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Oropharyngeal Candidiasis in HIV Suspected Patients Attending State Hospital Ijebu-Ode Ogun State Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OML and AO designed the study and performed the experimental part. Authors COA, STO and HIE wrote the protocol, wrote the first draft of the manuscript and managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: To determine the effect of CD4 count (a glycoprotein found on the surface of immune cells such as T helper cells, monocytes, macrophages and dendritic cells) on the Candida species associated with Oropharyngeal candidiasis among HIV suspected patients.
Place and Duration of the Study: State Hospital Ijebu Ode Ogun State Nigeria between February 2010 to August 2011.
Methodology: Outpatients attending State Hospital Ijebu Ode were screened for HIV infection using Determine kits, Stat-pak kits and Unigold test kits. Western blot was used to confirm HIV infection and to determine the predominance of HIV specific glycoproteins in HIV seropositive patients. A total of 350 samples of sputum and blood from the HIV seropositive individuals while 300 samples from the HIV seronegative individuals. Sputum samples were cultured on sabouraud dextrose agar, and the isolates were Gram stained

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while the yeast-like fungi were subjected to germ tube test. CD4 count in blood samples was determined using flow cytometry.
Results: HIV prevalence in females was 70.6% and in males was 29.4%. From three hundred and fifty patients suspected as HIV positive, seventy three had oral candidiasis (20.9%) while 277 (79.1%) were candidiasis negative. Higher oral candidiasis occurred in females (22.7%) than in males (16.5%). Candida alblicans was found to have higher occurrence of 86% among other Candida species. There is no significant association between the occurrence of oral candidiasis and the age of HIV subjects. There was higher occurrence of cases of immune depression (<350 CD4 count) in HIV seropositive (56.3%) than in HIV seronegative (0%) subjects. Candida infections occur when CD4 count was 200-500 cell/µl and usually represent the first indication of immune suppression. Decrease in CD4 count led to increase in occurrence of Candida species. The lowest number of Candida species was recorded when CD4 count was above 300 and Candida alblicans is the most predominant species isolated in this study.
Conclusion: The result of this study shows that HIV infection led to decrease in CD4 count which in turn promotes candidiasis.

Keywords: HIV-Subjects; oropharyngeal; candidiasis; Diagnostic kit.

1. INTRODUCTION

Human population with opportunistic fungal infection is assuming an alarming proportion over the last three decades; among such population are transplant recipients, cancer patients, human immunodeficiency virus patients and others who receive immunosuppressive treatments [1,2].

Breaking down of anatomical barriers for example the skin and immune system of individual is the common factor responsible for invasion of infection [3].

Over the years, fungal infections were regarded as insignificant in the clinical world. In the last two decades, an increased incidence of invasive fungal infection has created major challenge for health care providers owing to the high mortality among infected patients [1]. *Candida albicans* and other opportunistic yeast pathogens are normal flora of the skin, oral cavity and female genital tract of both immunocompetent and immunosuppressive individuals [4].

Candida in immunocompromised patients has been known to predispose the patients to disease development [5]. This abrogates the host defenses and invasiveness of the organisms causing disease. *Candida* spp are commensals of the human host and must be able to compete with other microorganisms at various sites of the body [6]. The most common fungal infection in patients with HIV infection is candidiasis, since it originates from the normal flora of the host and not from an external source [7]. *Candida* spp. has been shown to persist on mucosal surface of the host and its inability to colonize body site result in its removal. Adhesins and virulence of *C. albicans* promote host recognition and colonization which enables the organism to produce digestive enzymes thereby causing infection [8].

In many cases, *Candida* spp. could be from respiratory tract leading to major death among people infected with human immunodeficiency virus [9]. In human gastrointestinal tract, *Candida* is regarded as normal and natural flora and may be obtained from the two thirds

mouths of competent individuals and one third of those who are suffering with HIV diseases [10]. The most predominant causative agent of mucocutaneous candidiasis is *Candida albicans.* Others that are not usually occurring are *C. tropicalis, C. glabrata, C. parapsilosis* and *C. krusei*. *C. dubliniensis* and *C. albicans* are macroscopically similar and may cause approximately 15% of infections formerly assigned to *C. albicans* [11,12]. *C. dubliniensis* is usually isolated from HIV patients, although it is presently not differentiated from *C. albicans* considering their clinical manifestation.

The most initial manifestations of HIV-induced immunodeficiency to be recognized were oropharyngeal candidiasis (OPC) [13]. This practically affects large number of people with untreated HIV infection, presenting months or years before more severe opportunistic illnesses [14]. Oropharyngeal candidiasis may be a sentinel event indicating the presence of progression of HIV disease [15,16.] which is usually not associated with severe morbidity.

The objective of this study therefore is to determine the prevalence of *Candida* species associated with suspected HIV patients and its relationship with CD4 counts in Ijebu-Ode, South Western Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out in State Hospitals Ijebu-Ode from February 2010 to August 2011 in Ogun State, South Western Nigeria. A total of six hundred and fifty subjects of age range19-69 year were recruited for the study after obtaining ethical approval from authority of State Hospital Ijebu Ode, where these subjects were patients. HIV seronegative individuals served as controls.

2.2 Sample Collection

Three hundred and fifty (350) HIV seropositive and three hundred (300) HIV seronegative persons were recruited into the study. Each patient was requested to produce sputum and void it into a clean, dry, wide opened, leak proof labeled container. Tourniquet was tied round the arm of the subjects to make available a prominent vein in order to collect blood samples. The area was swabbed round with cotton wool soaked in 70% alcohol, and allowed to dry. Then 3-4 ml of venous blood were collected from each subject via veno puncture into EDTA vacuum tubes and mixed by inversion several times. These were labeled, dated and kept for analysis.

2.3 HIV Screening

Rapid HIV screening was carried out on the blood samples collected using Determine test kit (Aleere Medical Ltd Japan) [17], Uni-gold test kit [18] and Stat pak test kit [19]. Reactive results were confirmed using western blot according to the manufacturer's instructions.

2.4 Western Blotting Technique

This is for the detection of IgG antibodies in human serum or plasma directed against HIV1/2 antigens.

One milliliter of wash buffer was added to each active channel of the incubation tray. The labeled strips were placed individually into the wells using forceps, with the strip number facing up. The tray with the content was incubated for 1min. on rocking platform until strips were homogeneously wet. The wash buffer was aspirated away, and then 1ml of sample diluents was added to each active channel. After which10µl of each test samples or HIV 1/2 controls or human IgG negative control was added to the appropriate individual active channels and the tips were rinsed in wells to facilitate mixing. The set-up was incubated for 2 hours on rocking platform and the liquid content was aspirated while the active channels were rinsed with 1ml of wash buffer; rocked for 3 minutes and then aspirated. The washing step was repeated twice. 1ml of anti-human IgG conjugate was added to each active channel and incubated for 15 minutes on a rocking platform and washing step was repeated three times. After which 1ml of substrate solution was added to each active channel and incubated for 6-8 minutes on the rocking platform to initiate the colour reaction. All active channels were aspirated and rinsed with two brief changes of distilled water to stop colour development. The strips were transferred (face) with forceps to a paper towel and air-dried. The dried strips provide a permanent record of the test result. The bands on the positive controls strips were identified by aligning them with the marching bands on the reference card provided with the kit. The bands on the test strips were identified by comparing them to the bands on the positive control strips. The intensity of the test strips bands were compared with the p24 bands on the weakly reactive control. If the test strip band was darker than the p24 bands (HIV protein antigen bands which are the first marker to be detected in the serum within two weeks of infection) on the weakly reactive control, it was scored as present, if the band was lighter, it is scored as absent.

2.5 CD4 Cell Count Determinations

Blood of the patients were collected into EDTA containing tubes and arranged serially in a rack after which rohren tube was labeled appropriately. The whole blood samples in EDTA containing tubes were mixed properly and 20µl of the whole blood were dispensed into the labeled Rohren tubes with corresponding sample identity. 20µl of the CD4+ monoclonal antibody (ANOGEN-YES BIOTECH LABORATORIES LTD.) were added and mixed gently. The mix was incubated for 15minutes at room temperatures in the dark (i.e. away from light) and 800 µl of the dilution buffer were added and mixed gently. 840µl of the blood preparation were analyzed with the cyflow with an excitation light source of 532nm (green solid state laser). CD4 count was carried out and recorded. After use the cyflow was cleaned with 800 µl of distilled water, 800µl of decontaminant solution and 800µl cleaning detergent.

2.6 Cultivation of Sputum

The sputum samples were inoculated on sabouraud dextrose agar (Oxoid) plates and incubated at 37ºC for 24-72h and representative colonies were repeatedly streaked on sabouraud dextrose agar (Oxoid) to obtain pure cultures.

Speciation of *Candida* species was carried out using CHROMagar method, urease test and sugar fermentation profiling.

2.7 Germ Tube Test

Human serum (0.5ml) was pipette into a small test tube. Using a sterile wire loop, the serum was inoculated with a yeast colony from the cultured plate. The tube was placed in an

incubator at 35-37ºC for 2-3h. With a Pasteur pipetted, a drop of the serum yeast culture was transferred to a glass slide and covered with a cover slip. The preparation was examined using 10x and 40x objectives with the iris diaphragm closed sufficiently to give good contrast. Sprouting yeast cells that were tube-like outgrowths from the cells (known as germ tube) were observed. When sprouting yeast cells were seen, the culture was reported as *Candida albicans* positive. However, when the yeast cells did not show sprouting, the culture was reported as yeast other than *Candida albicans* [20].

2.8 Urease Test

On a prepared slant of Christensen Urea agar (Oxoid), colonies of isolated *Candida* spp. were inoculated and incubated at 30ºC for 48h. Colour change from yellow to pink indicated urease positive.

2.9 Sugar Fermentation

Candida colonies were inoculated into each peptone broth (1%w/v peptone and 0.5% Sodium Chloride) with Andrade indicator (0.05w/v) containing 2% each of carbohydrate (glucose, sucrose, lactose, galactose, inositol, cellobiose, raffinose and palatinose) and incubated at 37ºC for one week and examined at 48h interval for acid (pink colour) production.

3. RESULTS

Age distribution and percentage of HIV seropositive and negative patients are shown in Table 1. From positive samples one hundred and forty eight patients were from age range 31-40 representing 42%. The least was from age range 11-20 which had only four samples representing 1.1%. While from the controls one hundred and twenty six samples were from age range 31-40 and the least was from age range 61-70.

Results revealed the occurrence of higher cases of immune depression (<350CD4 count) in HIV positive (56.3%) than in HIV negative (0%) subjects, therefore there is a strong association between CD4 and HIV status.

The relationship between the prevalence of HIV and sex distribution revealed that from three hundred and fifty positive samples, two hundred and forty seven were from females and one hundred and three were from males. From the control, one hundred and six were from the females and one hundred and thirty four were from males.

The relationship between the prevalence of HIV and marital status of subjects where compared in the Table 2. Two hundred and twenty one samples were positive from married while the least was from unmarried.

Influence of the occupation on the prevalence of HIV was assessed (Table 3). Analysis showed that the unskilled labored had the highest with one hundred and sixty seven out of three hundred and fifty and the least was from the students.

Table 1. Age distribution (%) of HIV seropositive/seronegative subjects

Subjects	Age (Year) range						
	11-20	21-30	31-40	41-50	51-60	61-70	Total
HIV Positive	4(1.1)	99(28.3)	148(42.3)	73(20.9)	19(5.4)	7(2.0)	350
HIV Negative	14(4.7)	108(36.0)	126(42.0)	32(14.0)	14(4.7)	6(2.0)	300
Total	18(2.8)	207(31.8)	274(42.2)	105(16.2)	33(5.1)	13(2.0)	650

Table 2. Prevalence of HIV and marital status among the subjects

Subject	Marital status				Total
	Unmarried n (%)	Married n (%)	Widows' n (%)	Divorced n (%)	
HIV Positive	32 (39.0)	221(52.5)	36(64.3)	61(67.0)	350
HIV Negative	50 (61.0)	200(47.5)	20(35.7)	30(33.0)	300
Total	82 (100.0)	421(100.0)	56(100.0)	91(100.0)	650

Table 3. Influence of occupation on prevalence of HIV among the subjects

Subject	Occupation					
	Skilled labour n (%)	Artisan's n (%)	Unskilled n (%)	Student n (%)	Unemployed n (%)	Total
HIV positive	56(46.7)	99(56.6)	167(63.5)	13(18.8)	15(65.2)	350
HIV Negative	64(53.3)	76(43.4)	96(36.5)	56(81.2)	8(34.8)	300
Total	120(100.0)	175(100.0)	263(100.0)	69(100.0)	23(100.0)	650

Table 4 showed influence of patient education on the prevalence of HIV. One hundred and fifty were positive mainly from Secondary School Students and the least were from the uneducated patients.

Table 4. Influence of education on prevalence of HIV among the subject

Subjects	Education				
	Uneducated n (%)	Primary n (%)	Secondary n (%)	Tertiary n (%)	Total
HIV Positive	25(64.1)	94(60.3)	150(57.3)	81(42.0)	350
HIV Negative	14(35.9)	62(39.7)	112(42.5)	112(58.0)	300
Total	39(100.0)	156(100.0)	262(100.0)	193(100.0)	650

The sensitivity of the test kits is shown in Table 5. Western blot had hundred percent sensitivity closely followed by Determine kit which had99.75% while both Stat-pak kit and Uni-Gold kit had 99.4% sensitivity and their value of specificity were found to 100%.

Relationship between predominant of glycoprotein in HIV seropositive is shown in Table 6. P_{24} had 99.1% of HIV glycoprotein and closely followed by GP_{160} with 97.1% and the least were P_{51} and P_{31} that had 84.9% each.

On CHRO Magar *C. albicans* appeared light green in colour and smaller in size, *C. glabrata* appeared white, *C. tropicalis* was blue, *C. krusei* appeared light pink while *C. dubliniensis* was deep green and bigger in size than *C. albicans*. However germ tube test revealed that *C. albicans* and *C. dubliniensis* were positive while *C. glabrata, C. krusei* and *C. tropicalis* were negative. All the *Candida* species isolated in this work fermented glucose, their sugars

fermentation profiling varied from one species to the other. Only *C. krusei* was positive for urease test others were negative (Table 7).

The number and occurrence of *Candida* species in HIV seropositive showed that *C. albicans* has 86% occurrence followed by *C. krusei*and *C. tropicalis* with 4% occurrence each while *C. glabrata* and *C. dubliniensis* had 3% occurrence each. Relationship between HIV positivity and oral candidiasis was determined and showed that seventy three (20.9%) of the patients had oral candidiasis out of three hundred and fifty samples also oral candidiasis and gender of HIV positive patients relationship revealed that higher occurrence of oral candidiasis were found in females (22.7%) than in males (16.5%).

Relationship between the occurrence of oral candidiasis and ages of HIV positive subject showed that no significant association exists between the occurrence of oral candidiasis and ages of HIV subjects. However, the relationship between occurrence of *Candida* species and CD4 count is shown in Table 8. As the CD4 count increases the occurrence of *Candida* species decreases. 101-200 CD4 range had highest occurrence of *Candida* species and closely followed by 0-100 CD4 range while above 300 CD4 count, the lowest occurrence of *Candida* species was recorded.

Table 5. Sensitivity and specificity of some diagnostic kit with respect to western blot (gold standard)

Reactivity of Kit	Stat-Pak (%)	Determine (%)	Unigold (%)	Western blot (%)
Sensitivity	99.4	99.7	99.4	100.0
Specificity	100.0	100.0	100.0	100.0

Table 6. Predominant of glycoprotein in HIV seropositive subjects

HIV glycoprotein	n	(%)
P17	322	(92.0)
P24	347	(99.1)
P31	297	(84.9)
P41	310	(88.6)
P51	297	(84.9)
P66	308	(88.0)
Gp120	238	(68.0)
Gp160	340	(97.1)

Table 7. Morphology and biochemical characterization of *candida* species

Test	*C. alblicans*	*C. tropicalis*	*C. glabrata*	*C. krusei*	*C. dubliniensis*
Colour	**Light green**	**Blue**	**White**	**Light green**	**Deep green**
Size (mm)	**0.5**	**1.0**	**1**	**1.1**	**1.2**
Germ tube test	+	-	-	-	+
Urease	-	-	-	+	-
Glucose	+	+	+	+	+
Galactose	-	+	-	-	-
Inositol	-	-	-	-	-
Lactose	-	-	-	-	-
Cellobiose	-	+	-	-	-
Sucrose	-	+	-	-	-
Raffinose	-	-	-	-	-
Palatinose	+	-	-	-	-

Table 8. Relationship between CD4 count and occurrence of candida species

CD4 range	Candida species				
	Candida alblicans	***Candida glabrata***	***Candida krusei***	***Candida tropicalis***	***Candida dubliniensis***
0-100	17	1	1	1	2
101-200	27	-	2	2	-
201-300	16	1	-	-	-
301-400	3	-	-	-	-
Total	63	2	3	3	2

4. DISCUSSION

This study describes the prevalence of Oropharyngeal candidiasis and HIV among different ages and gender in patients attending State Hospital Ijebu Ode. The prevalence of HIV was found to be higher in female than male which agrees with the finding of *Obuekwe and Onunu* [21], which indicates that females are more predisposed to HIV infection than males. It has been reported that female (women) are responding to health promotion more positively and coming forward for treatment much more frequently than males [22].

The highest age range of HIV infected were between 31-40 (42.3%),which is the most active sexual life of individuals, the age group is associated with working class. It was observed in marital status that highest prevalence of HIV were among the divorce as reported by Sunili Solomon et al. [23], which suggest that they engage with multiple sexual partners. However in occupation the highest prevalence of HIV was found among unemployed which might be as a result of poverty, engaging themselves in commercial sex working in other to make ends meet, while the least was observed in students which are dependent, lacking nothing or little. HIV infection and literacy level have shown a high prevalence among the less educated in the study. The highest prevalence was found among uneducated individuals and least among individual with tertiary education which is in agreement with Matthew and Kamal [24], Hyde and White [25] and Singh et al. [26].

The 3 kits used were found to be 100% specific while Determine kits was found to be more sensitive than Stat pak and Unigold when compared with western blot technique, which was the gold standard. Overall test sensitivity or specificity may be improved by using test combinations under one or more decision rules for resolving discordant results.

The highest protein band found in western blot technique was p24, which is in accordance with most fourth generation test kits.

C. albicans, *C. glabrata*, *C. tropicalis*, *C. krusei* and *C. dubliniensis* were isolated from suspected HIV patients in this work and revealed 20.9% prevalence of oral-candidiasis within the population study. However 84% prevalence was reported by Neil, [27] while Enwuru et al. [28] reported 34.2% in Lagos Nigeria. This result shows that oropharyngeal candidiasis is presently an important opportunistic disease among HIV/AIDS patients. The morphology and biochemical properties of *Candida* species agreed with the findings of Bhavan et al. [29].

Candida albicans (85.7%) was the most frequently isolated species among HIV infected individuals, this is in agreement with the findings of Liu et al. [30] and Rejane et al. [31] who reported 52.4% and 57.4% respectively. Enwuru [28] reported 40.5% prevalence of *Candida*

alblicans among HIV infected patients. Jabra-Rizk et al. [10] reported that all species of *Candida* isolated from oral thrush of people living with HIV or having AIDS are potentially pathogenic. The remaining 14.3% of *Candida* are *C. glabrata*, *C. tropicalis*, *C. krusei* and *C. dubliniensis*, which is in agreement with findings of Bassetti et al. [32] and Peman et al. [33]. In this study higher occurrence of oral Candidiasis in females was observed, which was also reported by Obuekwe and Onunu [34] in Benin-Nigeria, Khongkunthian et al. [35] in Thailand, Butt et al. [36] in Kenya, Shiboski*et al*. [37] in San Francisco. However some studies have shown a higher prevalence among males, e.g. Patton et al. [38] in USA, Bendick et al. [39] in Cambodia and Jonsson et al. [40] in Zimbabwea.

HIV infection lowers the CD4 counts, as indeed shown in the study where higher proportion of immune depression in HIV positive with CD4 counts of less than 350 were found. Infections with *Candida* appear when CD4 count is 200-500cell/ul and usually represent the first indication of immune suppression [41]. However, in the endemic area where this research was carried out the CD4 counts of <350 is significant in immune depression. The relationship between CD4 count and prevalence of oral candidiasis in HIV seropositive patients showed that there was a reduction in CD4 count in HIV positive individual which was also supported by Bharathi and Rani [41] whereas in HIV seronegative patients the reverse was the case.

5. CONCLUSION

Oropharyngeal HIV suspected individuals were infected with *Candida* species and this leads to immune deterioration as was evident by lower CD4 count, which is highly predictive markers of severe and disease progression in HIV infected individuals.

CONSENT

All authors declared that the patients' consents were obtained before commencement of the study.

ETHICAL APPROVAL

All authors' hereby declare that all experiment has been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standard laid down by the States Hospital Ijebu Ode, Ogun State, Nigeria.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Richardson MD, Warnock DW. Fungal infection: Diagnosis and management, 3rd edition oxford Blackwell Publishing; 2003.
2. Binder U, Lass-Flörl C. Epidemiology of invasive fungal infections in the Mediterranean Area. Medit J Hemat Infect Dis.; 2011. 3: e20110016, DOI 10.4084/MJHID.2011.016.
3. Maertens J, Vrebos M, Boogaerts M. Assessing risk factors for systemic fungal infections. Eur J cancer care. 2001;10:56-62.
4. Ferreira C, Silva S, Faria-Oliveira F, Pinho E, Henriques M, Lucas C. Candida albicans virulence and drug-resistance requires the O-acyltransferase Gup1p. BMC Microbiology. 2010;10;238-251.
5. Khaled Abu-Elteen H, Mawieh Hamad A. Changing Epidemiology of Classical and Emerging Human Fungal Infections: A Review Jordan Journal of Biological Sciences. 2012;5(4):215–223.
6. Sanchez-Vargas LO, Oritez-Lopez NG, Villar M, Moragues MD, Aguirre JM, Chashat-CruzM, Lopez-Ribot J L, Gaitan – cepeda LA, Quindos G. Oral Candida isolates colonizing or infecting human immunodeficiency virus-infected and healthy person in Mexico. J Clin Microbial. 2005;43:4159-62.
7. Selik RA, Starcher ET, Curran JW. Opportunistic disease reported in AIDS patients: Frequencies, associations, trends. AIDS. 1988;1:175-82.
8. Abegg MA, Lucietto R, Alabarse PVG, Mendes MFA, Benfato MS. Differential Resistance to Oxidants and Production of Hydrolytic Enzymes in Candida albicans. Mycopathologia. 2011;171:35–41.
9. Faith M, Durden, Boni Elewski M. Seminars in Cutaneous Medicine and Surgery: HIV infection and Dermatologist. 1997;16(3):200-212.
10. Jabra–Rizk MA Falker WA, JrMerz WG, Baqui AA, Kelley JI, Meiller TF. Retrospective Identification and characterization of Candida dubliniensis isolates among Candida albicansclinical laboratory isolates from human immunodeficiency virus (HIV)-infected and non-HIV-infected individuals. J clin Microbiol. 2000;38:2423-6.
11. Tintelnot K, Haase G, Seibold M, Bergmmann F, Staemmler M, Franz T, Naumann D. Evaluation of phenotypic markers for selection and identification of Candida dubliniensis. J Clin Microbial. 2000;38:1599-608.
12. Schorling SR, kortiga HC, Froschb M, Muhlschlegel FA. The role of *Candida dubliensis* in oral candidiasis in human immune deficiency Virus-infected individuals. Crit Rev Microbial. 2000;26:59-68.
13. Gottlieb MS, Schroff R, Schanker HM, Weisman JD, Fan PT, Wolf RA, Saxon A. Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexualmen: Evidence of a new acquired cellular immunodeficiency. N. Engl. J. Med. 1981;305:1425-31.
14. Masur H, Michelis MA, Greene JB, Onorato I, Stouwe RA, Holzman RS, Wormser G, Brettman L, Lange M, Murray HW, Cunningham-Rundles S. An outbreak of community-acquired Pneumocystis carinii pneumonia: Initial manifestation of cellular immune dysfunction. N. Engl J. Med. 1981;305:1431-8.
15. Klein RS, Harris CA, Small CB, Moll B, Lesser M, Friedland GH Oral candidiasis in high-risk patients as the initial manifestation of the acquired immunodeficiency syndrome. N. Engl. J. Med. 1984;311:354-8.
16. Katz MH, Greenspan D, Westenhouse J, Hessol NA, Buchbinder SP, Lifson AR, Shiboski S, Osmond D, Moss A, Samuel M. Progression to AIDS in HIV-infected homosexual and bisexual men with hairy leukoplakia and oral candidiasis. Aids. 1992;6:95-100.

17. Available: WWW.aleere.com.determinetest.
18. Available: WWW.trinitybiotech.com.
19. Available: WWW.chembio.com
20. Phan GT, Belangen PH, Filler SG. Role of hyphal formation in Interactions of Candida albicans with endothelial cells. Infect. Immun. 2000;68(6):3485–3490.
21. Obuekwe ON, Onunu AN. Hiv-related oral disease in Benin City Nigeria West Afr. J Med. 2002;21:9-11.
22. Zakrzewska JM. Women as dental patients are there any gender differences? Int Dent J. 1996;46:548-557.
23. SuniliSolomon SS, Hawcroft CS, Narasimhan P, et al. Comorbidities among HIV infected injection drug users in Chennai, India Indian J Med Res. 2008;127(5):447–452.
24. Matthew Jukes and Kamal Desai. Education and HIV/AIDS A report prepared for the UNESCO Global Monitoring. Available: http://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=7&ved=0CFQQFjAG&url=http%3A%2F%2Fportal.unesco.org%2Feducation%2Fen%2Ffile_download.php%2F6c5eeae6d430e16f1fc891d55e026769Jukes_%2BM_3.doc&ei=NXN3U_hI8ODO4D9gIAJ&usg=AFQjCNE1W3JPVnofdseiptm3F2vhp000Q&bvm=bv.66917471,d.ZWU page. 2005;1-17.
25. Hyde S, White S. Challenges of education and cultural diversity in the work place. AIDS Health Promot Exch. 1993;2:4-7.
26. Singh S, Fukuda H, Ingle GK, Tatara K. Knowledge attitude the perceived risks of infection and sources of information about HIV/AIDS among pregnant women in an urban populationof Delhi. J. Commun. Dis. 2002;34:23-24.
27. Neil MC, Nash SL, Hajjeh RA, Phean MA, Com L, Pikaytis BD, Warnnic DW. Trend in mortality due to invasive mycotic diseases in the United States 1980-1997. Clinical Infect. Dis1. 2001;33(5)-641-7.
28. Enwuru CA, Ogunledu A, Idika N, Enwuru NV. Susceptibility Profile of Yeast-like organismsisolated from HIV/AID patients, using NCCLS Macrodilution method compared with Agar difution technique. African Journal of clinical and Environmental Microbiology. 2008;8(2):88-96.
29. Bhavan PS, Rajkumar R, Radhakrishnan S, Seenivasan C, Kannan S. Culture and identification of Candida Albicans from vaginal ulcer and separation of enolase on SDS-PAGE. Inter. J. Bio. 2010;2(1):84-93.
30. Liu X, Liu H, Guo Z and Luan W. Association of asymptomatic oral candidal carriage, oral candidiasis and CD4+ lymphocyte count in HIV-positive patients in China. Oral Dis. 2006;12:41-44.
31. Rejane PN, Maria AC, Guilherme MC, Ohane MCM. Yeasts isolated fromclinical samples of AIDS patients. Braz Journal of Microbiology. 2002;33(4):1517-8382.
32. Bassetti M, Filippo A, Laura N, Emanuele M, Maria Pia M, Michele M, Barbara R, Franco BP, Giancarlol and Claudio V. Incidence of candidaemia and relationship with fluconazole use in an intensive care unit. J Antimicrob Chemother. 2009;64(3):625-629.
33. Peman J, Bosch M, Canton E, Viudes A, Jarque I, Gomez-Garcia M, Garcia-Martinez JM, Gobernado M. Fungemia due to Candida guilliermondii in a pediatric and adult population during a 12-year period. Diag Microbiol Infect Dis. 2008;60:109-112.
34. Obuekwe ON, Onunu AN. Hiv-related oral disease in Benin City Nigeria West Afr. J Med. 2002;21:9-11.
35. Khongkunthian P, Grote M, Isaratanan W, Plyaworawong S, Reichart PA. Oral manifestation in HIV-positive adults from Northern Thailand. J Oral Pathol Med 2001;30:220-223.

36. Butt FM, Chindia ML, Vaghela VP, Mandalia K, et al. Oral manifestations of HIV/AIDS in a Kenyan provincial hospital East Afr Med J. 2001;78(8):398-401.
37. Shiboski CH, Hilton JF, Neuhaus JM, Canchola A, Greenspan D. Human Immune Deficiency Virus- related oral manifestations and gender. A longitudinal analysis. The University of California, San Francisco Oral AIDS Center Epidemiology Collaborative Group. Arch Intern Med. 1996;156:2249-2254.
38. Patton LL, McKaig RG, Strauss RP, Eron JJ Jr. Oral manifestations of HIV in a southeast USA population: Oral Diseases. 1998;4:I64-169.
39. Bendick C, Scheifele C, Reichart PA. Oral Manifestation in 101 Cambodians with HIV and AIDS. J Oral Pathol Med. 2002;31:1-4.
40. Jonsson N, Zimmerman M, Chidzonga MM Jonsson K. Oral manifestations in 100 Zimbabwean HIV/AIDS patients referred to a specialist Centre: Cent Afr J Med. 1998;44:31-34.
41. Bharathi M, Rani AU. Pathogenic fungal isolates in sputum of HIV positive patients. J AIDS HIV Res. 2011;3:107-113.

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