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Fungal Infections Epidemiology in A Population of PLHIV Hospitalized in the Centre Hospitalier Universitaire De Libreville / Gabon

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ABSTRACT

Opportunistic infections are important causes of death in HIV-infected patients. Moreover, management of the patients depends on knowledge of current epidemiology, risk factors, diagnosis and appropriate treatment. The aim of this study is to determine fungal opportunistic infection and associated factors among HIV-infected patients hospitalized in the infectious diseases ward of the Centre Hospitalier Universitaire de Libreville in Gabon. A cross-sectional study was carried out from April to October 2021. The study population was composed of patients admitted to the infectious diseases ward. Data including patients' demographics and clinical characteristics, comorbidities, existing ART or antifungal therapies were collected. For each participant, samples have been collected and laboratory tests were performed to detect Candida and Cryptococcus infections.

Of the 255 patients, 72.5% (n=185) were female and 86.2% (n=220) adults. Most of the patients were at WHO clinical stage I (45.5%) and III/IV (42.4%). More than half of the patients (n=122) had a CD4 cells count ≤ 200. ART intake was frequently reported by the participants (n=216) and one-third received antifungal therapy. Among patients, 211 had OIs; 29 (11.4%) were co-infected. Oropharyngeal candidiasis was diagnosed in 80.3% (n=205) of the inpatients. Neuromeningeal cryptococcosis affected 24.6% (n=35) of the participants, almost (82.8%; n=29/35) had candidiasis. A decreasing CD4 cell count was significantly associated with higher prevalence of Oropharyngeal candidiasis and coinfections..

Keywords

Oropharyngeal candidiasis, Neuromeningeal cryptococcosis, HIV, Gabon.

Introduction

Acquired immunodeficiency syndrome (AIDS) is still a growing public health concern. Indeed, near of 40 million of people are living with HIV worldwide at the end of 2022, and most of them are located in Africa [1].

The advent of antiretroviral therapy (ART) has significantly improved the expectancy and quality of life for many individuals with HIV [2,3]. AIDS is now considered as a chronic and controllable disease because of ART. Nevertheless, this disease is characterized by a profound immunodepression, which renders infected people more susceptible to opportunistic infections (OIs). The most common are tuberculosis (TB) and fungal infections. Among these last, the ones due to *Candida* species, *Cryptococcus neoformans* (*C. neoformans*), and *Aspergillus fumigatus* account for most of the life-threatening infections in immunocompromised individuals [4-7]. Although, *C.albicans* has been considered as the most commonly isolated organism, some studies have shown that

non-albicans *Candida* (NAC) species, including *C. tropicalis*, *C. krusei*, and *C. glabrata*, significantly account for infections [8-11]. Indeed, the rise of 'non-albicans' species of *Candida*, or other species of Cryptococcus which may be resistant or less sensitive to antifungal agents have been reported [10,11]. The interaction between HIV infection and other infectious diseases is a critical factor influencing clinical outcomes [12-14]. Fungal infections contribute to high mortality, even among patients on antiretroviral therapy in low-resource regions [2,9]. Candidiasis occurs in various clinical entities from mucocutaneous to invasive forms and *Cryptococcus neoformans* (*C.neoformans*) mainly induces Neuromeningeal Cryptococcosis (NMC) [13,14]. The analysis of OIs may provide useful information on circulating pathogens and causes of death in HIV-infected patients. Moreover, management of the patients depends on knowledge of current epidemiology, risk factors, diagnosis and appropriate treatment.

In Gabon, valuable data are necessary for the implementation of an algorithm based on the detection of Oro-Pharyngeal Candidiasis and Neuromeningeal Cryptococcosis for the management of fungal infections in HIV infected patients. The aim of this study is to provide an overview of fungal opportunistic infection and associated factors among HIV-infected patients hospitalized in the infectious diseases ward of the Centre Hospitalier Universitaire de Libreville in Gabon.

Material and Methods

Study Period and Site

Study Site and Population

A cross-sectional study was carried out from April to October 2021. The study population was composed of patients admitted to the infectious diseases ward in the Centre Hospitalier Universitaire de Libreville (CHUL), the biggest hospital of Libreville, the capital city of Gabon. PLHIVs aged above 18 years and clinically suspected of OPC and NMC were included. The admitted patients completed and gave a written informed consent. Data including patients' demographics and clinical characteristics, comorbidities, existing ART or antifungal therapies were collected. In addition, HIV-specific information such as CD4 cell count was also documented. After the interview of each participant, samples have been collected and diagnosis of candidiasis and Cryptococcus infection were performed in the Department of Parasitology, Mycology and Tropical Medicine of the Université des Sciences de la Santé, (Gabon). Each patient received a unique code for identification.

Sampling and identification of *Candida* sp

All participants received an oral mucosal examination. Oral samples were collected using sterile swabs and then transferred into transport medium. Swab samples were taken by rubbing the entire surfaces of the lesion. the swabbed areas sampled depended on the size of the lesion. Swab samples were plated and cultivated on Chromatic™ *Candida* (LIOFILCHEM), a selective and differential chromogenic medium for *Candida* species. Chromatic™ *Candida* detects a specific enzymatic activity, which leads to the presumptive identification of several clinically important *Candida* species including *C. albicans* based on colony colour and morphology after an incubation of 18-24 hours at 37°C.

Sampling and *Cryptococcus* Infection Diagnosis

For *Cryptococcus* antigen detection, each participant provided a single urine specimen collected in the morning. The clean and sterile urine container were identified and transferred to the laboratory in an icebox for processing within two hours. The antigen detection was done using the Pastorex Crypto plus (Bio-Rad). Briefly, this qualitative and semi-quantitative agglutination test which detects the capsular glycuronoxylomannan polysaccharide of *Cryptococcus neoformans* in biological samples including urine, was used according to the manufacturer's instructions. Briefly, the urine is diluted in a glycine buffer. Afterwards, 40 µl of the sample is added on a circle on the agglutination map and mixed with a drop of *Cryptococcus* latex which is a latex particles sensitized with an anti-glucuronoxylomannan monoclonal antibody (mouse), in a glycine buffer. A negative control was done by mixing 40µl of dilution buffer with a drop of latex. The card is then placed on a shaker for 5 minutes (160rpm), at room temperature (+14-30 °C). The agglutination results visible to the naked eye were obtained after five minutes.

Statistical Analysis

Data were collected onto Microsoft Excel by double entry procedure. Statistical analyses were done using Stat view 05. Prevalence of OIs were assessed. Association between the presence of OIs and baseline characteristics was analysed using Chi2 or Fisher exact test. All reported p-values were two-tailed, and a p-value < 0.05 was considered statistically significant.

Ethical Considerations

This study was approved by the scientific and ethics committee which was the legal entity to authorize research activities during the COVID-19 epidemic phase, under the number PROT00064/2020/P/CSCOVID/P. An authorization was also obtained from the CHUL Director of Medical Affairs. A written informed consent was obtained from each patient. The diagnosis of fungal infections was free of charges and the results were sent to the ward physicians for patient appropriate management.

Results

Patients characteristics

Of the 255 patients, 72.5% (n=185) were female and 86.2% (n=220) were adults aged of 55 years old or less. According to the marital status, the level of education and the professional activity: 81.2% were single, 62.7% had a secondary school level and 77% had an employment ($p>0.05$). Considering their habits or lifestyle, less than two third (63.9%) of the participants did not use alcohol while, almost 90% of them did not smoke.

The frequency of patients at WHO clinical stage III/IV was high (42.4%). Half of the participants (n=125) were infected since 6 years or more and almost one third (n=81) discovered their HIV infection within the last 2 years (Table 1). CD4 cell count was available for 222 patients. More than half of the patients (n=122)

had a CD4 cells count ≤ 200. ART intake was frequently reported by the participants (n=216). The most frequent therapy used was TDF-3TC associated with DTG (64%) or EFV (4.3%) or LPV/r (1.2%). One third (n=87, 34.2%) of the PLHIV were under antifungal therapy.

Among the patients presenting with other conditions, 2.3% were diabetic. Kaposi sarcoma was reported in 4 patients while cerebral toxoplasmosis was found in 35 patients (13%) and tuberculosis in 47 (18%). Almost 10% (n=25) of patients had malaria. Anaemia was found in 19 PLHIV in the study. Similar proportion of participants had respiratory infections (n=17, 6.7%).

Table 1: Patients characteristics according to the presence or absence of opportunistic Mycosis (oral candidiasis and neuromeningeal cryptococcosis)

	All	NMC n (%)	OPC-NCM n (%)	OPC n (%)	No opp mycosis n (%)	P-value
Gender, n (%)						**0.66**
Female	185(72.6)	3(50.0)	21(72.4)	129(73.3)	32(72.7)	
Male	70(27.4)	3(50.0)	8(27.6)	47(26.3)	12(27.3)	
Age groups, n (%)						**0.55**
> 55 years	35(13.7)	1(16.6)	5(17.3)	24(13.7)	5(11.3)	
< 55 years	220(86.3)	5(83.4)	24(82.7)	152(86.3)	39(88.6)	
Marital status, n (%)						**0.2**
Single	207(81.2)	4(66.7)	27(93.1)	145(82.4)	31(70.4)	
Married	39(15.3)	2(33.4)	2(6.9)	26(14.8)	9(20.5)	
Widowed	9(3.5)	0(0.0)	0(0.0)	4(3.6)	4(9.1)	
Occupation, n (%)						**0.82**
Worker	110(43.2)	4(66.8)	11(37.9)	75(42.6)	20(45.5)	
Unemployed	84(32.9)	1(16.6)	12(41.4)	56(31.8)	56(34.1)	
Manager	61(23.9)	1(16.6)	6(20.7)	45(25.6)	9(20.4)	
Education, n (%)						**0.87**
Primary	62(24.4)	2 (33.3)	7 (24.1)	42 (23.8)	11(25.0)	
Secondary	160(62.7)	4 (66.7)	19 (65.5)	112 (63.6)	25 (56.8)	
Higher	33(12.9)	0 (0.0)	3 (10.4)	22 (12.5)	8 (18.2)	
ART, n (%)						**0.4**
Yes	216(84.7)	6(100.0)	26(89.6)	145(82.4)	39(88.6)	
No	39(15.3)	0(0.0)	3(10.4)	31(17.6)	5(11.4)	
HIV diagnosis, n (%)						**0.04**
1-2 years	81(31.7)	0(0.0)	10(34.4)	61(34.7)	10(22.7)	
3-5 years	49(19.2)	4(66.6)	6(20.7)	28(15.9)	11(25.0)	
≥ 6 years	125(49.1)	2(33.4)	13(44.9)	87(49.4)	23(18..3)	

***NMC:** Neuromeningeal cryptococcosis,
***OPC**: oropharyngeal candidiasis,
***No opp mycosis**: No opportunistic mycosis

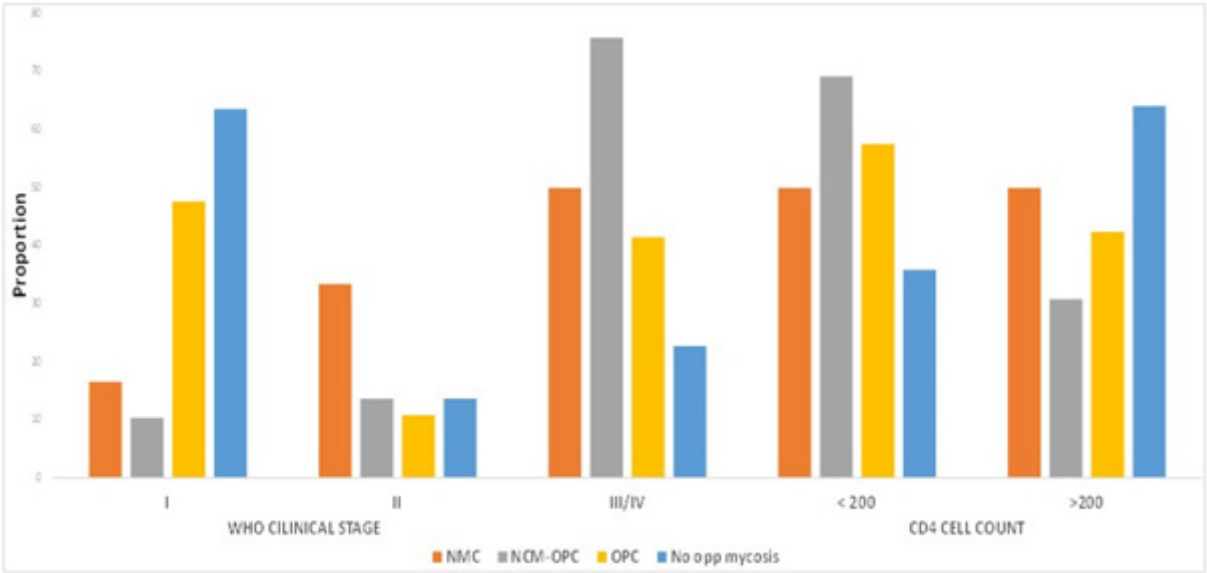

Figure 1: Distribution of Prevalence of OIs according to WHO clinical stages and CD4 counts cells.

Prevalence of Opportunistic Infections (OIs)

Among patients, 211 had OIs; 29 (11.4%) were co-infected. Oropharyngeal candidiasis (OPC) was diagnosed in 80.3% (n=205) of the inpatients. *Candida albicans* (*C.albicans*) was detected in 132 patients and non *C.albicans* infection was found in 73. Neuromeningeal cryptococcosis (NMC) affected 24.6% (n=35) of the participants, more than 80% (82.8%; n=29/35) had candidiasis.

Single infection was diagnosed in 182 inpatients including 6 with NMC and 176 with OPC. The frequency of single and co infection did not vary according to the age (p=0.9) (Table 1).

Occurrence of OIs in patients was related to the HIV infection diagnosis period. The association between OIs and CD4 cells count was found. Patients without OIs had more frequently higher CD4 cell count (>200). A decreasing CD4 cell count was significantly associated with higher prevalence of OPC and coinfection NMC-OPC. Coinfected participants had more frequently CD4 cells count ≤ 200 (69.2%; n=18/26) (p=0.03) (Table 1, Figure1). Nevertheless, prevalence of NMC increased with HIV clinical stage. Three quarter (n=22/29) of coinfected patients were at WHO stage III/IV (p=0.0002). Among the patients with HIV infection since 6 years or more, almost half had OPC (n=87/176, 49%) or were coinfected (n=13/29, 44.8%), while only 2 (33%; n=2/6) of them had only NMC (p=0.04). Tuberculosis was two times less frequent in patients with OPC (n=29/176; 16.4%) compared to those with co infection (31%) or single NMC infection (33.3%).

Discussion

Opportunistic fungal diseases remain a health problem in sub-Saharan Africa, notably in people living with HIV (PLHIV) [1]. In this study, the prevalence of *Cryptococcus neoformans* and *Candida* sp was determined in hospitalized patients in CHUL. Oral candidiasis associated with HIV infection occurs frequently and could be considered as an initial manifestation of the disease.

The majority of HIV seropositive patients were less than 55 years and most of them were female and single. These patients admitted at hospital were mainly at WHO clinical stage III/IV. As reported by others, HIV infected patients at WHO III/IV stage had frequently low CD4 cell count [15]. Half of the participants admitted at hospital have been diagnosed HIV infection since at least 6 years; half had a CD4 count cell ≤200. Altogether, these data suggest a lack of compliance of the participants for their treatment and for their follow-up care.

Indeed, more than 90% of the patients had OIs. In the present study, oropharyngeal candidiasis and Cryptococcal meningitis were found in 80.3% and 24.6% of the inpatients, respectively. High prevalence of oropharyngeal candidiasis has also been reported in previous studies carried out years ago in Tehran, Iran (74.5% in 2008-2009) and in Treichville, Cote d'Ivoire (79.4% in 2011) [16,17]. Infections were mainly due to *C.albicans*, non albicans species and *C.neoformans*. In a previous study performed in Gabon in 2010, opportunistic infections were identified in 458 patients. Pulmonary tuberculosis, herpes zoster, cerebral toxoplasmosis, oral candidiasis, and severe pneumonia accounted for 9% to 22% of the patients. The less frequent infections were Cryptococcal meningitis and pneumocystosis which were found in 0.44% and 0.21% of the patients, respectively [18]. In India, in a tertiary care hospital, among OIs, the most frequently found were due to *Candida* species (86.0%), followed by Aspergillus (6.5%) and Cryptococcus (3.3%) [19]. In Iran, *Candida* species recovered from oral cavity of HIV/AIDS patients were *Candida albicans* the commonest of Candida species, followed by *C. dubliniensis, C. tropicalis, C. glabrata, C. kefyr* and *C. Africana* [20]. Non albicans species have been reported in previous studies to be strongly pathogens [21]. In some studies, the emergence of 'non-albicans' species of *Candida*, and other species of *Cryptococcus* which may be resistant or less sensitive to antifungal agents has been highlighted.

The high proportion of oropharyngeal candidiasis, among these patients in the CHUL, underlines the need to increase the mindfulness of clinicians in diagnosis and early treatment of these infections in order to improve and/or to adopt an appropriate management of the patients especially in resource limited countries. Almost all of the patients of the CHUL were under ART, notably TDF-3TC and more than one third were under antifungal therapy. All the patients with NMC were under ART and more than 80% of those with OPC or coinfected received antiretroviral treatment. Such high prevalence of OIs in these patients may be due in defaulting from care as reported by Sabin Nsanzimana et al. [22]. A greater understanding of adherence to interventions (treatment and care) for these patients in critical situation is essential. As previously reported, oropharyngeal candidiasis is an important clinical marker of the progression of HIV disease, being predominant in more than 50% of HIV positive person, as found here and by others, as well as a clinical surrogate for severe CD4+ depletion [23].

Indeed, in the present study, patients had frequently low CD4 cell count. It was obvious among coinfected patients. These last represented less than 15% of the participants but the increase of their proportion was inversely correlated with CD4 rate. Some authors showed that patients who presented HIV associated oral candidiasis without any type of Anti-Retroviral Therapy (ART) had a median CD4+ count lower than 200 cells/mm3, an associated inverse relationship between CD4+ count and the prevalence of OC could be inferred [24,25]. However, the absence of OPC does not necessarily exclude severe immunosuppression. Three quarters of co-infected patients were at stage III/IV AIDS. This is in agreement with the WHO classification, which associate opportunistic infections and the stage III/IV. The main limitations of our study may be the lack of diagnosis of other pathogens inducing OIs, and therefore a wide description and identification of the spectrum of the pathogens in these patients.

Conclusion

A wide spectrum of fungal infections was observed amongst HIV-infected patients. Oral candidiasis and Neuromeningeal cryptococcosis are the commonest fungal infections in these

patients, with non negligible prevalence of coinfections, suggesting the importance of early detection and of the management of people living with HIV, particularly those with signs of advanced immunosuppression. Reinforce the patient awareness for their adherence to care is crucial.

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