Assessment of Cytological Features of Sputum Samples Obtained From Immunocompromised Patients Coinfected with Some Pulmonary Pathogens in Kano State, Nigeria

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Abstract: Patients with advanced stages of Human immunodeficiency virus (HIV) infection, or Acquired immunodeficiency syndrome (AIDS), are vulnerable to secondary opportunistic infections. The aim of the study was to assess the cytological features of sputum samples obtained from patients co-infected with pulmonary pathogens in Kano. Sputum samples were collected from HIV infected patients attending Aminu Kano Teaching Hospital and digested using potassium hydroxide and epithelial and non-epithelial cells were harvested, smeared and fixed using alcohol for Papanicolaou staining method for cellular morphology. The sputum samples were also smeared using Ziehl-Neelsen (ZN) stain for *Mycobacterium tuberculosis*, and another smear was stained using Grocott's Methanamine Silver (GMS) staining method for fungi. A total of 65 HIV infected patients were studied. Majority (53.8%) of the participants, were males and 46.2% were females. Cellular features of the sputum samples revealed the presences of mild inflammatory changes in 21.5% of patients, moderate inflammation in 1.5%, negative findings in 61.5%% and unsatisfactory smears in 15.4%. The commonest opportunistic pathogens detected were *Candida species* (24.5%), *Mycobacterium species* (20%) and *Aspergillus species* (9.2%). The study identified that majority of the cytological changes observed in the sputum samples of the HIV infected patients studied were inflammatory changes, which might be due to the presence of *Mycobacterium turberculosis*, *Candida species* and *Aspergillus* species. Hence, the cytological features of the sputum samples could play an important role in detecting the pulmonary pathogens.

Keywords: Cytological-features, Immunodeficiency, Pulmonary Pathogens.

INTRODUCTION

Microorganisms take advantage of the opportunity offered by a weakened immune system in Patients living with advanced stages of HIV infection (AIDS) and are vulnerable to secondary infections (Saidu *et al.,* 2009). Atypical infections begin to occur as disease advances and they are characterized by a late response to antibiotic therapy and reactivation of old latent infections (Bezabhe, 2016). Some opportunistic infections and certain cancers are AIDS-defining conditions, including pneumocystis pneumonia, neurotoxoplasmosis, atypical or disseminated pulmonary tuberculosis, cryptococcal meningitis and cytomegalovirus retinitis (Boyton, 2007). Lungs are often the main site of AIDS-related infections and cancers, and patients are treated the same as immunocompromised patients. However, it should be remembered that the disease may progress faster in these patients, thus increasing the mortality rate when diseases are undiagnosed or not adequately treated (Santos, 2019).

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 2008). 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.,* 2008).

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.,* 2008). Sputum cytology 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. Determination of opportunistic infections in HIV infected patients will be used in management of patients and forms the basis for further research on opportunistic pathogens and their cytopathic effects.

Due to the excessive use of broad spectrum antibiotics, long- term use of immunosuppressive agents and the increasing population of terminally ill and immunocompromised patients, pulmonary infections have emerged as world-wide healthcare problems in recent years among immunocompromised patients (Bhagavath *et al.,*2003). Therefore, pulmonary infections tend to be also one of the challenges of HIV patients which lead to cytological changes in lung tissue, effective management and diagnosis of these infections must be taken into consideration. The most characteristic feature in glandular cells is the presence of cilia on the apical surface, anchored into a terminal bar or plate. Cilia stain bright red and the terminal bars are seen as a distinct thickening of the apex. Goblet cells which are mucus producing cells are approximately the same length but usually wider than the ciliated cells with a basal placed nucleus and a distended supranuclear cytoplasm that is tightly packed with faintly basophilic vacuoles representing packages of mucus (Bibbo and Wilbur 2008).

Pulmonary infections affecting immunocompromised patients have been associated with high rate of mortality in previous years. This study will improve the 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 knowledge of this study can be used to verify the cytological changes in immunocompromised patients, thus improving healthcare in general.

The aim of this study was to assess the cytological features of sputum samples obtained from immunocompromised patients co-infected with pulmonary pathogens in Kano, Nigeria.

MATERIAL AND METHODS

A cross sectional study was conducted among the Adult HIV-infected patients attending Aminu Kano Teaching Hospital located within Kano metropolis, Kano State, Northwestern Nigeria. It is a referral Centre for both private and public health institutions in and around Kano. Ethical approval for this research was obtained from the Ethics Committee of Aminu Kano Teaching Hospital. The patient's socio demographic and clinical data were obtained both by direct interview.

All adult HIV infected Patients attending Aminu Kano Teaching Hospital, Kano serve as the study population. The sample size was determined using an estimated prevalence of 4.0% (Karuga, 2014). Fifty nine (59) sample size was obtained but rounded up to 65 in order to increase precision and minimize error. A structured questionnaire was administered to all participants who consent to the study.

Sputum samples were collected from the selected patients and was digested by adding 10 percent Potassium hydroxide (10% KOH) to the sputum in a ratio of one part to ten part 10% KOH to eliminate the slimy nature of sputum and enhance harvesting of epithelial and non-epithelial cellular elements (Chakraborty and Nishith 2008). The setups were allowed to stand for 30 minutes and then centrifuged at 1500rmp for 5 minutes. The supernatant was discarded and the deposits were smeared on clean grease free slides (Chakraborty, 2000).

Three smears were prepared; one was immediately fixed in 95% alcohol for Papanicolaou staining method for cellular morphology (Demay, 1996). The smears were primarily stained in Heamatoxylin, counterstained in O.G 6 and E.A 50 respectively; the smears were then dehydrated and finally mounted in DPX. Two smears were allowed to air dried. One air dried smear was stained using Ziehl-Neelsen (ZN) stain for *Mycobacterium tuberculosis* (Bibbo and Wilbur 2008). The other air dried smear was stained using Grocott's Methanamine Silver (GMS) staining method for fungi (Drury and Wallington 1980). All stained smears were examined microscopically with x10 and x40 objectives. The results obtained were expressed as percentages and presented on tables, charts and photomicrographs.

RESULTS

A total of sixty five (65) immunocompromised patients were enrolled in this study, out of which 53.8% were males and 46.2% were females (Table 1). According to age group, 40%, 30.8%, 27.7% and 1.5% participants fell within the age range of 31-40, 41-50, 21-30 and ≥50 years respectively (Table 1). The population of married participants were 90.8%, 6.2% were separated and 3.1% were widowed (Table 1). Table 1 show that 44.6% had secondary level education, 27.7% had primary level education, and 15.4% had no formal education while only 12.3% had tertiary level education.

The cytological changes associated with opportunistic pathogen among HIV patients was revealed in Table 2. Mild inflammatory changes were seen in 14 (21.5%) of the patients, moderate inflammatory changes were seen in 1 (1.5%) of the patients, normal finding in 40 (61.5%) and unsatisfactory smear (acellular) in 10 (15.4%).

Table 3 revealed that 16 (24.6%) of the sputum samples were positive for *Candida* species, 13 (20%) were positive for *Mycobacterium tuberculosis* and 6 (9.2%) were found to be positive for *Aspergillus* species.

Table 1: Socio-demographic distribution of the study participants

Variables	Frequency (n)	Percentage (%)
Gender		
Male	35	53.8
Female	30	46.2
Total	65	100
Age group		
21-30	18	27.7
31-40	26	40.0
41-50	20	30.8
≥ 51	1	1.5
Total	65	100
Marital status		
Married	59	90.8
Widowed	2	3.1
Separated	4	6.2
Total	65	100
Educational level		
Primary	18	27.7
Secondary	29	44.6
Tertiary	8	12.3
None	10	15.4
Total	65	100

Table 2: Cytological changes of sputum samples obtained from HIV patients co-infected with pulmonary pathogens

Variable	Frequency (n)	Percentage (%)
Mild inflammation seen	14	21.5
Moderate inflammation seen	1	1.5
Normal	40	61.5
Acellular	10	15.4
Total	65	100

Table 3: Frequency of Occurrence of Organisms in HIV patients Co-infected with Pulmonary pathogens

Organism	No positive	% Occurrence
Mycobacterium tuberculosis	13	20
Aspergillus spp.	6	9.2
Candida spp.	16	24.6
Negative smear	30	46.2
Total No Examined	**65**	

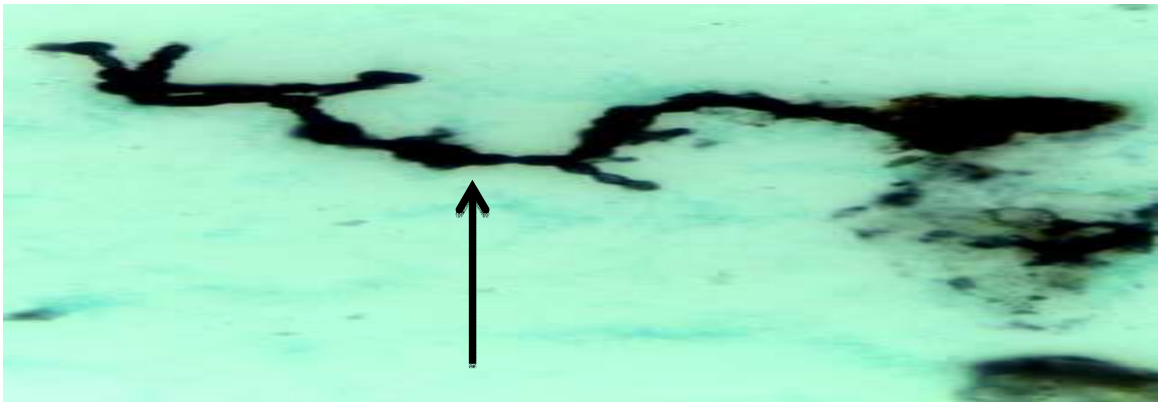

Figure 1: A photomicrograph showing *Aspergillus* hyphae and inflammatory changes. GMS stain (X40)

Figure 2: A photomicrograph showing *Candida* spore. GMS Stain (X40).

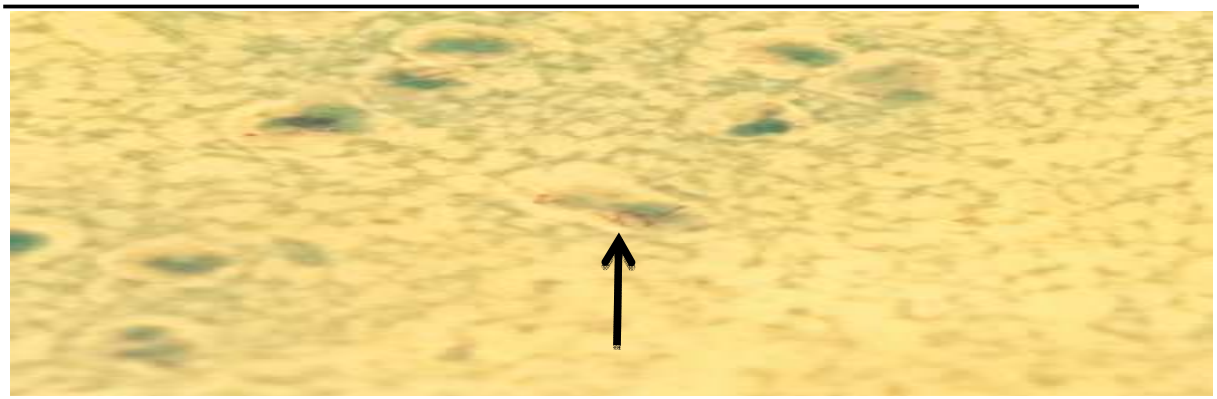

Figure 3: A photomicrograph showing mild inflammation and *Mycobacterium tuberculosis.* ZN stain (X40)

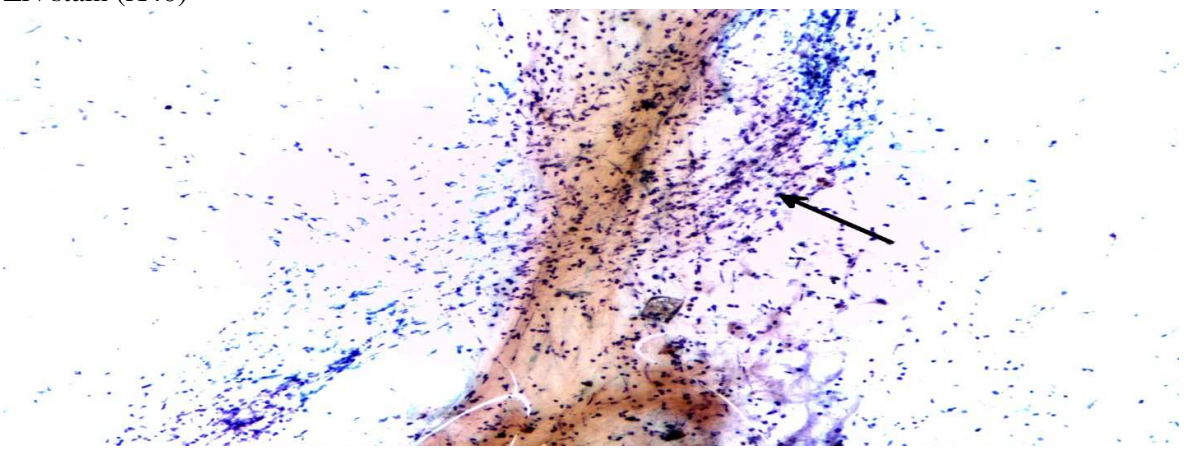

Figure 4. A photomicrograph of sputum cytology showing mild inflammation in HIV patient. Papanicolou stain (X40)

DISCUSSION

This study demonstrates that pulmonary sputum cytology analysis used in the study, remains a noninvasive procedure, simple to process, accurate and cost effective method in which multiple specimens can be collected for a variety of investigation.

The findings of the study, indicated that majority of the patients were between the age ranges of 31 to 40 years, which account for about 40% of the subject population. This agrees with the findings of Saha *et al.* (2011) in which, majority of the patients in their study were within the same age range. Majority of the patients, 44.6% had secondary level education; this is contrary with Kirunga *et al.* (1997) from Uganda who reported 51% of participant only completed primary education.

Pulmonary aspergillosis has recently been described as an emerging infection in patients with HIV but the pathological changes have not been well documented (Nash *et al.,* 1997). The exposure to *Aspergillus species* is universal, though invasive aspergillosis is rare in general, it is commonly found in immunocompromised cases like in HIV infection (Padayatchi, 2011). Our results also suggested that the overall prevalence of *Aspergillus species* is 6 (9.2%), this is lower than the previous reported from Maiduguri, Northeastern Nigeria which has a frequency of 46% (Adisa *et al.,* 2013). Our finding is also in contrast to the study by Bharathi and Rani, (2002) in India who reported 16% which was higher than the findings of this study but close to that reported by Anupriya *et al.* (2007) who reported a frequency of 8.3%.

Candidiasis is the most common reported opportunistic fungal infection in HIV infected patients.

It has been found to be the most common fungal isolation in HIV/AIDS and is of prognostic value only as its presence indicates progression of immunodeficiency (WHO, 2009). In this study, the frequency distribution of *Candida species* was found to be 24.6% which agrees with the findings of Saini *et al.* (2007), Mulla *et al.* (2007) and Khan *et al.* (2012) were they reported 23%, 26% and 24%. Our study disagree with the findings of Esebelahie *et al*. (2013) in Nigeria who reported an overall prevalence of Candida colonization among HIV infected patients to be 52%.

Tuberculosis is one of the most common types of opportunistic infections (OIs) associated with HIV infection and this may be further compounded by socio-economic factors such as malnutrition, poor hygiene, poverty and unemployment (Saha *et al.,* 2011). Ziehl-Neelsen (ZN) method is recommended by the World Health Organization (WHO) for screening patients because it is simple and rapid (Daniel, 1999). Mycobacterium culture is the reference method for isolation of tubercle bacilli, but it is time consuming and requires specialized safety procedures in laboratories. This study utilized the conventional method in detection of Acid Fast Bacilli (AFB) in sputum and 13 (20%) positive smears were seen which agrees with the previous study by Yassin *et al.* (2014) from Ethiopia which suggested the prevalence of TB to be 19% among HIV infected individuals. The findings of this study is in contrast with the findings of Dauda (2010) from Nigeria, and Saini *et al.* (2012) from India which reported the prevalence of TB to be 38% and 44% in HIV infected subjects respectively which are significantly higher than the findings of this study.

Sputum cytology is an example of exfoliative cytology, which is based on spontaneous shedding of cells derived from the epithelial lining of an organ into a cavity from where they can be removed by noninvasive means (Ammanagi *et al.,* 2012). It is a simple, accurate, reliable, cost-effective and noninvasive procedure for the assessment of respiratory diseases, including preinvasive and invasive pulmonary malignancies (Ammanagi *et al.,* 2012). Cytological examination of sputum is accepted as a useful diagnostic tool in carcinoma of the lung (Ammanagi *et al.,* 2012). The most common cytological changes in this study were mild inflammatory changes in 14 (21.5%), and 1 (1.5%) had moderate inflammation. This agrees with the findings of Selvaggi and Gerber (1986) who reported mild inflammatory changes in HIV patients with pulmonary infections. Epithelial cells of the respiratory tract undergo morphologic changes in response to injuries caused by various agents (Hormoz, 2012).

Inflammatory changes can be of two types; acute and chronic inflammation. Acute inflammation is characterized by presence of neutrophils, histiocytes, debris, and necrosis, including pneumonia, abscess, and purulent bronchitis, resulting in tissue destruction. Chronic inflammation is characterized by presence of an abundance of immature lymphocytes and plasma cells. Immature lymphocytes could not be mistaken for malignant lymphoma. The key to this differential diagnosis is the same as for follicular cervicitis in the Pap smear and consists in finding a range of maturation of the lymphoid cells and tangible body macrophages (Demay, 1996). When inflammation is severe, a protein-rich exudate, with deposition of fibrin and finely granular precipitate, may occur. In addition, mucus, bacteria, inflammatory cells, and lysed cells may cause the "dirty background" of inflammation to closely resemble a tumor diathesis (Demay, 1996).

CONCLUSION

In conclusion, this study shows majority of the cytologic changes in the immunocompromised patients were mild inflammation and moderate inflammation which might be due to the infection with *Mycobacterium tuberculosis*, and *Aspergillus* species as the pathogens that were detected.

RECOMMENDATIONS

There is need to include mycology investigations (cytochemistry mainly GMS) as a routine investigations for HIV patients so as reduce the disease burden. Sputum cytology should be used for preliminary diagnoses of opportunistic pathogens before confirmatory test.

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