Journal of
Medical Research and Surgery

Research Article

Nkwoemeka NE, et al., J Med Res Surg 2020, 1:2

Fungal Isolates in Hiv Positive and Negative Subjects Attending Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Amaku, Awka, Anambra State, Nigeria

Ndidi Ethel Nkwoemeka[1], Chris Okwudili Anyamene[2], Izunna Somadina Okwelogu[3], Paschal Chiedozie Amakiri[4], Chisom Godswill Chigbo[2] and John Osilumie Dirisu[5]

[1]*Department of Microbiology/Natural Sciences,School of General Studies, University of Nigeria Nsukka, Enugu State, Nigeria*

[2]*Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University Awka, Nigeria*

[3]*Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka, Nigeria*

[4]*Chester Medical School, University of Chester, United Kingdom*

[5]*Department of Medical Laboratory Science, College of Medicine, Ambrose Alli University, Ekpoma, Nigeria*

Correspondence to: Paschal Chiedozie Amakiri, Chester Medical School, University of Chester, United Kingdom, **Tel:** +447721652992; **E-mail:** 1818021@chester.ac.uk

Received date: January 25, 2020; **Accepted date:** January 31, 2020; **Published date:** February 15, 2020

Citation: Nkwoemeka NE, Anyamene CO, Okwelogu IS, et al. (2020) Fungal Isolates in HIV positive and negative subjects attending Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Amaku, Awka, Anambra State, Nigeria. J Med Res Surg. 1(2): pp. 1-9.

ABSTRACT

Background: Fungal pathogens are the leading cause of severe healthcare-associated infections in immunocompromised patients, especially from low-income countries like Nigeria. Acquired Immunodeficiency Syndrome (AIDS) arising from HIV infection is reputed to be a key in immune suppression globally. Presently, Anambra state is number 5 in HIV prevalence in Nigeria. This study was aimed at screening patients from the Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, for fungal infections. Samples were collected with consideration to gender, age, occupation, education status and clinical presentations.

Methods: Sampling was done according to global best practices. Patients' consent and details were obtained by signing a consent form and filling of questionnaires respectively. Key questionnaire details included socio-demographic and personal details, history of present illness, clinical signs and symptoms. All patients were screened afresh for HIV-1 and HIV-2 using an immuno-chromatographic technique with Determine kit (Alere, Japan) and Stat-pack kit (USA). Specimens screened include Urine, Blood, Oropharyngeal swab, Skin scraping, and Sputum. All samples were labeled, transported and processed within 1-2 hours of collection.

Results: A total of 1200 participants (610 males and 590 females) were examined. About 16.5% and 54.41% of the subjects examined were positive for HIV infections and fungal infections respectively. Overall, 233 males (19.41%) and 420 females (35.0%) had fungal infections. Fungal infections were most prevalent in patients between 30-39 years, 17.75%, followed by 40-49 years (15.0%). Infection was least prevalent in patients between 15-29 years at 5.0%. Farmers had the highest prevalence rate of fungal infection, though not significant when compared with those of traders, artisans and public servants (X^2=0.621; df=2; P>0.05). *Candida* species were the most prevalent fungal pathogen, accounting for 609 (67.1%) followed by *Aspergillus* spp. 234 (25.8%) and *Cryptococcus* species 31 (3.4%). *Penicillium* and *Fusarium* spp. were the least prevalent pathogens (1.9%). Oropharyngeal candidiasis was the most common fungal infection (49.9%-17.4% HIV negative and 32.46% HIV positive patients); followed by Pneumocystis pneumonia (28.80%-9.50% HIV negative and 19.30% HIV positive patients). Similarly, Cryptococcal meningitis was the least recorded infection at 16.81% (6.43% HIV negative and 10.38% HIV positive patients). Significantly, fungal infections were more prevalent in HIV infected patients (62.14%) compared to HIV negative patients (33.33%).

Conclusion: This study has revealed that HIV positive and negative patients are often affected with fungal infections in Anambra State, Nigeria. This may have contributed to impaired immune system in patients and development of AIDS in HIV infected patients. Poor personal hygiene and intimate association with household pets among subjects examined were the suspected sources of infection. Therefore, there is an urgent need for improved surveillance and monitoring of fungal infections in the area and in other Teaching Hospitals in the country as well as treatments of infected subjects..

Keywords:

Fungal pathogens, Immunocompromised, HIV patients, Healthcare-associated infections.

Introduction

Fungi are neither plants nor animals. They were once thought to be plants but are now classified as their own kingdom. Fungal pathogens especially *Candida* species are the leading cause of severe healthcare-associated infections in immunocompromised persons globally [1]. Infectious tropical diseases especially from

fungal organisms have a huge effect in terms of mortality and morbidity and impose a heavy economic burden on affected countries. These diseases predominately affect the world's poorest people [2]. Fungal infections are increasingly becoming common and yet often neglected in developing countries [3,4].

The fungus *Candida albicans*, a common cause of infection in mucosal tissues, forms long filaments called hyphae, comprised of tubular cells that are required for virulence [5]. However, a protein fragment released by filaments of the fungus *Candida albicans* destroys host cells [6]. Fungi are major contributors to the opportunistic infections that affect HIV/AIDs patients especially in settings where resources are insufficient or are not provided at all [7]. Opportunistic fungi cause lethal systematic infections and impose high medical costs on health systems [8].

Oropharyngeal Candidosis (OPC) is the most prevalent opportunistic fungal infection that is commonly found in HIV-infected patients, even in the 21st century. Wibawa, et al. [9] pointed out that fungal infections remain a clinical challenge in HIV patients with severe immune suppression. Globally, the phenomenon of HIV/AIDS is best viewed as a pandemic affecting nearly all the countries of the world. According to the annual report of the Joint United Nations Programme on HIV/AIDS (UNAIDS), there are approximately 36.7 million people worldwide living with HIV/AIDS at the end of 2016. Of these, 2.1 million were children (<15 years old), there were 1.8 million new infections and 1.0 million AIDS-related deaths [10].

Nigeria is a large, multi-ethnic, multicultural, multiplex and diversely confounding society. HIV/AIDS in Nigeria is one of the HIV sub epidemics in a generalized epidemic setting [11]. HIV infection and AIDS remain a disease of public health concern in Nigeria. This is because Nigeria is the most populous country in sub-Saharan Africa and its one of the countries highly affected by the HIV/AIDS scourge [12]. The estimated number of people living with HIV in Nigeria as of 2016 was 3.2 million, giving a prevalence rate of 2.9% among the adult population, only 51% of those living with HIV are accessing Highly Active Anti-Retroviral Therapy (HAART) [10]. Approximately, 160,000 people died from AIDS-related illnesses in Nigeria [10]. Anambra state is number 5 state in terms of HIV prevalence in Nigeria (A high prevalence of 30% of fungal opportunistic infection was recorded in HIV subjects attending a Tertiary Hospital in South-East, Nigeria [13].

The present study sought to determine the incidence and prevalence of fungal infections in immunosuppressed patients at the Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka

Materials And Methods

Study area

The study was carried in Anambra State, the eighth-most populous State in Nigeria, located in the South-East geopolitical zone of Nigeria (National Population Commission, 2006). The area is located between latitude 6° 20' 00" North and Longitude 7° 00' 00" East. It has a population of 2,061,961.984 (2,117.984 males and 2,059,844 females), (NPC, 2006). Anambra State has a tropical climate. The average temperature is 25.9°C while the average rainfall in a year is 1386 mm. A survey of existing health care facilities showed that there are private hospitals and a major teaching hospital/HIV Centre in Awka. This served as a referral center for the state and her environs.

Study Population

The study population comprised of HIV-positive and HIV-negative patients attending Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Amaku Awka. The State Teaching Institution located in Anambra State and serving patients of all socio-economic status in South Eastern, Nigeria. The hospital offers both general and fertility health services for the people of Anambra and its environs.

Study Design

This study was done to determine fungal infections in HIV-Positive and HIV-negative patients. At enrolment, informed consent was obtained and each study participant was asked to complete a questionnaire that consisted of socio-demographic and personal details, history of present illness, clinical signs and symptoms, etc. Patients were grouped using the WHO staging system which ranges from the asymptomatic stage (Stage I) to mild symptoms (Stage II), advanced symptoms (Stage III), and severe symptoms (stage IV) [14]. The Simple Random Sampling without replacement described by Shelabh [15] was used. HIV Positive and HIV negative patients were selected among all the patients attending the hospital within the specified period. Descriptive statistics of socio-demographic variables and characteristics of the sample populations were expressed. Male and female subjects aged between 15 and >60 years old attending Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Amaku Awka were recruited. Patients younger than 15 years of age, individuals who refused to answer the questionnaires, individuals with a history of urinary tract infection symptoms, Diabetes mellitus, hypertension, renal diseases or sickle cell disease were not recruited.

Ethical Clearance

Ethical approval for this study was obtained from the Hospital Management Board's Ethics Committee, Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Amaku Awka.

Specimen Collection

The Specimen collection was done through the assistance of experienced Medical laboratory scientists.

HIV Status of patients

The HIV status of the patients was confirmed by guidelines provided by the National AIDS Control Organization (NACO, Strategy III) [16].

Results

A total of 1200 patients were examined and 198 (16.5%) tested positive to HIV while 1002 (83.5%) tested negative to HIV. A total of 81 male and 117 female patients, representing 13.2% and 9.75% respectively tested positive to HIV and fungal infections. Cumulatively, 198 (16.5%) and 653 (54.41%) tested positive to HIV and fungal infections. The age group of study subjects ranged from 15 to >60 years. Infections with respect to age range were highest for 30-39 years, with 213 (17.75%) fungal infection and closely followed by 40-49 years 180 (15.0%). The least age range was >60 years which recorded 90 (7.5%). Fungal infection occurred most in subjects whose occupation was predominantly farming 183 (15.25%). The highest education status with fungal infection were the illiterates 17.08% (202) (Table 1).

The distribution of different fungal isolates among various clinical samples is found on Table 2. Nine hundred and eight organisms were isolated. Differences in the isolation rate were statistically significant. *Candida albicans* had the highest frequency 50.4% (458) followed by *Aspergillus fumigatus* 14.1% (128). Others include *Cryptococcus neoformans* 2.0% (18) and *Penicillium marneffei* 1.9% (17). The organism with the lowest frequency was *Fusarium oxysporum* 0.8% (7). In this study, out of a total of 2106 clinical specimens screened, *Candida* spp. was the most prevalent organism isolated from all the specimens; oropharyngeal swab 18.5% (168), sputum 9.4% (85), blood 19.5% (177), stool 3.4% (31), urine 8.1% (74) and skin 8.1% (74) followed by *Aspergillus* and *Cryptococcus* species. The organisms with the least prevalent rate were *Fusarium* 1.9%(17) and *Penicillium* 1.9% (17) species which were isolated from skin specimen only. The highest number of clinical specimen screened was urine with the prevalence of 6.70% (141) in HIV positive patients 6.70% (141) and HIV negative patients 14.29% (301) followed by oropharyngeal swab 4.80% (101) and 15.19% (320) from HIV positive and negative individuals respectively. The least clinical specimen was skin scraping with the prevalence of 6.74% (142) from HIV positive patients and 2% (42) from HIV negative patients (Table 3).

Weight loss was the most frequent clinical presentation from patients with a prevalence rate of 4.33% (52) in males and females 6.0% (72), followed by an oral ulcer. Neck rigidity was the least clinical presentation recorded (Table 4).

Table 1: Socio-Demographic characteristics of subjects.

Characteristics	No. of subjects screened (%)	No. Tested +ve for HIV No. (%)	No. Tested -ve for HIV No. (%)	No. Tested +ve for fungal Infections No. (%)	No. Tested -ve for fungal Infections No. (%)
Gender					
Male	590 (49.2)	81 (6.75)	509 (42.41)	233 (19.41)	357 (29.75)
Female	610 (50.8)	117 (9.75)	493 (41.08)	420 (35.0)	190 (15.83)
Total	1200 (100)	198 (16.5)	1002 (83.5)	653 (54.41)	547 (45.58)
Age (YEARS)					
15-29	170 (14.2)	26 (2.17)	144 (12.0)	60 (5.0)	110 (9.17)
30-39	278 (23.2)	62 (5.17)	216 (18.0)	213 (17.75)	65 (5.42)
40-49	310 (25.8)	48 (4.0)	262 (21.8)	180 (15.0)	130 (10.83)
50-59	240 (20.0)	39 (3.25)	201 (16.75)	110 (9.17)	130 (10.83)
>60	202 (16.8)	23 (1.91)	179 (14.92)	90 (7.5)	112 (9.33)
Total	1200 (100)	198 (16.5)	1002 (83.5)	653 (54.41)	547 (45.58)
Occupation					
Housewives	148 (12.3)	13 (1.08)	135 (11.25)	32 (2.67)	116 (9.67)
Students	168 (14.0)	20 (1.67)	148 (12.33)	14. (1.17)	154 (12.83)
Farmers	218 (18.2)	53 (4.42)	165 (13.75)	183 (15.25)	35 (2.92)
Civil Servants	298 (24.8)	46 (3.83)	252 (21.0)	158 (13.17)	140 (11.67)
Traders	195 (16.3)	43 (3.58)	152 (12.67)	130 (10.83)	65 (5.41)
Artisans	173 (14.4)	23 (1.92)	150 (12.5)	136 (11.3)	37 (3.08)
Total	1200 (100)	198 (16.5)	1002 (83.5)	653 (54.4)	547 (45.58)
Education Status					
Primary	281 (23.4)	59 (4.92)	222 (18.5)	198 (16.5)	83 (6.91)
Secondary	371 (30.9)	39 (3.25)	332 (27.67)	140 (11.67)	231 (19.25)
Tertiary	335 (27.9)	32 (2.67)	303 (25.25)	110 (9.17)	225 (18.75)
Illiterates	213 (17.8)	68 (5.67)	145 (12.08)	205 (17.08)	8 (0.67)
Total	1200 (100)	198 (16.5)	1002 (83.5)	653 (54.4)	547 (45.58)
Religion					
Catholic	525 (43.8)	95 (7.9)	430 (35.8)	253 (21.08)	272 (22.66)
Protestants	309 (25.8)	50 (4.17)	259 (21.58)	180 (15.0)	129 (10.75)
Muslim	80 (6.7)	9 (0.75)	71 (5.92)	40 (3.33)	40 (3.33)

Pentecostal	175 (14.6)	24 (2.0)	151 (12.58)	95 (7.9)	80 (6.67)
Other	111 (9.3)	20 (1.67)	91 (7.58)	85 (7.08)	26 (2.17)
Total	1200 (100)	198 (16.5)	1002 (83.5)	653 (54.4)	547 (45.58)
Marital Status					
Single	255 (21.3)	51 (4.25)	204 (17.0)	200 (16.66)	55 (4.58)
Married	580 (48.3)	64 (5.33)	516 (43.0)	191 (15.91)	389 (32.42)
Separated/Divorced/ Widowed	365 (30.4)	83 (6.92)	282 (23.5)	262 (21.83)	103 (8.58)
Total	1200 (100)	198 (16.5)	1002 (83.5)	653 (54.4)	547 (45.58)
*Figures in brackets represent percentage prevalence of each group					

The system involvement in patients in relation to sex is presented on Table 5. The Gastrointestinal Tract (GIT) was mostly affected.The prevalence rate in male is 11.83% (142) and in female 14.75% (177) followed by the respiratory tract with male prevalence rate of 12.33% (148) and female 12.92% (155).The least was the skin with the prevalence rate of 2.5% (30) in male and in female 4.25% (51).

The HIV/ART status on admission is presented in Table 5. Cases of known diagnosis before visiting the hospital were 710 (59.20%) while a new diagnosis was 490 (40.8%). Those currently receiving ART 702 (58.5%) were higher than those who were not yet on ART 498 (41.5%). However, patients who have received ART for more than 24 weeks were more 450 (64.1%) than those who have received for just 24 weeks 252 (35.9%).

Table 2: Species distribution of different fungal isolates.

Fungal isolates	**No of isolates no (%)**	**Prevalence (%)**
A. niger	68	7.5
A. fumigatus	128	14.1
A. flavus	38	4.2
***Candida* spp**		
Candida albicans	458	50.4
C. tropicalis	90	10
C. krusei	40	4.4
C. glabrata	21	2.3
***Cryptococcus* spp**		
C. neoformans	18	2
C. gatti	13	1.4
***Penicillium* spp**		
Penicillium marneffei	17	1.9
Fusarium spp		
Fusarium solani	10	1.1
Fusarium oxysporum	7	0.8
Total	908	100

Table 3: Distribution of different fungal isolates among various clinical samples.

Clinical Sample	No. of the clinical sample from patients (%)	No. of the clinical sample from HIV positive patients (%)	No. of the clinical sample from HIV negative patients (%)	Fungal organisms isolated	Fungal organisms (%)
Oropharyngeal swab	421 (20.0)	101 (4.80)	320 (15.19)	*Candida* spp	168 (18.5)
Sputum specimen	385 (18.3)	92 (4.37)	293 (13.91)	*Aspergillus* spp	72 (7.9)
				Candida spp	85 (9.4)
				Cryptococcus spp	7 (0.8)

Blood specimen	343 (16.3)	144 (6.84)	199 (9.45)	*Candida* spp	177 (19.5)
				Cryptococcus spp	10 (1.1)
Stool specimen	331 (15.7)	60 (2.85)	271 (12.87)	*Aspergillus* spp	5 (0.6)
				Candida spp	31 (3.4)
Urine specimen	442 (21.0)	141 (6.70)	301 (14.29)	*Aspergillus* spp	88 (9.7)
				Candida spp	74 (8.1)
				Cryptococcus spp	14 (1.5)
Skin specimen	184 (8.7)	142 (6.74)	42 (2.0)	*Aspergillus* spp	69 (7.6)
				Candida spp	74 (8.1)
				Fusarium spp	17 (1.9)
				Penicillium spp	17 (1.9)
Total	2106 (100)	680 (32.3)	1426 (67.7)		908 (100)
*Figures in brackets represent percentage prevalence of each group					

Table 4: Frequency of Clinical Presentation in relation to sex.

Clinical Presentation	No. of participants Examined (%)	Male No. (%)	Female No. (%)
Weight loss	124 (10.3)	52 (4.33)	72 (6.0)
Oral ulcer	120 (10.0)	50 (4.17)	70 (5.83)
Fever	118 (9.8)	50 (4.17)	68 (5.67)
Headache	117 (9.8)	51 (4.25)	66 (5.5)
Loss of appetite	109 (9.1)	42 (3.5)	67 (5.58)
Cough	100 (8.3)	38 (3.16)	62 (5.16)
Dyspnea	101 (8.4)	43 (3.58)	58 (4.83)
Diarrhea	94 (7.8)	26 (2.16)	68 (5.67)
Neck rigidity	89 (7.4)	41 (3.41)	48 (4.0)
Others	129(10.8)	64 (5.33)	65 (5.42)
Asymptomatic	99 (8.3)	39 (3.25)	60 (5.0)
*Figures in brackets represent percentage prevalence of each group.			

Table 5: System Involvement in Patients.

System	No. of Patients (%)	Male No. (%)	Female No. (%)
Gastrointestinal Tract (GIT)	319(26.58)	142(11.83)	177(14.75)
Respiratory	303(25.25)	148(12.33)	155(12.92)
Central Nervous System	258(21.50)	108(9.0)	150(12.5)
Colorectal	142(11.83)	61(5.08)	81(6.75)
Genitourinary	97(8.08)	41(3.41)	56(4.67)
Skin	81(6.75)	30(2.5)	51(4.25)
Total	1200(100)	530(44.2)	670(55.8)
*Figures in brackets represent percentage prevalence of each group.			

Table 6: HIV /ART Status of patients on Admission.

HIV Status on Admission	No. of Patients	HIV +ve	HIV -ve
Known	710	119 (9.9)	591 (0.5)
New Diagnosis	490	79 (6.6)	411 (34.3)
Total	1200	198 (16.5)	1002 (83.5)
Currently Receiving ART			

No	498	124 (10.3)	374 (31.2)
Yes	702	74 (6.2)	628 (52.3)
Total	1200	198 (16.5)	1002 (83.5)
Duration of ART			
>24Weeks	450	131 (10.9)	319 (26.6)
<24Weeks	252	33 (2.8)	219 (18.3)
Total	702	198 (16.5)	1002 (83.5)
*Figures in brackets represent percentage prevalence of each group.			

Discussion

In this study, the number of patients who had fungal infections was more in HIV positive patients than in HIV negative individuals. This could be attributed to the fact that HIV infection reduces the immunity; CD4 count, in patients and leaves them susceptible to opportunistic infections, mostly fungal infections. This was similar to the results of other studies done by Chen, et al, (2013) and Maurya, et al., (2013) in Northern Taiwan and Northern India respectively, who reported that Oropharyngeal Candidosis (OPC) is an opportunistic fungal infection that is commonly found in HIV-infected patients [17,18]. However, the number of females 35% (450) who had fungal infections and subsequently positive to HIV were more compared to males 119.4% (233). Furthermore, majority of the patients who had fungal infection and subsequently HIV positive belonged to 30-39 years in the ratio of (3.43: 1), the most productive age group of the country, showing a female preponderance with a male to female ratio of (2.88:1; 3.59:1). Again, this is consistent with studies on HIV patients in India and Iran [19,20]. The preponderance of females may be due to the vulnerabilities created by the unequal cultural, social and economic status of the genders [21,22] or due to anatomical variations. Males give out while females receive during sexual acts making it more likely for the females to be infected [22].

Candida species 67.1% (609) were the most prevalent fungi isolated followed by *Aspergillus* spp. 25.8% (234) and *Cryptococcus* species 3.4%(31). This observation is similar to the studies of Jahromi, et al. [23], Pagano, et al. [24], Kashyap, et al. [25] and Parmar, et al. [26]. Similarly, *Candida* species (18.5%) was the most common organism isolated from oropharyngeal specimen making oropharyngeal candidiasis the most common opportunistic fungal infection. This is in line with other studies [27, 28]. The occurrence of oral candidiasis is recognized as an indicator of immune suppression and is often found in HIV infected patients with CD4 counts fewer than 200 cells/µL [29, 30]. *C. albicans* has been reported as the most pathogenic of *Candida* species [31-33].

Also, the present study highlighted that the prevalence of *Candida* species was significantly higher (ρ value, $P \leq 0.05$) in the HIV positive study group as compared to the HIV negative control group. This is similar to the results of other studies [17, 18].

In sputum specimen, *Candida* species 9.4% (85) predominates followed by *Aspergillus* species 7.9% (72). The least prevalent specie was Cryptococcus 0.8% (7).However, there was low prevalence of fungal infection in sputum compared to our study which is in line with Bharathi et al. [36,37].

According to the Ochiabuto et al. [38], a total of six different fungal isolates were recorded in their study from sputum specimens, with *C. albicans* isolates the highest. Among the *Candida* species, *C. glabrata* (7%) was isolated in this study. The report also stated that *C. glabrata* was also isolated [39,40].

Based on the site of sample collection, the *Candida* species (91.4%) was the most common pathogen isolated from hospitalized subjects followed by *Cryptococcus* species (3.74%). Prevalence of *Candida* infection was higher although a lot of variation in the prevalence and incidence of candidemia has been reported in different places in India varies from 1.6% to 6.9% [41-42].

In the stool specimen, *Candida* spp.prevalence rate was 3.4% (31) and *Aspergillus* spp. 0.6% (5) were isolated in this study, indicating that these yeasts can survive in the extreme conditions of the gastrointestinal tract. Recent studies have demonstrated an incidence of fungemia caused by *Aspergillus* spp between 2.3% and 5.34% in the USA and Europe, respectively [43].

In the urine specimen, *Candida* spp. 8.1%(74) *Aspergillus* spp. 9.7%(88) and *Cryptococcus* spp. 1.5%(14) were isolated in this study. A point prevalence survey done in 228 hospitals from 29 European countries stated that 9.4% of nosocomial Urinary tract infections were caused by *Candida* spp. and *Aspergillus* spp., though minimal. Depending on the population examined, *Candida* is reported in up to 44% of urine samples sent for culture. Two retrospective analyses were done in Israel and Italy found much lower rates (varying between 0 and 1.4%) in urine cultures [44].

Aspergillus species were isolated from sputum 7.9% (72), stool 0.6% (5), urine 9.7% (88) and skin specimen 7.6% (69). *Aspergillus* exposure is universal, though invasive *aspergillosis* is rare in general, it is commonly found in immunocompromised cases like in HIV infection [45]. According to Bharanthi et al. [46] out of 13 specimens isolated, *A. fumigatus* was isolated in 6 specimens; *A. niger* was isolated in another 6 specimens and *A. flavus* in one specimen. Since the findings of this study correlate significantly with the previous report stated, *Aspergillus* species could be said to be the second most prevalent species in HIV patients. Sturt [47] revealed that *Aspergillus* was cultured from the sputum of 45 patients with HIV, but only 5 patients were not infected with *Aspergillus* species. In a separate study of 40 AIDS patients with cultures of *Aspergillus* species, 24 of the cultures represented colonization. This finding suggests that the detection of *Aspergillus* by sputum culture, even in patients with advanced HIV infection, is equivalent to documenting invasive disease requiring antifungal therapy.

Cryptococcus neoformans was isolated from three specimens (sputum, blood, and urine) in this study. This finding is similar to that of a study carried out in Cameroon where *C. neoformans* had a rate of 2.04% in HIV patients [48]. *C. neoformans* was also isolated from 5 samples out of 100 samples from HIV patients in India [49]. This means that its prevalence is low in most localities. *Cryptococcosis* is an AIDS-defining illness, WHO stage IV and caused by *Cryptococcus neoformans*. It is an opportunistic fungal infection, often seen in the immunocompromised. It is the second commonest opportunistic infection after tuberculosis in South East Asia

In this study, the prevalence was slightly above the WHO estimate. Studies done by many researchers have placed the CD4 T+ cells at the manifestation of *Cryptococcus* disease at <100 cell/mm3. This research work corroborated this, as all the study specimens showed CD4<100 cells/mm3.

Penicillium marneffei was the third most common fungi isolated in this study. Penicilliosis is the third common opportunistic infection in patients with AIDS [51]. It is believed to hasten the emergence of AIDS and is always associated with low CD4 count typically less than 100 cells/µl [36]. It is very pathogenic and can mimic tuberculosis with 50% cases resulting in cough, dyspnea, and hemoptysis. According to an earlier study,by Bharanti and Usha, *Penicillium marneffei* was isolated from HIV positive individuals whose CD4 counts were less than 100 cells/µl [52].

Similar low incidences of Penicilliosis in HIV positive patients from Africa have been reported by Iroezindu et al. [53] (1.25%) and Alli et al. [54] (3.2%). The main reason for the low prevalence of *Penicillium marneffei* infection in Nigeria is the unavailability of high-quality diagnostic laboratories and low yield specimens, as bronchoalveolar lavage is not performed in most hospitals [53].

More so, the findings of Xiao et al.[29], revealed the prevalence of *Penicillium marneffei* (1.4%) to be almost similar to our findings (1.9%). He also reported that *P. marneffei* within an osteolytic lesion in an HIV-negative person is a rarely reported diagnosis. This patient's corticosteroid treatment for subacute thyroiditis, which affected the patient's immune function, possibly increased her chance of infection. The symptoms that lead to the diagnosis of *P. marneffei* infection are often non-specific and it tends to be regularly misdiagnosed. Cases of *P. marneffei* in non-HIV-infected patients are rare and other reports have shown that there are different clinical manifestations between HIV-positive and HIV-negative patients.

Presence of weight loss, oral ulcers, loss of appetite and neck rigidity seen in this study constitute clinical features of oral thrush and esophageal candidiasis. These features affect oral intake and and significantly affect the quality of life of such patients and are considered important markers of clinical disease progression and immunosuppression [54]. As seen with other studies [55], majority of the HIV positive patients in this study presented with more of these symptoms that HIV negative petients at the time of recruitment .However, according to Kaur et al., [56], the most common system involvement in clinical infection of HIV was Gastrointestinal (GI) and Respiratory tract which is similar to this study.

This study revealed that cases of the number of HIV patients before visiting the hospital were more than patients screened at the point of visiting the hospital. Similar results were also observed in the work Bharanthi, et al. [57] that more patients are aware of their HIV status.Moreso, those currently receiving ART were higher than those who were not yet on ART. However, patients who have received ART for more than 24 weeks were more than those who have received for just 24 weeks. This finding is consistent with previous reports from various countries. Ceballos et al., [58], in their studies of 154 patients who were on ART for a minimum of 6 months, reported a 30% reduction in the prevalence of fungal colonisation.

Conclusion

This study has revealed that HIV positive and Negative patients are often affected with fungal infections in COOUTH, Anambra State, Nigeria. This may have contributed to impaired immune system in patients and development of AIDS in HIV infected patients. The researchers have recommended the constant monitoring; treatment, and evaluation of the fungal infection in the area and in other Teaching Hospitals in the country should be encouraged. Also, both viral load and CD4 cell count monitoring be used in monitoring the risk of opportunistic infection (OI) and treatment failure. These can be used to significantly improve the life expectancy of patients living with HIV and fungi infections.

Sharing of unhygienic personal belongings with the infected patients and intimate association with pet animals could increase the mode of transmission. However, HIV/AIDS and fungi infections awareness campaigns among people in Anambra State must be given a particular attention to specific issues on their transmission, risk factors and management and starting antiretroviral treatment as soon as possible, and sustaining it as part of everyday routine, is the best way of ensuring that the immune system stays strong.

References

1. Brown GD, Denning DW, Gow NA, et al. (2012) Hidden killer: human fungal infections. Sci Transl Med. 4(165): pp. 165rv13.

2. De Rycker M, Baragana B, Bilbert IH (2018) Challenges and recent progress in drug discovery for tropical diseases. Nature. 559(7715): pp. 498-506.

3. Limper AH, Adenis A, Le AT, et al. (2017) Fungal infections in HIV/AIDS. The Lancet, 17(11): pp. 334-343.

4. Ocansey BK., Pesewu GA, Codjoe FS, et al. (2019) Estimated Burden of serious Fungal Infections in Ghana. J Fungi. 5(2): pp. 38.

5. Mayer FL, Wilson D, Hube B (2013) Candida albicans: pathogenicity mechanisms. Virulence. 4(2): pp. 119-128.

6. Mitchell A.P (2016) Microbiology: Fungus produces a toxic surprise. Nature. 532(7597): pp. 41-42.

7. Dunaiski CM, Denning DW (2019) Estimated Burden of fungal infections in Namibia. J Fungi. 5(3): pp. 75.

8. Nami Mohammadi R,Vakili M, Khezripour K, et al. (2019) Fungal vaccines, mechanism of actions and immunology: a comprehensive review. Biomed Pharmacother. 109(1): pp. 333-344.

9. Wibawa T, Praseno DP, Aman AT (2015) Virulence of Candida albicans isolated from HIV infected and non-infected individuals. Springer Plus. 4(1): pp. 408.

10. United Nations Programme on HIV/AIDS (UNAIDS) (2017) UNAIDS Data 2017. The HIV epidemic in Nigeria..

11. Parkhurst HO (2014) Structural approaches for prevention of sexually transmitted HIV in general populations: definitions and an operational approach. J Int AIDS Soc. 17 (1): pp. 19052.

12. United Nations Programme on HIV/AIDS (UNAIDS) (2016) AIDS Info. Accessed 14th September, 2017.

13. Enweani IB, Ochiabuato BOMT, Nwankwo A, et al. (2013) Opportunistic Fungal infections in HIV patients attending DOTS clinic in a Tertiary Hospital in South-East, Nigeria. Abstract of 113th Conference of American Society for Microbiology.

14. WHO (2008) WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children. Switzerland: World Health Organization.

15. Shelabh HC (2016) Introduction to Statistics and Data Analysis. Springer International Publishing Switzerland: pp. 319-461.

16. National Aids Control Organization. (2016) Overview of HIV epidemic in India; Annual report. 2: 336-393.

17. Chen J, Li W, Huang X, et al. (2013) Evaluating total lymphocyte count as a surrogate marker for CD4 cell count in the management of HIV-infected patients in resource-limited settings: a study from China. PLoS One. 8(7): pp. 6974

18. Maurya V, Srivastava A, Mishra J, et al. (2013) Oropharyngeal candidiasis and candida colonization in HIV positive patients in Northern India. J Infect Dev Ctries 7(8): 608-613.

19. Badiee P, Alborzi A, Davarpanah MA (2010) Distributions and antifungal susceptibility of Candida species from mucosal sites in HIV positive patients. Arch Iran med 13(4): pp. 282-287.

20. Srirangaraj S, Venkatesha D (2011) Opportunistic infections in relation to antiretroviral status among AIDS patients from south India. Ind J Med Micro 29(4): pp. 395-400.

21. Damtie D, Yismaw G, Woldeyohannes D, et al. (2013) Common opportunistic infections and their CD4 cell correlates among HIV-infected patients attending at antiretroviral therapy clinic of Gondar University Hospital, Northwest Ethiopia. BMC Res Notes. 6(534).

22. Jha UM, Raj Y, Venkatesh S, et al. (2014) HIV epidemic among men who have sex with men in India: National scenario of an unfinished agenda. HIV AIDS (Auck). 6(1): pp. 159-170.

23. Jahromi SB, Khaksar AA (2005) Deep-seated fungal infections in immunocompromised patients in Iran. Iran J Allergy Asthma Immunol. 4(1): pp. 27-32.

24. Pagano L, Caira M, Candoni A, et al. (2006) The epidemiology of fungal infections in patients with hematologic malignancies: the SEIFEM-2004 study. Haematologica. 91(8): pp. 1068-1075.

25. Kashyap B, Das S, Kaur IR (2012) Fungal profile of clinical specimens from a tertiary care hospital. Asian Pac J Trop Biomed. 2(1): pp. S401-S405.

26. Parmar R, Sharma V, Thakkar C, et al. (2012) Prevalence of opportunistic fungal infections in HIV positive patients in tertiary care hospital in Rajkot. Nat J Med Res.2(4): pp. 463-465.

27. Wadhwa RK, Agarwal SK, Jain S, et al. (2007) AIDS-related opportunistic mycoses seen in a tertiary care hospital in North India. J Med Micro. 56(8): pp. 1101-1106.

28. Jain S, Singh AK., Singh RP, et al. (2014) Spectrum of opportunistic fungal infections in HIV-infected patients and their correlation with CD4+ counts in western India. J Med Micro Infec Dis. 2(1): pp. 19-22.

29. Xiao J, Gao G, Li Y, et al. (2013) Spectrums of opportunistic infections and malignancies in HIV-infected patients in tertiary care hospital, China. PLoS One. 8(1): pp. e75915.

30. Mulla SA, Patel MG, Vaghela G, et al. (2007) A study of opportunistic infection in HIV-seropositive patients. Ind J Commun Med. 32(3): pp. 208-209.

31. Campos LN, César CC, Guimarães MD (2009) Quality of life among HIV-infected patients in Brazil after initiation of treatment. Clinics (Sao Paulo, Brazil). 64(9): pp. 867-875.

32. Nejad Mohammad E, Rabirad N, Hadizadeh MR, et al. (2013) The Prevalence of Tb in HIV Patients and Risk Factor with Frequent Referral (Iran, 2009-10). Iran Red Crescent Med J. 15(1): pp. 58-61.

33. Mane A, Kulkarni S, Ghate M, (2017) Characterization of vaginal lactobacilli from HIV-negative and HIV-positive Indian women and their association with genital HIV-1 shedding. J Med Microb. 66(1): pp. 1471-1475.

34. Nayak AP, Green BJ, Beezhold DH (2013) Fungal hemolysins. Med Mycol. 51(1): pp. 1-16.

35. Coco A, Kleinhans E (2008) Prevalence of primary HIV infection in symptomatic ambulatory patients. Ann Fam Med. 3(5): pp. 400-404.

36. Bharathi M, Rani AU (2011) Pathogenic fungal isolates in sputum of HIV positive patients. J AIDS HIV Res. 3(6): pp. 107-113.

37. World Health Organization (2009) Laboratory Manual for diagnosis of Fungal Opportunistic infections in HIV/AIDS patients.

38. Ochiabuto OMTB, Nwankwo A, Enweani IB, et al. (2014) Fungal Isolation in HIV Patients and CD4 Count. Int STD Res Rev. 2(2): pp. 111-122.

39. Sailaja VV, Pai LA, Madhur V (2004) Lower respiratory tract infections in patients with HIV infection. Ind J Med Microb. 22(1): pp. 28-32.

40. Aruna A, Arora U, Bajaj R, et al. (2005) Clinico-microbiological study in HIV sero-positive patients. Ind Acad Clin Med. 6(2): pp. 142-145.

41. Sardi JC, Scorzoni L, Bernardi T, et al. (2013) Candida species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. J Med Microb. 62(1): pp. 10-24.

42. Deorukhkar S, Saini S (2015) Virulence factors attributed to pathogenicity of non albicans Candida species isolated from human immunodeficiency virus infected patients with oropharyngeal candidiasis. AnnPath Lab Med. 3(1): pp. 62-65.

43. Wirth F, Goldani LZ (2012) Epidemiology of Aspergillus spp in HIV Patients in Europe: an emerging pathogen. Interdiscip Perspect Infect Dis. 465717.

44. Achkar JM, Fries BC (2010) Candida infections of the genitourinary tract. Clin Microb Rev. 23(2): pp. 253-73.

45. Pruthvi BC, Vikram S, Suman SK., et al. (2006) Spectrum of Clinical Presentation and Opportunistic Infections in HIV: An Indian Scenario, 13 the International Congress on Infectious

Diseases. pp. e484.

46. Bharanthi B, Swamicloss D (2010) Incidence of Bacterial and fungal co-infection in some HIV infected Indian population. Ind J Biotech. 3(2): pp. 199.

47. Sturt AS, Dokubo EK, Sint TT (2010) Antiretroviral therapy (ART) for treating HIV infection in ART-eligible pregnant women. Cochrane Database Syst Rev. 17(3): pp. CD008440.

48. Gupta S, Madhur P, Maskey D, et al. (2007) Immune restoration syndrome with disseminated penicillium marneiffei and cytomegalo virus co-infection in an AIDS patient. AIDS ResistTher. 4(1): pp. 21-25.

49. Youngabi KA, Mbachasm WF, Nubia KK (2009) Yeast Strains Isolated from HIV Seropositive patients in Cameroon and their Sensitivity to extracts of eight Medicinal Plants. Afr J Microb Rev. 3(4): pp. 133-136.

50. Banerjee U (2005) Progress in diagnosis of opportunistic infections in HIV/AIDS. Ind J Med Res. 121(4): pp. 395-406.

51. Ampel NM (2005) Coccidiomycosis in persons infected with Type I HIV. Clin Infec Dis. 41(1): pp. 1174-1178.

52. Bharanthi M, Usha A (2011) Pathogenic fungal isolates in sputum of HIV positive patients. J AIDS HIV Res. 3(6): pp. 107-113.

53. Iroezindu MO, Ofondu EO, Hausler H, et al. (2013) Prevalence and risk factors for opportunistic infections in HIV patients receiving antiretroviral therapy in a resource-limited setting in Nigeria. J AIDS Clinic Res. S3:002.

54. Zanoni BC, Gandhi RT (2014) Update on opportunistic infections in the era of effective antiretroviral therapy. Infect Dis Clin North Am. 28(3): pp. 501–518.

55. Bishnu S, Bandyopadhyay D, Samui S, et al. (2014) Assessment of clinico-immunological profile of newly diagnosed HIV patients presenting to a teaching hospital of eastern India. Ind J Med Res. 139(6):903–912.

56. Kaur R, Dhakad MS, Goyal R, et al. (2016) Spectrum of Opportunistic Fungal Infections in HIV/AIDS Patients in Tertiary Care Hospital in India. Can J Infect Dis Med Microbiol. 46(2); 338-341.

57. Bharanthi B, Swamicloss D (2010) Incidence of Bacterial and fungal co-infection in some HIV infected Indian population. Ind J Biotech. 3(2): pp. 199.

58. Ceballos-Salobrena A, Gaitan-Cepeda LA., Ceballos-Garcia L (2000) Oral lesions in HIV / AIDS patients undergoing highly active antiretroviral treatment including protease inhibitors: a new face of oral AIDS? AIDS Patient Care STDS. 14 (12): pp. 627–635.