Journal of Advances in Medicine and Medical Research

30(1): 1-8, 2019; Article no.JAMMR.49823
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

Isolation of *Candida albicans* among HIV Patients Presenting with Oral Thrush in Abuja, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author YT designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors UAB and AMA managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMMR/2019/v30i130157
Editor(s):
(1) Dr. Kalpy Julien Coulibaly Department Environnement et Santé (Intérim), Institut Pasteur de Côte d'Ivoire, Université Félix Houphouet-Boigny, Abidjan, Côte d'Ivoire.
Reviewers:
(1) Kaunara. A. Azizi, Tanzania.
(2) Ronald Bartzatt, University of Nebraska, USA.
Complete Peer review History: http://www.sdiarticle3.com/review-history/49823

Original Research Article

Received 08 April 2019
Accepted 24 June 2019
Published 01 July 2019

ABSTRACT

Background: *Candida* infections are known contributors to the high morbidity and mortality rates seen in HIV positive patients.
Methodology: This was a descriptive cross-sectional study. The study was carried out at the Microbiology research laboratory, department of Medical Microbiology, University of Abuja Teaching Hospital, Gwagwalada. The population is made up of two hundred and ten (210) patients who presented with oral thrush between fifteen years and seventy years which comprises of 160 HIV seropositive and 50 non age and sex matched HIV seronegative patients. Culture, Microscopy and ELISA methods were used for isolation of *Candida albicans*. An interviewer-administered, structured questionnaire was used as the study tool.

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Results: The mean age for the isolation of *Candida albicans* was 30 ± 18.7 years, with the highest proportion of isolates within the age range of 21-30 years accounting for 27.1% of the study population recruited and the lowest proportion of isolates being 41-50 years accounting for 8.0%. From the 210 subjects with *Candida albicans* in the study, one hundred and sixty (160) representing 76.2 were isolated from HIV seropositive clients and *Candida albicans* isolation rate among HIV seronegative population was 23.8%.

Conclusion: The sensitivity, specificity and positive predictive value of using Grams reaction methods in the diagnosis of *Candida albicans* was 22.9%, 95.2% and 82.6%. The sensitivity, specificity and positive predictive value of using ELISA methods was 25.7%, 86.7% and 65.9%. In this study, there was preponderance of *Candida albicans* isolate among the young and the old in HIV seropositive patients but largely isolated from older patients among HIV seronegative patients.

Keywords: Isolation methods; Candida albicans; oral candidiasis; HIV patients.

ABBREVIATIONS

HIV : Human Immunodeficiency Virus
ELISA : Enzyme Linked Immunosorbent Assay

1. INTRODUCTION

The yeast *Candida* being the main cause of candidiasis is a commonly isolated pathogen from immunocompromised patients.It is the major cause of death in immunocompromised patients, especially in under developed countries [1,2]. Oral candidiasis is a common condition in HIV-AIDS patients, caused by commensal yeasts which may colonize the mucous membranes of the mouth causing morbidity due to several factors including immunosuppression, smoking, poor nutrition and the use of antibiotics [2,3].

Sub-Saharan Africa has 23.5 million cases of HIV and is home to 92% of the world's HIV-positivepregnant women of whom 24% died of pregnancy related complications [1,3]. One of the most common HIV-associated opportunistic infections is candidiasis, primarily caused by *Candida albicans*or *Candida dubliniensis*. *Candida* infection can present as pseudomembranous candidiasis (thrush), characterized bywhite pseudomembranes in the oral mucosa and/or upper digestive tract; acute atrophic-candidiasis. HIV-related *Candida* infections were found to beassociated with a higher patient mortality in developing countries than in developed countries [2,3,4].

To date, several *Candida* species have been found to be opportunistic especially in HIV/AIDS patients [1,5]. Microscopy of *C. dubliniensis* reveals the presence of*Candida* species which includes pseudohyphae and chlamydospores [5,6]. Other than microscopy, culturing techniques can be used in the differentiation of *C. albicans*and *C. dubliniensis*bygrowing the organisms in Sabouraud agar plates at 45°C [1,3,7,8] and also growing theorganisms in tobacco agar (a mixture of tobacco and agar) at 28°C for 48-72 hours. It has been suggested that the use of oral antifungals in oropharyngeal candidiasis must be reserved especially for HIV immunocompromised cases where response to antifungal treatment is limited [9,10,11,12]. ELISA (Enzyme Linked Immunosorbent assay) can also be used to isolates candida species which are more developed for *Candida albicans* compared to other species [10,12].

In the course of this work, there is paucity of information regarding the laboratory characterization of *Candida albicans* in the tropics and the need to examine and analyze various laboratory diagnostic techniques in other to proffer the fast, cheap and accurate method. Therefore the aim of the study was to isolate and characterize *Candida albicans* among HIV patients with oral thrush and to evaluate the diagnostic tools.

2. MATERIALS AND METHODS

2.1 Study Design

This was a descriptive cross-sectional study which spanned over forty-eight month (August 2016 to September 2018.

2.2 Study Area

The study was carried out at the Microbiology research laboratory, department of Medical

Microbiology, University of Abuja Teaching Hospital, Gwagwalada. The hospital is 330 bedded with two research laboratory which service all the clinical departments. It also receives sample from other hospital in Gwagwalada and neighbouring state such as Nassarawa, Kaduna, Niger and Kogi state. Abuja is the administrative capital of Nigeria, hosting the national Parliaments and the Presidency. This is a cosmoplolitan city by all standards with several nocturnal recreation areas [13].

2.3 Study Population

The population is made up of two hundred and ten (210) patients who presented with oral thrush between fifteen years and seventy years, comprises of 160 HIV seropositive and 50 non age and sex matched HIV seronegative patients recruited from general outpatients department. The study protocol was strictly adhered to, which include counseling of patients. The purpose of this work was explained to the participants before they fill in the consent form. An interviewer-administered, structured questionnaire was used as the study tool. This form contained bio-demographic data, diagnosis and laboratory result.

2.4 Inclusion Criteria

i. Adult of both sexes aged fifteen to seventy years with symptom of oral thrush.
ii. HIV seropositive patients irrespective of their CD4+ status
iii. HIV seronegative patients.

2.5 Study Sample Size

The minimum sample size was calculated using the following formula [14]

$$N = \frac{Z^2 p q}{d^2}$$

Where:

N= Minimum sample size

Z= the standard normal deviation corresponding to 95% levels of significance (1.96)

A total of 210 patients were recruited for this study.

2.6 Laboratory Procedure

2.6.1 Specimen collection, transportation and processing

The throat was swab using single use spatula to scrub and placed in a universal container for onward transportation to the laboratory [15]. Blood specimen was collected in EDTA bottle and centrifuged to release the serum.

2.7 Determination of HIV

All the patients that enrolled for the study were screened at the counseling and testing units. Finger pricks were used to aseptically collect blood samples for HIV serology, using HIV rapid test kits. They were either used singly or in combination with other test kits. The positive results were repeated with other test kit to confirm their HIV positive status. The former gave room for participants to supply information on the type of medication (if any), source of the drugs, and duration of administration and hygiene ethics.

2.8 HIV Test Kits

Determine HIV $^{1}/_{2}$: Determine HIV $^{1}/_{2}$ is an immunochromatography test for the detection of antibodies to HIV -1/HIV -2. Manufactured for Inverness medical Jape Co. Ltd.By ABOTT JAPAN CO. LTD. The chase buffer was stored at 2-30°C.

Chembio HIV $^{1}/_{2}$ Stat Pak: Chembio HIV $^{1}/_{2}$ Stat Pak essay employed a combination of antibody binding protein which was conjugated to colloidal gold dye particle, antigens to HIV ½ which were bound to the solid phase. The sample being tested and the running Buffer were applied through the sample window. The running Buffer facilitates the lateral flow of the specimen through the membrane and promoted binding the antibodies to the antigens. If present the antibody binds to the gold to the gold conjugated antibody binding protein. In a reactive sample the dye conjugated immune complex migrated to the nitro cellulous membrane and was captured by the antigens immobilized in the area producing a pink/purple line [15].

In the absence of HIV1/2 antibodies, there was no pink/purple line in the test area. The sample continues to migrate along the membrane and produced a pink/purple line in the control area

containing immunoglobulin G antibodies. The procedure control served to demonstrate that the specimen and the reagents was applied properly and have migrated through the device. Chembio HIV 1/2 Stat Pak was manufactured by Chembio Diagnostic System Inc.3661 Horseblock Road, Medford NY 11763, USA. The HIV 1/2 Sat Pak test device was stored at temperature between 8-30°C in the vial desiccant.

2.9 Gram Procedure (Microscopic)

For simple microscopic methods, normal saline was dropped on the glassslide, emulsify with the specimen and cover it with a cover slip and viewed under high power field for the presence of yeast cells. Using wireloop, bursen flame, crystal violet, lugol's iodine, and acetone, safranin, microscope, cottonwool, oil immersion, sterile water, and timer to process the specimens, the outcome was Gram positive large yeast cells.

2.10 Culture Methods

Culture methods involve the use of Sabouraud's selective media (Sabouraud dextrose agar) chromogenic agar(Oxoid*Candida* agar, Oxoid, UK). The inoculated plates were incubated at 37°C for 24 to 48 hrs. Sabouraud dextrose agar allow for eevaluation of the shape and morphology of growth while Chromogenic media allows for the differentiation of different *Candida* species based on the color of the colonies, which is a simple, rapid but accurate test based on the ability of *Candida albicans* to cleave chromogenic substrate in the media using the enzymes hexosaminidase and confirmation of presumptive clinical *Candida albicans*isolates was done using the germ tube test which relies on the ability to form short lateral filaments (tubes). This was quality controlled using control strain of *Candida albicans*ATC*C* 90028.

2.11 ELISA Methods

Break off to expose the wells needed (number of samples plus 2 for controls) and place in holder. Two 2 drops of the emulsify thrush was added to each test well. Then incubate for 30 minutes at room temperature (15-25°C), then washed. Two 2 drops of Reagent 1 was added to each well. It was incubating for another 5 minutes, then washed. Two (2) drops of Reagent was added to each well, and incubated for 5 minutes, then washed. Two (2) drops of Chromogen was also added to each well and incubated for 5 minutes. Finally, drops of Stop solution was added to each well and mixed gently by tapping the side of the strip holder with index finger and the result read visually or at 450/620-650nm. Interpretation of result was done using ELISA Reader. Zero reader on air. Wells read at 450/620-650nm. Absorbance reading of 0.15 OD units and above indicates the sample contains *Candida albicans* antigen and so recorded as REACTIVE.

3. RESULTS

This study was carried out among 210 participants between the ages of fifteen years and seventy years comprising of one hundred and sixty HIV seropositive patients and fifty seronegative patients recruited after determination of their status but none withdrew from the study. In total there were 86 males (40.9%) and 124 females (59.1%) and the male to female ratio (M:F) was 1:1.5. *Candida albicans* were isolated from 59 males HIV seropositive compared to isolation *Candida albicans* from 91 HIV seropositive females, the difference in the isolation of *Candida albicans* in terms of gender was statistically significant and there was positive association between the isolation rate of *Candida albicans* and gender (p=0.001, Pearson Chi-Square= 27.261, df= 1, positive Spearman Correlation= 0.529 Table 1). The mean age for the isolation of *Candida albicans* was 30 ± 18.7 years, with the highest proportion of isolates within the age range of 21-30 years accounting for 27.1% of the study population recruited and the lowest proportion of isolates being 41-50 years accounting for 8.0%. However, this distribution of isolation was not statistically significant (p=0.085, Table 2).

From the 210 subjects with *Candida albicans*in the study, one hundred and sixty (160) representing 76.2 were isolated from HIV seropositive clients while 50 representing 23.8% were isolated from HIV seronegative population. Of the 160 *Candida albicans* isolated among HIV seropositive, 36 were isolated from the 61 – 70 years age group giving a age specific prevalence rate of 22.5%; 21 *Candida albicans* were isolated (13.1%) from the 15-20 years age group. The highest proportion of candida isolate among seropositive patients was within age group 21-30 years while the least proportion was in age group 41-50 years accounting for 29.4% and 7% respectively (Table 1). Among, HIV seronegative patients 10 *Candida albicans* were isolated from the 21 – 30 years age group giving a age specific prevalence rate of 20.0%; 11

Candida albicans were isolated (22.0%) from the 31-40 years age group. *Candida albicans* were frequently isolated from 61-70 years age group with age specific rates of 32.0%. The difference in the isolation rate of *Candida albicans* among HIVseropositive population and HIV seronegative patients was statistically significant (p= 0.003, Pearson Chi-Square= 1.417, df= 1, Table 1).

In comparing the three methods (Grams, culture and ELISA methods) used in the identification of the *Candida albicans*among the HIV seropositive patients, the percentage detection rates were: 30.0% (Grams reaction), 36.3% (Culture methods) and 33.7% (ELISA methods). Among HIV seronegative individuals, the isolation rate of *Candida albicans* were 20.0% (Grams reaction), 24.0% (Culture methods) and 56.0% (ELISA methods). The lowest isolation rate was observed using the Grams methods in both groups (Table 3).

Furthermore, the sensitivity, specificity and positive predictive value of using Grams reaction methods in the diagnosis of *Candida albicans* was 22.9%, 95.2% and 82.6%. *Candida albicans* were isolated from the throat swab specimens collected using Sabouraud dextrose agar culture methods with specificity, sensitivity and positive predictive value of this methods was 27.6%, 94.3% and 82.9%. In comparison, the sensitivity, specificity and positive predictive value of using ELISA methods was 25.7%, 86.7% and 65.9% (Tables 4, 5, 6).

Table 1. Distribution of oral candidiasis among age group

Age group	Total (%)	HIV/AIDS +ve (%)	HIV/AIDS -ve (%)
15-20	24 (11.4)	21 (13.1)	3 (6)
21-30	57 (27.1)	47 (29.4)	10 (20)
31-40	40 (19.0)	29 (18.1)	11 (22)
41-50	17 (8.0)	7 (4.4)	10 (20)
51-60	20 (9.5)	20 (12.5)	0 (0
61-70	52 (24.8)	36 (22.5)	16 (32)
Total	210 (100)	**160 (76.2)**	**50 (23.8**

Table 2. Gender and distribution of *Candida albicans*

Gender	Total (%)	HIV +ve samples (%) N=160			HIV –ve samples (%), N=50		
		Grams	Culture	ELISA	Grams	Culture	ELISA
Male	86 (40.9)	12 (25)	13 (22.4)	34 (63)	5 (50)	12 (58.3)	15 (53.6)
Female	124 (59.1)	36 (75)	45 (77.6)	20 (37)	5 (50)	5 (41.7)	13 (46.4)
Total	**210**	**48 (30)**	**58 (36.3)**	**54 (33.8)**	**10 (20)**	**17 (24)**	**18 (56)**

CD+ Count N=160

Table 3. Isolation methods for *Candida albicans*

Factor	Grams (%)	Culture (%)	ELISA (%)
HIV/AIDS +ve N=160	48 (30)	58 (36.3)	54 (33.7)
HIV/AIDS –ve N=50	10 (20)	12 (24)	28 (56)
Total	58 (27.6)	**70 (33.3)**	**82 (39.1)**

Table 4. *Candida albicans* isolated via grams

GRAMS	HIV +VE	HIV –VE	TOTAL
Positive	48	10	58
Negative	162	200	362
TOTAL	210	210	420

Table 5. *Candida albicans* isolated via culture

Culture	HIV +VE	HIV –VE	Total
Positive	58	12	70
Negative	152	198	350
TOTAL	210	210	420

Table 6. *Candida albicans* isolated via ELISA

ELISA	HIV +VE	HIV –VE	TOTAL
Positive	54	28	82
Negative	150	182	332
TOTAL	210	210	420

4. DISCUSSION

The ability to make quick diagnosis is very important in order to reduce man-hour loss. The laboratory isolation of causative agent of oral thrush is critical in prediction of clinical cure when the organism and indeed the species is identified and communicated to the physician. This is more expedious among HIV/AIDs patients in our facility. Although, *Candida albicans* is the most prevalent cause of oral thrush but, simple and cost effective methods are needed to make laboratory diagnosis. This study examines three laboratory techniques and compared them with the standard (Chromogenic *Candida albicans* media plus germ tube test) in our resource limited environment.

From the study, *Candida albicans* were isolated in high rates from the young and the old in both HIV seropositive and HIV seronegative, 21 (13.1%) and 3 (6.0%) among the young ones respectively, 36 (22.5%) and 16 (32.0%) among the older age groups respectively. This is not consistence with other study [2,10,12]. This difference might be due to the methodology employed in this study. In other studies, the sample size of both serogroups were equal. Immunity of the patients might play a central role with regards to age specific prevalence rate [4,7]. Among the younger age group (15-20 years age group), reduced T-cell immunity among adolescence HIV patients might account for higher isolation rate of *Candida albicans* than HIV seronegative adolescence. But, among the older ones, waning immunity contribute largely increased isolation rate of *Candida albicans* (61-70 years age group). In this study, *Candida albicans* were more isolated among sexually active young adult in both sero-groups.

In relation to gender, *Candida albicans* were mostly isolated from the females than males; this assertions was consistent with other previous study [1,10]. These might be due to interaction of estrogen hormone and the immune systems.

Early recognition of this opportunistic fungal infection in the mouth will definitely avoid grave discomfort especially among HIV seropositive individuals. Culture methods using sabouraud dextrose agar media can also be used in the initial identification of *Candida albicans*, this media is less expensive than chromoagar. Most of the studies analyzed primed in the use of Sabraoud dextrose agar and confirm any isolates from the media with germ tube test. But, this method requires the use of incubator, steady power source, and advance manpower to pour the prepared sabouraud dextrose agar media aseptically and for reading of the plate. And in most cases antifungal susceptibility testing are infrequently and not routinely done after pouring and setting of the culture dish. Interestingly, in developed society laboratory work concentrated on the use of high technologic such as PCR but these methods may not be applicable to us in the developing world, where financial resources are scarce.

Culture methods are available in most clinical and research laboratories but this method is more cumbersome to process, time consuming and the small impurities may contaminate the plate, with sensitivity of 27.6%, this technique provide excellent screening tool when compared to other methods and can provide useful data for epidemiological studies. This technique has a low detection limit, and does not give information of on-going active infection and previous infection [8,10].

The detection and laboratory diagnosis of *Candida albicans* by ELISA method from this study have high sensitivity among HIV seropositive and HIV seronegative patients. Low positive predictive value of 65.9% is low compare with 82.6% and 82.9% positive predictive value using microscopy and culture diagnostic methods respectively. The test is not expensive and available to patients. The test can serve as point of care apparatus and the turnaround time is high. However, this technique does not tell us if an infection is active or the antigen presence is as a result of a previous infection. In comparison with the two other methods used in this study, ELISA method has a better detection rate, 33.7% among HIV seropositive and 56.0% among HIV seronegative patients. This outcome was similar to other studies around the world [3,6,7].

Grams method had showed a lower sensitivity, hence not fit for screening tool especially for HIV

patients who have oral thrush. Apart from microscopy problems of interobserver variability, grams reagents for microscopy are made of simple dye and alcohol which are inexpensive [11,12].

The efficacy of the methods in this study have revealed that there is no one method that is fool prove, therefore, the combination of any two of the three methods will be the best option. This is very important in the sense that no patients with oral thrush should have delayed diagnosis.

5. CONCLUSION

Candida albicans is an opportunistic fungal infection isolated from patients with oral thrush and are commonly seen in immunosuppress state such as HIV infection. This study revealed preponderance of *Candida albicans* among the young and the old in HIV seropositive patients and largely isolated from older patients among HIV seronegative patients, but regular and rapid investigation in diagnosis *Candida albicans* in the laboratory should be encouraged for patients presenting with oral thrush.

6. RECOMMENDATION

1. Elderly patients with thrush, irrespective of the HIV status should be evaluated for *Candida albicans*
2. HIV patient with thrush should have a laboratory test in order to ascertain the species.

CONSENT

As per international standard patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per university standard guideline participant consent and ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle3.com/review-history/49823