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Prevalence of Candidiasis and Associated Site of Infection among HIV Patients Attending Federal Medical Center, Azare

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Abstract

Patients with Human Immunodeficiency Virus (HIV) are at the greatest risk of being infected with various opportunistic infections, as their immune systems become so weak to fight against the infectious agents. Candida species are among the commonest opportunistic pathogens affecting people with weakened immune systems. Candidiasis remains the major challenge of public health important to the HIV patients. There is scarcity of information regarding the impact of Candidiasis in public health in Northeastern part of Nigeria, especially Bauchi State. This study aimed at determining the prevalence of Candidiasis and the associated site of infection in the HIV patients. Clinical samples such as blood, urine, sputum and oral swab were collected from 300 HIV patients attending Federal Medical Center Azare, Bauchi State. The pathogens were isolated on Sabouraud Dextrose Agar and identified using Corn meal agar, Germ tube test, ChromagarCandida, Gram staining and Lactophenol cotton blue. Four Candida species were isolated among which Candida albican (51%) was the most prevalent followed by Candida glabrata (12%). The isolates colonized the oral cavity of 51.11% of the patients making oral candidiasis most common among the patients. The oral candidiasis is the most troublesome form of candidiasis among HIV patients, and has been caused mostly by Candida albican.

Key words- Opportunistic infections, Candidiasis, Site of Infection, HIV, Bauchi

INTRODUCTION

Human immunodeficiency virus (HIV) is a retrovirus from the genus Lentivirus which is attacking and directly or indirectly destroying CD4+ T cells. Mammals may become infected with such viruses, which are responsible for the long-duration illnesses with a long incubation period (Alimonti, Ball and Fowke, 2003). Opportunistic fungal infections were reported to be the most common complication of human immunodeficiency virus (HIV) infection, and the development of opportunistic infections in HIV patients lies upon the exposure of the patients to the pathogens, virulence of the pathogens, the immunity of the patients, and the use of antimicrobial agents for prevention against infectious agents (Kong *et al.*, 2007). Most of these infections are responsible for an increased hazard of death in the HIV seropositive patients. The patients who are suffering most, from such kinds of infections may have interruptions in antiretroviral therapy leading to more rapid progression of HIV disease. Seriously immunocompromised patients may suffer from various kinds of opportunistic infections that greatly influence their well-being, health care expenses and quality of life (Kong *et al.*, 2007). Numerous fungal species are capable of causing disease in humans, animals and plants. Infections caused by them are highly prevalent in humans, as it was estimated that greater than 1 billion people worldwide have infections caused by these organisms. Several fungal species were reported to cause diseases in humans (Havlickova, Czaika and Friedrich, 2008). Due to the immunocompromization, the HIV patients are vulnerable to the various kinds of opportunistic infections caused by different species of pathogenic organisms including protozoa, fungi, viruses, and bacteria (Zanoni and Gandhi, 2014). Fungal opportunistic infections are the major cause of morbidity and mortality in HIV patients (Durden and Elewski, 1997). *Candida* species are common commensals in the general population and may account for about 75% of the pathogens were isolated from the oral cavity and genital tracts of the HIV seropositive patients. Higher rates of mucosal carriage of *Candida* in HIV seropositive men and women were severally reported, with mucosal candidiasis representing the primary pathological challenges. Oropharyngeal *Candidiasis* was reported to be the commonest

opportunistic infection affecting HIV-seropositive individuals, occurring in about 80-90% of patients (Merenstein *et al.*, 2013). Oesophageal *Candidiasis* was reported in up to 23% of the HIV patients. It was recently reported that the prevalence of oesophageal *Candidiasis* raised up to 50% (Grabar *et al.*, 2008; Lortholary *et al.*, 2012). *Candida vulvovaginitis* in HIV patients was reported to have similar frequencies with that observed in the immunocompetent. Candidemia emerged as the major threat to the patients living with HIV; however, numerous researches provided clues on how to handle the issue (Grabar *et al.*, 2008; Pappas *et al.*, 2016). *Candida albicans* remains the most frequent cause of *Candidiasis* globally but the distribution of *Candida* species varies geographically. Oral candidiasis has been reported as the most common fungal opportunistic infection among such group of patients. It has been reported that almost all HIV patients are colonized with *Candida* and up to 90–95% develop clinical lesions as the disease progresses (Pfaller *et al.*, 2011; Wadhwa *et al.*, 2007). It has also been noticed that low CD4 counts and high HIV viral load significantly correlate with oral *Candidiasis* in HIV patients. It was suggested that, diabetes mellitus, head and neck cancer, smoking, the use of oral prostheses, age, race, poor nutritional status (Gottfredsson *et al.*, 1999), reduced salivary flow, the use of antibiotics, pregnancy, low CD4+ count, high HIV viral load and lack of qualitative antiretroviral therapy were the major risk factors of *Candidiasis* in the HIV seropositive patients (Gottfredsson *et al.*, 1999; Liu, Guo and Luan, 2006). This study aimed at determining the prevalence of *Candidiasis* and its site of infection in the HIV patients. The study yielded information on the impact and prevalence of Candidiasis in people living with HIV, as well as the sites of infection in the patients, so as to provide an effective management and control for the infections and improve health status of the patients.

MATERIALS AND METHODS

Study area

The research was conducted at the Federal Medical Center Azare Bauchi State, which is one of the standard tertiary medical centers owned by the Federal Government of Nigeria, providing all kinds of medications and treatments. The center is located at No. 5 Sule Katagum road Azare Bauchi State Nigeria.

Study Population

This research is cross-sectional, in which data were randomly collected from the HIV patients enrolled in Federal Medical Center Azare at a single point in time. The study population includes HIV patients of all ages and sexes attending the Center with signs and symptoms of Candidiasis, but all HIV patients without signs and symptoms of the infection were excluded.

Sample Size

The sample size was obtained using the formula below:

$n = Z^2P(1-P)/e^2$ (Jaykaran and Tamoghna, 2013)

Therefore the total of 300 patients was enrolled for this study, Z equals to 1.65 at 90% confidence level, standard deviation of 50%, and 5% margin of error were used for this research.

Ethical approval

The ethical approval was obtained from the Research and Ethics Committee, Federal Medical Center Azare, Bauchi State Nigeria, with reference number FMCA/COM/36. The patients enrolled in the research were informed about the investigation, and their voluntary participation in the work was solicited and obtained.

Sample Collection

Urine, blood, oral swab, and sputum were collected from 300 patients attending Federal Medical Center Azare and transported to the Microbiology Laboratory Bauchi State University Gadau for investigation. Isolation of the *Candida* Species

The pathogens were isolated on Sabourauds dextrose agar (SDA). The prepared medium was inoculated with the test specimen and incubated at 35^{o}C with periodic checking for fungal growth (Naveena and Joy, 2014).

Identification of the pathogens

Fungal isolates were identified by Gram staining, lactophenol cotton blue preparation, sub-culturing the organisms on corn meal agar and chromagar Candida. Also by using germ tube test (Naveena and Joy, 2014)

Potassium hydroxide (KOH) preparation

Samples of a clinical specimen was dropped on a clean dry microscope slide, and 10 % KOH was added. This was then gently heated and examined under microscope.

Gram staining

The agar smear was prepared and heated gently to get it fixed. The slide was flooded with 0.5 % crystal violet and left for 30 sec. then rinse gently with water. The slide was then flooded with Lugol's iodine and allowed to remain for 30sec. and wash off with water. This was followed by decolourization with 95% ethanol and rinse with water.

The slide was then flooded with 0.1 % safranin and allowed to stand for about 1min. Finally the slide was washed with water and blotted dry. The slide was examined using an oil immersion objective for cell morphology and Gram reaction.

Lactophenol cotton blue (LCB)

One drop of the lactophenol cotton blue stain was placed on a clean slide. A small portion of the test organism was picked up and mixed with the stain. A cover slip was gently pressed on the slide to avoid air bubbles and to make a thin mount. The prepared slide was examined under low power (x10) with reduced lighting, and then switched to high power (x40) to confirm the presence of suspected fungal structures.

Chromagar candida

Ten grams (10g) of Chromopeptone, 20.0 g of Glucose, 2.0 g Chromogen Mix and 15.0 g Agar was dissolved in 1000 ml of distilled water. This was autoclaved at 121ºC for 15 minutes and allowed to cool before pouring it into a plate. The plate was inoculated with the test organism, incubated for 20 to 48 hours at 35˚C and observed for the appearance of different colours.

Corn meal agar

Three consecutive parallel scratches were made into the surface of the Corn Meal Agar plates through to the bottom. Using an inoculating loop, a streak was made across the three scratches. A coverslip was flamed and placed over the center of the inoculation scratches. A positive control of a known chlamydospore producing isolate was prepared. The plates were incubated at 25˚C and examined at 24, 48 and 72 hours for production of chlamydospores.

Germ tube test

The germ tube medium (human serum) was inoculated with *Candida* specie, and Incubated at 37ºC for 2 to 4 hours. After incubation, a drop of serum was withdrawn from the test tube and placed on to a glass slide. The slide was covered with a cover slip, and examined for presence of germ tubes.

RESULTS

This work revealed that, four *Candida* species were isolated from the various clinical samples. The species were *Candida albicans, Candida glabrata, Candida dubliniensis* and *Candida tropicalis*. *Candida albican* was isolated from 51% of the samples, *Candida glabrata* from 12%, *Candida dubliniensis* from 8% while, 4% of the samples were tested *Candida tropicalis* positive (table 1).

Moreover, the study revealed that, the pathogens were isolated from 19.11%, 29.78% and 51.11% of the blood, urine and oral swab of the patients respectively, but none was isolated from the sputum (table 2).

Table 1: Distribution of *Candida* species in HIV infected individuals attending Federal Medical Center Azare

Isolates	Positive	Percentage (%)
C. albican	153	51
C. glabrata	36	12
C. dublinensis	24	8
C. tropicalis	12	4
Total	225	75

Table 2: Identification of the infection site in the patients

Samples	Frequency	Percentage
Blood (Candidaemia)	43	19.11
Urine (Urogenital)	67	29.78
Sputum (Respiratory tract)	0	0.00
Oral swab (Thrush)	115	51.11
Total	225	100

DISCUSSION

Cases of *Candida albican* were reported in most of the patients enrolled (51%). This indicated that, *Candida albican* was the most prevalent pathogen. This is inline with the findings of Ugwa E A., (2015) who stated that, the most frequent cause of the Vulvovaginal Candidiasis was *Candida albican* with the total number of 316 (84.5%) cases out of 374 patients. Kwawukume *et al.*, (2002) reported 25% while Nwadioha *et al.*, (2013) reported the cases of the infection in 61% of the patients enrolled. Additionally, Onifade and Olorunfemi (2005), reported a prevalence of 81.5% in their study participants.

Also, the study is in agreement with the results of Merenstein *et al*. (2013), Pfaller *et al.,* (2011) and Wadhwa *et al.,* (2007) who reported different species of Candida in people with weak immune systems among which *Candida albican* emerged most prevalent specie. However, the study revealed that *Candida glabrata* emerged as second most prevalent (12%), followed by *Candida dublinensis* (8%) while *Candida tropicalis* was rare among the patients. The study agreed with the findings of Ngwa *et al*., (2020) who reported that, cases of *Candida glabrata* were noticed in 16.9%, while *Candida tropicalis* was recorded from 6.4% of the patients.

On the other hand, the study reported oral Candidiasis (51.11%) as the most troublesome form of Candidiasis among the HIV patients. This is in line with the result of Ngwa *et al* (2020) who reported the cases of oral candidiasis in 42.86% of the HIV patients enrolled in their study. Likewise, Merenstein *et al*. (2013), Pfaller *et al.,* (2011) and Wadhwa *et al.,* (2007), reported higher number of cases of oral Candidiasis in the patients.

Moreover, the urogenital tracts of 29.78% of the patients were reported to have been involved. This is in line with the finding of Ibrahim *et al*., (2013) who reported cases of vaginal Candidiasis in 41% of the patients. While only 19.11% of the patients were suffering from Candidaemia. The respiratory tract of none of the patients was involved, as no any specie was detected from the sputum of the patients. The current study disagreed with the result of Jyotsna *et al*., (2016) who reported respiratory tract Candidiasis in 31.7% of the patients.

CONCLUSION

Oral candidiasis remains the most common type of *Candida* infection among HIV patients attending Federal Medical Center Azare, Bauchi State.

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