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PULMONARY CANDIDIASIS AND CD₄ COUNT IN HIV POSITIVE PATIENTS SEEN IN JOS, NORTH CENTRAL NIGERIA

Peter YJ,¹ Isa AH,² Anzaku AS,³ Builders MI⁴

¹Department of Medical Microbiology and Parasitology, ²Department of Haematology and Blood Transfusion, ³Department of Obstetrics and Gynaecology, ⁴Department of Pharmacology and Therapeutics, College of Health Sciences, Bingham University Teaching Hospital, Jos.

Corresponding author: Dr. YJ Peter. drjonahp@gmail.com. Department of Medical Microbiology and Parasitology, College of Health Sciences, Bingham University Teaching Hospital, Jos.

ABSTRACT

Background: Accurate and reliable diagnosis of HIV opportunistic infections plays a central role in effective HIV intervention programmes. Pulmonary infections are the leading cause of morbidity and mortality in HIV infected individuals.

Objectives: We set out to determine the prevalence of Pulmonary candidiasis by isolating *Candida* species from the sputum of HIV sero-positive patient's presenting to hospital with complaint of cough for more than two weeks and related the level of CD₄ count to Pulmonary candidiasis.

Methods: Using sterile wire loop, each sputum sample was inoculated into duplicate SDA (Thermo Scientific, UK); one tube without antibiotics, another tube supplemented with Chloramphenicol (0.05%) and Cycloheximide (0.5%) antibiotics. The patient's CD₄ count was determined using a Cyflow machine (PARTEC[®], Germany).

Result: Fifty two (54.2%) female and 44(45.8%) male HIV positive subjects were compared with a control group made up of, 52(54.2%) female and 44(45.8%) male HIV negative subjects. Twenty one (21.9%) HIV positive subjects had *Candida* species in their sputum samples compared to 12(12.5%) in the HIV negative subjects. Among HIV positive subjects, 17(17.7%) had *Candida albicans* isolated from their sputum, 11(11.5%) of whom had a CD₄ count of <200 cells/ul.

Conclusion: We concluded that, there is a risk of pulmonary candidiasis occurring in HIV infected patients with CD₄ count <200cells/ul and that, *Candida species* contributes to chronic cough experienced by HIV infected patients. We recommend that HIV positive patients who have chronic cough and whose CD₄ count is <200cells/ul be placed on systemic antifungal medication.

Keyword words: Candida, Cough, Nigeria, Sputum

CANDIDOSE PULMONAIRE ET NUMERATION LYMPHOCYTES CD₄ CHEZ LES PATIENTS VIH OBSERVES AU NORD - CENTRALE DE JOS, NIGERIA.

Peter YJ,¹ Isah AH,² Anzaku AS,³

¹Département de Microbiologie et Parasitologie Médicale, ²Département d'Hématologie et de transfusion sanguine, ³Département et de gynécologie de Bingham, Jos.

Auteur Correspondant : Dr Y.J Peter . Département de Microbiologie et de Parasitologie Médicale, Collège des Sciences de la sante, l'Universitéhôpital d'enseignement de Bingham, Jos. drjonahp@gmail.com

RÉSUMÉ

CONTEXTE : Diagnostic précis et fiable des infections opportunistes joue un rôle central dans l'efficacité du programme d'intervention de VIH. Les infections pulmonaires sont les causes principales de morbidité et mortalité chez les individus infectés par le VIH.

OBJECTIFS : Nous avons cherché à déterminer la prévalence de Candidose Pulmonaire en isolant les espèces de *Candida* des expectorations des patients VIH séropositifs présentant à l'hôpital les plaintes de toux pendant plus de deux semaines et lie le niveau de numération lymphocytes CD₄ a la Candidose Pulmonaire.

METHODES : Utilisant boucle de fil stérile, chaque échantillon d'expectoration a été inoculé en double exemplaire SDA (Thermo Scientific, UK) ; un tube sans antibiotiques, un autre tube complété d'antibiotiques Chloramphénicol (0,05%) et Cycloheximide (0,5%). La numération lymphocytes CD₄ du patient a été déterminé en utilisant un Machine CyFlow (PARTEC[®], Germany).

RESULTAT : Cinquante - deux (54,2%) sujets féminins et 44(45,8%) sujets masculins VIH positifs ont été comparés à un groupe contrôlé composé de 52(54,2%) sujets féminins et 44(45,8%) sujets masculins VIH

négatifs. Vingt et un (21,9%) sujets VIH positifs ont eu des espèces *Candida* dans leurs échantillons d'expectoration comparativement à 12 (2,5%) dans les sujets séronégatifs. Parmi les sujets séropositifs, 17 (17,7%) ont eu *Candida albicans* isolé de leur expectoration, 11 (11,5%) d'entre eux ont eu la numération lymphocytes CD₄ de <200 cellules/ul.

CONCLUSION : Nous avons conclu qu'il ya une risque de Candidose Pulmonaire se produisant chez les patients infectés par le VIH de numération lymphocytes CD₄ de <200 cellules/ul et que, *Candida species* contribue à une toux chronique vécué par les patients infectés par le VIH. Nous recommandons que les patients séropositifs qui ont une toux chronique et dont la numération lymphocytes est <200 cellules/ulètre place sur l'antifongique systématique médicament.

Mots - Clés : *Candida*, La toux, Nigeria, Expectoration.

INTRODUCTION

Candida species have been increasingly recognized as a source of fungal pneumonia in patients with acquired immune deficiency syndrome (1,2). Candidiasis is a common endogenous opportunistic yeast infection (3). Candidiasis may present as an acute, chronic, superficial or disseminated infection (2). It is a worldwide infection affecting all age, sex and occupational groups. Pulmonary infections are the leading causes of morbidity and mortality infections in HIV infected individuals (4,5). Pulmonary candidiasis is characterized by low grade fever, cough with mucous and sometimes bloodstained sputum as well as pleural effusion. Of the causative agents, the most common is *Candida albicans* (3). While other *Candida* yeast may occasionally cause clinical disease, *Candida albicans* is the organism isolated from most patients (6). *Candida* species are true opportunistic pathogens that exploit recent technological advances to gain access to blood circulation and deep tissues (7,8).

Soon after the Acquired Immunodeficiency Syndrome (AIDS) was first described in 1981, it became clear that opportunistic infections (OIs) occurred with remarkable frequency and caused substantial morbidity and mortality among patients with AIDS (9,10). Many of the OIs in adults are usually secondary to activation of "innocent" pathogens, which were commensals existing passively at a time when host immunity was intact, before the acquisition of HIV infection (11). Clinical AIDS is a common finding in many HIV health care facilities in Nigeria (12,13).

Among the various OIs, respiratory infections account for up to 70% of AIDS defining illnesses and their relative importance differs in different parts of the world (14,15). Respiratory OIs are a common manifestation of HIV/AIDS in Nigeria (16). Sixty percent of patients seen in the Jos University Teaching Hospital (JUTH) with AIDS presented with respiratory tract infections, ranging from sinusitis, to upper and lower respiratory tract infections (17).

A diagnosis of *Candida* pneumonia is difficult to establish in immune compromised patients (1). Making a diagnosis of candidiasis may often be

difficult, but the risk factors associated with the condition are well known and are commonly found in the intensive care units (18). The presence of one or more risk factors should therefore heighten clinical suspicion (14). Clearly, a thorough understanding of the natural history of AIDS related OIs and a comprehensive analysis of the pace and quality of immune recovery in each patient is required for optimal management of this condition (15).

In tropical medical practice, chronic cough, fever and weight loss presenting in a clinic, raises a suspicion of pulmonary tuberculosis (PTB) until ruled out, given the prevalence of PTB in the environment. Therefore sputum acid fast bacilli (AFB) examinations as well as chest x-ray (CXR) are ordered for; the patient is often started on anti-tuberculosis therapy immediately. In the absence of determination of other cause, even when sputum returns negative for AFB, treatment is continued empirically anyway (17). When the response to the anti-tuberculosis treatment is poor or absent, the tendency is more likely to be to doubt the patients drug compliance, genuineness of the drugs used or suspect drug resistant PTB. Cases like this are common in tropical medical practice and pulmonary mycosis is rarely thought of as a likely cause of these symptoms (18,19).

The HIV/AIDS patients presenting with respiratory tract symptoms like cough, breathlessness and chest pain will need detailed investigations including sputum microscopy (Gram and AFB staining), culture and chest X-ray (CXR).

To date, few, if any, rigorous studies on the causes of chronic cough in African, treatment-naive, HIV infected patients have been performed (20,21). As regards the diagnosis of pneumonia, there is little information about the role of *Candida* species isolated from respiratory samples, and criteria for the diagnosis of *Candida* species pneumonia are still to be defined (22,23). Such analyses are helpful for determining the preventive medications and treatment needed for HIV- infected persons (24).

The aim of this study was to determine the prevalence of *Candida* species in the sputum of HIV sero-positive patients and relate their Cluster

of Differentiation (CD₄) cell count to presence or otherwise of *Candida* species in the subject.

MATERIALS

Patients from 18 years old who presented to JUTH ARV treatment clinic and who have been coughing for at least two weeks were referred to a TB Reference Laboratory for sputum AFB microscopy, culture and sensitivity. These were the candidates that were approached to be recruited and asked for consent to be included in this study.

At the TB reference laboratory, patients included were selected statistically, using simple random sampling method (25,26). Patients on anti-tuberculosis or anti mycoses treatment, were excluded as well as any patient who had ever smoked or is currently smoking cigarette, any who presents with pedal edema or history of heart failure. Written informed consent was obtained and it was made clear to each patient that he/she was free to opt out from the study at any time.

Sputum samples were obtained from 96 HIV sero positive patients (case) who have been coughing for at least two weeks. A matching patient based on sex and age group presenting on the same day, who is HIV negative from other clinics presenting to the TB reference laboratory, at the same period are selected as a control. Patients who later failed to produce up to three fresh sputum samples as instructed were excluded. One hundred and five patients who fulfilled the inclusion criteria were recruited for the study; the extra 9 patients were added to make room for attrition during the study. Baseline blood samples were taken for CD₄ lymphocyte count. Ethical clearance for the study was obtained from the Jos University Teaching Hospital's Ethics Committee.

Two hundred and eighty eight (96x3) sputum samples and 96 baseline blood samples for CD₄ count were obtained from these patients. Similarly 288 sputum samples were collected from HIV negative control patients who had chronic cough, fever and weight loss.

LABORATORY PROCEDURE

The patients' confidence was boosted through explanation of the reasons for the collection of the sputum. Three separate sputum specimens were collected from each patient (spot, morning, spot) (27,28). A gram stained smear was immediately prepared from each freshly produced sputum sample submitted to assess the quality of the sputum before it was accepted for the study. Then using a sterile wire loop, the sputum was inoculated into duplicate Sabouraud Dextrose agar tubes (SDA, Thermo scientific, UK); a tube without antibiotics, a tube supplemented with

Chloramphenicol (0.05%) and Cycloheximide (0.5%) antibiotics. Yeast growth to be analysed is collected and emulsified in sterile water to McFarland 0.5 equivalent. The analytic profile index (API) 20C Aux (Biomérieux, France) was then used to identify the yeast encountered (29). This was guided by the accompanying manufacturers database (V4.0) – with the API looked up and the numerical profile determined from the list of provided profiles.

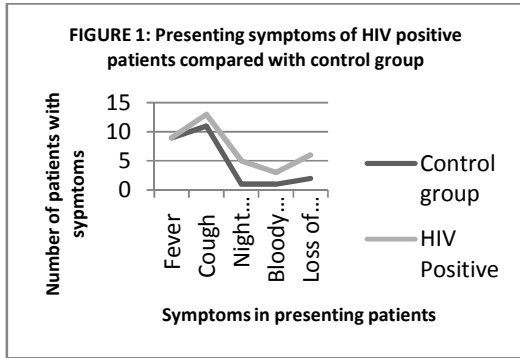
Using lavender coloured EDTA vacutainer, about 5mls of blood was withdrawn using the cubital fossa vein of each of the selected patient. The patient's CD₄ count was determined using a Cyflow machine (PARTEC[®], Germany), observing peaks generated and the count read after the run from the Cyflow monitor screen. Data was analysed using SPSS statistical package. Proportions were compared using X² test, differences at the 5% level being regarded as significant (25,26).

RESULTS

Fifty two (54.2%) females and 44 (45.8%) males HIV positive patients were compared with a control group made up of, 52(54.2%) females and 44(45.8%) males HIV negative patients. Among the cases 49 (50.7%) females and 34 (35.6%) males were below the age of 45 years. Above the age of 45 years, 3 (3.1%) females and 10 (10.4%) males presented. Cases between the ages of 30-39 years formed the largest age group presenting with 25 (26.0%) females and 20 (20.5%) males.

Of the cases studied, 95 (99.0%) presented with cough compared to 93 (96.9%) in the control group, 75 (78.1%) presented with fever compared to 73 (75.0%) in the control group, 6 (6.3%) with Loss of weight compared to 46 (47.9%) in the control group, 4 (4.1%) with Bloody sputum compared to 20 (20.8%) in the control group and 3 (3.1%) with Night sweats compared to 41 (42.7%) in the control group.

Twenty one (21.9%) HIV positive patient's sputum samples had *Candida* species compared to 12 (12.5%) samples in the control group. Seventeen (17.8%) patient's sputum samples out of the 96 HIV positive patient's samples had *Candida albicans* compared to 10 (10.4%) in the control group. All the 17 HIV positive patients who had *Candida albicans* isolated, from their sputum presented with cough, 69.2% presented with fever and only one patient presented with Bloody sputum (Figure 1).



Nine (9.4%) of the HIV positive patients who had *Candida albicans* isolated from their sputum were between the ages of 30-39 years, all were females. At $P < 0.05$ these results are statistically significant. The age group of 30-39 contributed 9 (9.4%) out of 45 (46.9%) of patients from whom *Candida albicans* was isolated. This was followed by 4 (4.1%) out of 18 (18.8%) patients within the age group of 30-34 (Table 1).

TABLE 1: Distribution of *Candida albicans* isolates within the age grouping of HIV positive patients.

Age group	With Candida Total (%)	Without Candida Total (%)
15-19	0 (0.0)	0 (0.0)
20-24	3 (3.1)	10 (10.4)
25-29	3 (3.1)	12 (12.5)
30-34	4 (4.2)	13 (13.5)
35-39	5 (5.2)	21 (21.8)
40-44	0 (0.0)	11 (11.4)
45-49	2 (2.1)	7 (7.3)
50+	0 (0.0)	5 (5.2)
Total	17 (17.7)	79 (82.3)

Among the 17(17.7%) HIV positive patients who had *Candida albicans* isolated from them, 11 (11.5%) had a CD_4 count < 200 cells/ul (8 females, 3 males). At $P < 0.05$ these results are statistically significant (Table 2).

TABLE 2: Distribution of CD₄ count by *Candida albicans* isolated among HIV positive patients

Candida albicans isolates from

CD ₄ count(Cell/ul)	Males	Females	Total (%)
<200	3	8	11 (64.7)
200-500	4	2	6 (35.3)
Total	7	10	17 (100)

X² = 6.9 d.f. = 1 P < 0.05

DISCUSSION

The study set out to determine the prevalence of Pulmonary candidiasis by isolating *Candida* species from the sputum of HIV sero-positive patient's presenting to hospital with complaints of cough for more than two weeks and relate the level of CD₄ count to Pulmonary candidiasis. There were more female HIV positive patients reporting to clinic than were male patients. The most common presenting symptom among Pulmonary Candidiasis patients was cough, closely followed by fever and loss of weight.

The main finding in this study was that HIV positive patients in Jos had *Candida* species in their sputum, compared to the HIV negative control group. This figure varies from findings of other parts of the world, where similar studies were carried out. A recognized difficulty in studies of this type is the method of specimen collection and the sterility of the sputum container used (4,7). In our study the patients were provided with sterile bottles and only the patients whose three sputum sample pairs yielded *Candida* species were accepted as positive for *Candida species*.

Our finding is much lower than the 35.5% *Candida* species isolated by Nwabuisi and Ologe in Ilorin, Nigeria (30). Castro and Martinez in 2008 detected 47.5% candidiasis in Brazil, and 60% was reported in the USA (2,14). The finding is however higher than that found in Taiwan, and India (31,32).

Fungi isolated from sputum may represent either pathogens or saprophytes (32). The prevalence and prognosis of pulmonary fungal infection has been difficult to evaluate since diagnoses was seldom confirmed (31).

This study agrees with established finding of other studies that *Candida albicans* is the most commonly isolated strain of *Candida*, both as a colonizer in the general population and as a pathogen in patients infected with HIV (3,4,32).

In this study the median CD₄ count among HIV positive patients was lower than found in Zaire (15). Many (64.7%) of our patients who had CD₄ count of <200cell/ul, had chronic cough and their sputum yielded *Candida albicans* (12,15). This finding in our study suggests that the severity of HIV infection is directly proportional to the degree of immune deficiency as indicated by the CD₄ count of the patient (8,11,14).

CONCLUSION

We concluded that a high number of HIV infected patients had *Candida species* isolated from their sputum and had CD₄ count <200cells/ul. This suggests that, *Candida species* contributes to chronic cough experienced by HIV infected patients and that, the most predominant *Candida* species isolated was *Candida albicans*.

LIMITATIONS

The limitation of the study was that only HIV positive patients who presented to the clinic were captured in the study, other HIV positive patients who were too sick were admitted directly for in-patient care from the emergency care unit.

RECOMMENDATIONS

We recommend that HIV positive patients who have chronic cough and whose CD₄ count is <200cells/ul be commenced on prophylactic systemic antifungal medications until their CD₄ count reaches 500cells/ul.

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