammation was not observed in teachers or civil servants. Three cases 3(2.7%) of inflammation were seen in the sputum smears of students, along with 13(11.7%) non-inflammatory cases (X^2=0.3390; P>0.05), which indicate no significant association. For smoking status, inflammation was observed in 3(2.7%) sputum smears and 18(16.2%) non-inflammatory cases. Inflammation was also observed in the sputum smears of 14(12.6%) non-smokers, and 76(68.5%) sputum smears did show inflammation, with (X^2=0.021; P>0.05), which also indicate no statistically significant association.

On cytological assessment, sputum smear consists of sheets of squamous cells with normal vesicular nuclei. There are also small groups of columnar cells with hyperchromatic nuclei. In the background there are some polymorphonuclear leucocytes (×400 magnification) (Figure 1-4).

Table 1: Demographic characteristics of the participants (n=111)

Demographics		Frequency (Percentage)
Gender	Male	57 (51.4)
	Female	54 (48.6)
Age Group	11-20	18 (16.2)
	21-30	17 (15.3)
	31-40	28 (25.2)
	41-50	36 (32.4)
	51+	12 (10.8)
Occupation	Farmer	94 (84.7)
	Teacher	-
	Civil Servant	1 (0.9)
	Student	16 (14.4)
Marital Status	Single	31 (27.9)
	Married	80 (72.1)
	Widow	-
	Widower	-

Table 2: Knowledge and awareness of the participants on tuberculosis (n=111)

Questions on knowledge of TB		Frequency (Percentage)
Knowledge of TB	Yes	19 (17.1)
	No	92 (82.9)
Family history of TB	Yes	3 (2.7)
	No	108 (97.3)
Mode of transmission	Coughing	8 (7.2)
	Sneezing	3 (2.7)
	Consumption of unpasteurised milk	56 (50.5)
	I do not know	44 (39.6)
Prior treatment of MTB	Yes	2 (1.8)
	No	109 (98.2)

MTB=*Mycobacterium tuberculosis*

Table 3: Behavioural factors and clinical conditions of the participants (n=111)

Behavioural factors		Frequency (Percentage)
Smoking Status	Yes	22 (19.8)
	No	89 (80.2)
Duration of smoking	1-5 Years	8 (7.2)
	6-10 Years	6 (5.4)
	11-15 Years	3 (2.7)
	16-20 Years	5 (4.5)
	Cannot remember	89 (80.2)
Alcohol Consumption	Yes	50 (45.0)
	No	61 (55.0)
Duration of alcohol consumption	1-5 Years	8 (7.2)
	6-10 Years	20 (18.0)
	11-15 Years	4 (3.6)
	16-20 Years	21 (18.9)
	Cannot remember	58 (52.3)
Clinical symptoms		
Reported symptoms	Profuse night Sweating	0 (0.0)
	Productive cough	75 (67.6)
	Coughing without blood	36 (32.4)
	Coughing with blood	-
	Weight Loss	-
Underlying conditions	HIV/AIDS	-
	Diabetes mellitus	-
	Kidney disease	-
	None	111 (100.0)

Table 4: Distribution of various bacteria in sputum specimens (n=111)

Microorganisms	Frequency (Percentage)
Mycobacterium tuberculosis	-
Gram Positive Cocci in Chain	86 (77.5)
Gram Positive Cocci in Cluster	17 (15.3)
Gram Positive Diplococci	2 (1.8)
Gram Negative Bacilli	6 (5.4)

Table 5: Abnormalities observed in the sputum smears (n=111)

Abnormality	Frequency (Percentage)
Premalignant	1 (0.9)
Malignant	-
Inflammation	17 (15.3)
No Abnormality	93 (83.8)

Table 6: The relationship between demographic factors and inflammation

Demographic factors		Inflammation		X^2	P value
		Inflammed	Non-inflammed		
		Frequency (Percentage)	Frequency (Percentage)		
Gender	Male	9 (8.1)	48 (43.2)	0.020	0.887^{NS}
	Female	8 (7.2)	46 (41.4)		
Occupation	Farmer	14 (12.6)	80 (72.1)	0.339	0.844^{NS}
	Teacher	-	-		
	Civil Servant	-	1 (0.9)		
	Student	3 (2.7)	13 (11.7)		
Smoking Status	Yes	3 (2.7)	18 (16.2)	0.021	0.884^{NS}
	No	14 (12.6)	76 (68.5)		

Chi-square tests were done to reach P value; NS=not significant.

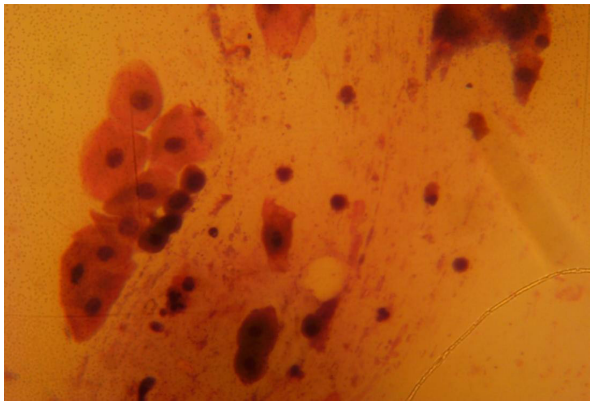

Figure 1: Sputum smear consists of sheets of squamous cells with normal vesicular nuclei. There are also small groups of columnar cells with hyperchromatic nuclei. In the background there are some polymorphonuclear leucocytes (×400 magnification).

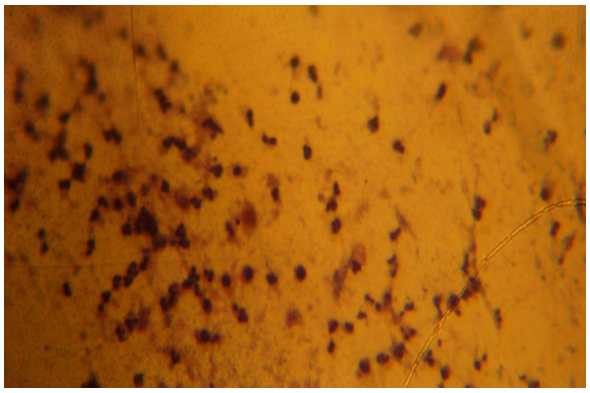

Figure 2: Sputum smear consists of few squamous and columnar epithelial cells with generally unremarkable nuclei. At background are numerous polymorphonuclear leucocytes (×400 magnification).

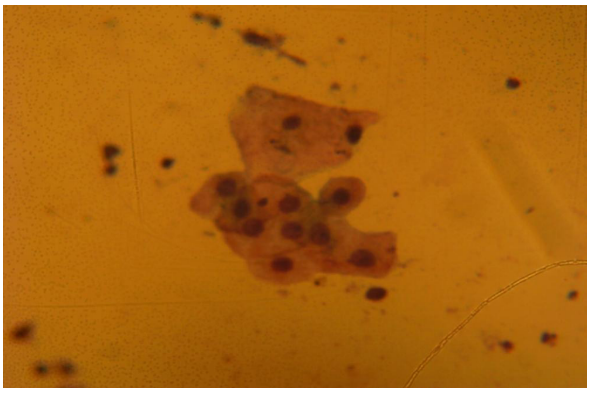

Figure 3: Sputum smear consists of sheets of squamous cells in clusters. Vesicular nuclei show some variation in sizes and some of the nuclei show hyperchromasia. At the background there are few polymorphonuclear leucocytes (×400 magnification).

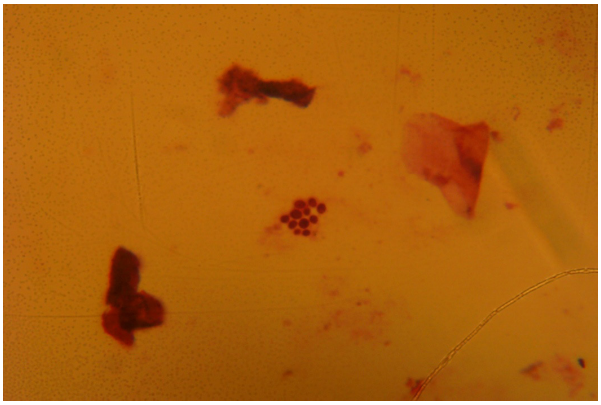

Figure 4: Sputum smear consists of sheets of squamous cells and cluster of columnar epithelia cells with nuclei showing obvious variation in size and marked hyperchromasia indicative of some neoplastic changes (×400 magnification).

DISCUSSION

On sputum specimens of initially suspected cases, no case of MTB was identified. The distribution of *Mycobacterium tuberculosis* (MTB) in this study disagrees with that of a study conducted by Dahal et al.[24] in north-central Nigeria, which showed a prevalence of 7.8% and that of Agabi et al.,[25] with a prevalence of 12.2%. The distribution in this study suggests that the studied population or sample group had no active tuberculosis infections at the time of investigation. The zero distribution of *Mycobacterium tuberculosis* in the specified area could be attributed to socioeconomic factors such as improved living conditions, access to healthcare, and good nutrition, which can play a crucial role in preventing TB. Moreover, Wua settlements have a low population density, which may also reduce the likelihood of TB transmission compared to more crowded areas. Genetic factors or a historical lack of exposure to the bacterium in the community might contribute to the absence of TB cases. Most isolates 86(77.5%) were identified as Gram-positive cocci arranged in chains, which is characteristic of Streptococcus species. These bacteria are commonly found in the oral cavity, respiratory tract, skin, and intestines of humans and can cause a range of infections, from mild throat infections to more severe conditions such as pneumonia or bacteraemia. The high distribution suggests these organisms are the primary pathogens or commensals in the sample environment. The second most common type of isolate was Gram-positive cocci in clusters 17(15.3%), typically indicative of *Staphylococcus*

species. Notably, *Staphylococcus aureus* can be pathogenic, causing infections like pneumonia, abscesses, or sepsis. The cluster formation is a hallmark of Staphylococci, distinguishing them from Streptococci, which form chains. This finding suggests a significant but lesser presence of *Staphylococcal species*, which may be opportunistic or commensal organisms. The presence of Gram-positive diplococci at 2(1.8%) suggests species such as Streptococcus pneumoniae, known for causing pneumonia, meningitis, and other respiratory infections. While less prevalent in this study, their presence could still be significant in clinical infections. The predominance of Gram-positive cocci indicates that the bacterial infections were mainly caused by *streptococcal and staphylococcal species*. These findings suggest that clinical or environmental samples were dominated by typical bacterial pathogens, potentially reflecting common infections like respiratory rather than TB. A small percentage distribution of the isolates 6(5.4%) were Gram-negative bacilli, which include a broad range of bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. These organisms are often associated with more severe infections, including pneumonia, urinary tract infections (UTIs), and hospital-acquired infections. Their lower distribution indicates a secondary or opportunistic role in the microbial population under study.

On cytological examination of the sputum smears, sheets of squamous cells with normal vesicular nuclei were found. There were also small groups of columnar cells with hyperchromatic nuclei. In the background there are some polymorphonuclear leucocytes. PAP stain of sputum smears of 93(83.8%) of the individuals in Wua specimen who participated in the research showed no abnormality as showed in Table 5. Some sputum smears consist of few squamous and columnar epithelial cells with generally unremarkable nuclei. At background are numerous polymorphonuclear leucocytes. As presented in Table 5, the sputum smears of 17(15.3%) participants showed severe inflammation of the respiratory tract, indicated by profuse infiltration of leucocytes. Some of the inflammatory cases (2.7%) recorded were among smokers, and smoking could be one of the causes of the inflammation. This finding is similar to that of Yan et al., as they reported that smoking causes inflammation in the body by triggering an increased abundance of proinflammatory bacteria. Non-smokers exhibited higher amounts of anti-inflammatory microorganisms than smokers; these microorganisms can create short-chain fatty acids, which limit inflammation.[26] The correlation between smoking and inflammation strengthens the case for smoking being a primary irritant in these individuals. However, since inflammation was observed in both smokers and non-smokers, other causes, such as bacterial, or viral infections, as well as conditions like asthma or COPD, should be considered. Further investigations are needed to ascertain the specific causes of the inflammation, which may be due to bacteria, fungi, viral infections, chronic obstructive pulmonary disease (COPD), asthma, interstitial lung diseases, and inhalation of irritants. Some sputum smears consist of sheets of squamous cells and cluster of columnar epithelia cells with nuclei showing obvious variation in size and marked hyperchromasia indicative of some neoplastic changes. 1(0.9%) of pre-malignant cases were recorded, which may be a result of familiarity since the survey form of the dysplastic individual showed no smoking status. No risk factors for lung cancer were recorded in the survey. Our finding of premalignant case 1(0.9%) is dissimilar to the findings of the study done by Neumann et al., as they found a prevalence of 74.6% of premalignant lesions; they reported such a high prevalence of premalignant cases because the study was conducted among patients with lung cancer.[27] There is no common literature on premalignant cases in sputum smears within the limit of my literature review to further support the finding.

CONCLUSION

To conclude, the study revealed that *Mycobacterium tuberculosis* (MTB) was not present in any of the samples. Instead, the most common bacteria were Gram-positive cocci in chains, Gram-positive cocci in clusters, Gram-negative bacilli, and Gram-positive diplococci. No isolates of fungi were also found. Notwithstanding, without additional culture-based identification, the presence of these microorganisms in sputum – a non-sterile sample – has no therapeutic value. Cytological examination of the sputum smears revealed cases of normal exfoliated cells. Nevertheless, some samples displayed evidence

of inflammation, such as a high level of leucocyte infiltration and respiratory tract inflammation. A minor percentage of premalignant alteration was also found by the investigation, indicated by clusters of columnar epithelia cells with nuclei that showed clear size variation and noticeable hyperchromasia, as well as sheets of squamous cells.

Conflict of Interest: The authors declare no conflict of interest.

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Ethical Approval: Ethical Review Committee of Dalhatu Araf Specialist Hospital, Lafia, Nasarawa State, Nigeria, approved ethical permit for this study.

Authors' Contribution: All the authors were equally involved in concept, design of the study, questionnaire formulation, data collection, compilation and analysis as well as manuscript preparation, critical review and final submission.

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