Candida albicans infection among HIV positive and HIV negative women- Case study at Butare University Teaching Hospital (CHUB), Southern province of Rwanda


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ABSTRACT

Background: Candida albicans still major leading global health challenge especially in immunocompromised individual particularly living in low-income countries with limited access to the antifungal drugs. Though the incidences and prevalence of opportunistic infection have been reduced due to the availability and strict adherence to the antiretroviral therapy, candidiasis remains the most frequent fungal infection with high morbidity and mortality and It is still a neglected topic in the research sectors of developing world including Rwanda. This study aimed to determine the prevalence of candida albicans infection among HIV positive and HIV negative women attending Butare University Teaching Hospital in the southern province of Rwanda.

Methods: A descriptive cross-sectional study was conducted with 306 female participants aged between 17-64 years old. Conventional sampling method was used to obtain the desired population and an interviewer structured questionnaire was administered to gather social demographic information and associated risk factors. both urine and a drop of blood for HIV were requested for testing. HIV testing followed the national HIV testing policy.

Results: Among 306 women participated; the average age range was 30-40 ages. The overall prevalence of candida albicans was 10.5% and was highly isolated in HIV positive women 21(13.7%) than in HIV negative women 11(7. 2%). Pregnancy and antibiotics usage were statistically significant associated with candidiasis with p-value <0.05 while age was not statistically associated with candida albicans infection with p-value>0.05.

Conclusion: The availability and strict adherence to antiretroviral therapy to people living with HIV have tackled the opportunistic infections like candidiasis. However, poor hygiene still the major health challenge for both groups. Hence, improved hygiene and early treatment with an improved diagnostic test to the risk population should highly be importance.

Key words: Prevalence, Candida albicans infection, Butare University Teaching Hospital.

INTRODUCTION

Fungal infections continue to pose significant morbidity and mortality due to the increasing number of at-risk population across the world particularly in developing countries1-2. Globally, an estimated 37.9 million people were living with HIV in 20183. HIV/AIDS officially recognized as epidemic since the 1980s with the increase of immunocompromised individual that has exposed them to numerous opportunistic infections including candida infection of fungal aetiology and accompanied with massive population death4-5. Candida species including C. albicans, C. tropicalis, C. glabrata, C. kruzei, C. dublinensis, C. guilliermondii, C. parapsilosis, C. kefyr, and C. pelliculosa are the most frequent fungal species of the human microbiota and these species asymptotically colonizes many areas of the body, predominantly the gastro-intestinal and genit-urinary tracts of healthy individuals6-7. However, in the condition that could impair the host immune system, C.
Candida albicans can change from normal commensal state to a pathogenic state by penetrating the mucosal surface and cause a deep-seated invasive and life-threatening infection\(^6\). The pathogenic state of C. albicans is highly associated with its ability to undergo phenotyping switching from different cellular form like yeast cell to hyphae cell depending on environment growth conditions\(^9\),\(^10\),\(^11\).

C. albicans remains the most frequent pathogenic candida species responsible for almost fungal morbidity and mortality in the developing world and causing the increased cost of care and duration of hospitalization\(^12\). The non-albicans candida species have significantly emerged to cause infection in patients living with HIV due to the fact that these non-albicans species are less susceptible to the commonly used antifungal drugs which hinder their effective treatment\(^13\). Although C. albicans remains the dominant species causing Blood Stream Infection, the frequency of occurrence varies throughout the world from a low of 37% in Latin America to a high of 70% in Norway\(^14\). The recent global incidence was estimated to be 700,000 cases of invasive candidiasis and reported to have a mortality rate of 38% to 44% in developing countries with resource-limited settings where conventional diagnostics test is slow, expensive or delay to access the antifungal drugs in all countries and a high burden of HIV coinfection\(^15\).

In Africa and the Middle East, Candida albicans is highly isolated to cause invasive and superficial candidiasis with the prevalence of 55.18% and 76.92% respectively\(^16\). This was mostly seen in Sub-Saharan Africa and central Africa where Candida albicans infections rate was (22.74%), followed by the South African region (22.44%). With regard to C. albicans invasive infections still prevalent in Sub Saharan and central Africa at (12.34%), followed by the Middle East (6.05%)\(^16\). Vaginal C. albicans infection has been implicated most often in a number of candida infections which affects approximately two-thirds of the women of reproductive age globally. Candida may become worse in a patient with the compromised immune system, such as HIV/AIDS patients or those that are on immunosuppressive therapies, broad-spectrum antibiotics users and pregnant women, this is a potentially life-threatening leading to systemic fungal infection\(^17\).

In Rwanda, Candida infection and other fungal aetiology disease are still under-diagnosed with routine diagnostic tests and rarely considered as a global health challenge particularly in immunocompromised individuals that could result in drug resistance. Moreover, there is limited data with no published information mainly in research sectors oriented on candidiasis highlighting the burden of the aforementioned infection in HIV positive or HIV negative individuals that could contribute to the ineffectiveness of treatment if not properly managed. Thus, this study is aimed to determine the prevalence of Candida albicans infection among HIV positive and HIV negative women attending Butare University Teaching Hospital in the southern province of Rwanda.

**METHODS**

**Design and Study area**

This was a hospital-based descriptive cross-sectional study conducted from May to September 2019 at Butare University Teaching Hospital. It is located at Mamba, Butare Cell, Huye District in the Southern province of Rwanda, a National Referral Hospital which serves more than 3,772,230 people from Southern Province and others from different locations of Rwanda.

**Sample size determination & Sampling**

A convenience sampling strategy was used for participant's selection. By referring to the prevalence of Candida albicans 27.5% in the previous study conducted in Uganda\(^18\). The minimum sample size was calculated using Fisher’s formula \(n=z^2p(1-p)/d^2\) which yields a minimum sample size of 306\(^19\). Where \(n=\)sample size, \(z=\)statistic for the level of confidence i.e.1.96, \(P=\)expected prevalence (27.5%) and \(d=\)allowable error i.e. 0.05.

**Data collection and processing**

Participants meeting the inclusion criteria and willing to participate in the study were conveniently recruited from the ART (Antiretroviral Therapy) clinic and OPD (Outpatients Department) service. The study was mainly intended to isolate the pathogenic microorganism, therefore those who were using the antifungal treatments were excluded from the study.

The informed consent forms were given together with a semi-structured questionnaire to gather demographic information. The participants from the ART clinic were given urine containers and requested to bring urine sample whereas those from OPD department were requested to give blood for HIV status testing as an addition to urine samples. The HIV status testing followed the national HIV testing policy by use of Alere® HIV combo for screening and Stat Pak for confirmation. The participants confirmed positive HIV test result was counselled by Hospital counsellors of the ART clinic. All 306 urine samples were streaked over Sabouraud Dextrose Agar media -SDA and incubated aerobically at 37°C for 3-4 days. Yeast growths were then identified by colonial morphology characteristic of Candida on above-mentioned media. For confirmation of Candida albicans using germ tube test, a single colony from SDA was
isolated, transferred and emulsified in human serum for 4 hours at 37°C.

**Data analysis method**
The data were entered into Microsoft Excel (Microsoft Corp, Santa Rosa, CA, USA) and exported for statistical analysis in IBM SPSS Statistical developer version 21. Relative frequency of demographic characteristics was summarized using descriptive statistic. Correlation of dependent and independent variables was analyzed using Chi-square test. The level of statistical significance was set at 95% confidence level where \( p < 0.05 \).

**Ethical considerations**
Ethical approval was obtained from the Institution Review Board, University of Rwanda/ College of Medicine and Health Sciences and Ethical Committee of Butare University Teaching Hospital with reference numbers CMHS/IRB/219/2019 and RC/UTHB/057/2019 respectively. The participants were explained about the study, only participants with signed informed consent form were enrolled in the study. Moreover, Ethical clearance was distributed to the head of the respective department.

**RESULTS**
The study included 306 women participants of the average age range of 31-40 years old of which 153 were HIV positive and 153 were HIV negative women from ART clinic and OPD department respectively (Table 1). *Candida* species were isolated in 59(19.2%) out of total population which account 32(54.2%) of germ tube formers isolated as *Candida albicans* and 27(45.7%) germ tube test negative isolated as *Candida non-albicans* (data not shown). *C. albicans* was highly isolated among ART participants 21(13.7%) compared to OPD participants 11(7.2%) (Table 1). The overall prevalence of *C. albicans* was 10.5% while *Candida non-albicans* accounted for 8.8% (Table 1). However, *Candida albicans* was significantly associated with antibiotics usage and pregnancy status in both HIV positive and HIV negative women with \( p < 0.05 \) (Table 2).

**TABLE 1. Demographic characteristic of study population in relation to Candida species**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Microorganisms isolated</th>
<th>Participants n</th>
<th>C. albicans (%)</th>
<th>C. non albicans (%)</th>
<th>Negative (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20]</td>
<td></td>
<td>7</td>
<td>0(0%)</td>
<td>1(14.3%)</td>
<td>6(85.7%)</td>
<td></td>
</tr>
<tr>
<td>[21-30]</td>
<td></td>
<td>87</td>
<td>16(18.4%)</td>
<td>8(9.2%)</td>
<td>63(72.4%)</td>
<td></td>
</tr>
<tr>
<td>[31-40]</td>
<td></td>
<td>123</td>
<td>7(5.7%)</td>
<td>14(11.4%)</td>
<td>102(82.9%)</td>
<td>0.084</td>
</tr>
<tr>
<td>[41-50]</td>
<td></td>
<td>55</td>
<td>7(12.7%)</td>
<td>2(3.6%)</td>
<td>46(83.6%)</td>
<td></td>
</tr>
<tr>
<td>&gt;50</td>
<td></td>
<td>34</td>
<td>2(5.9%)</td>
<td>2(5.9%)</td>
<td>30(88.2%)</td>
<td></td>
</tr>
<tr>
<td>HIV status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td>153</td>
<td>21(13.7%)</td>
<td>8(5.2%)</td>
<td>124(81%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td>153</td>
<td>11(7.2%)</td>
<td>19(12.4%)</td>
<td>123(80.4%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>306</td>
<td>32(10.5%)</td>
<td>27(8.8%)</td>
<td>247(80.7%)</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 2. Distribution of Candida albicans among study population associated with pregnancy and antibiotics usage status**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Participants n</th>
<th>C. albicans (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnant</td>
<td>Yes</td>
<td>5</td>
<td>3 (60%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>148</td>
<td>18 (12.2%)</td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibiotics Usage</td>
<td>Yes</td>
<td>19</td>
<td>7 (36.8%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>134</td>
<td>14 (10.4%)</td>
</tr>
</tbody>
</table>
DISCUSSION

*Candida albicans* infection remains a major public health concern particularly among immunocompromised individuals in developing countries with limited resources including Rwanda. Understanding its prevalence and associated risk factors can be a major contribution to the effective control and preventive measures. The present study revealed 10.5% as the prevalence rate of *Candida albicans* in the entire study population and was the most isolated species considered with other *Candida* species. This is in accordance with the works reported elsewhere in a different study which stated that *C. albicans* is the most frequent colonizer and responsible for almost clinical manifestation of candidiasis.20,21.

The prevalence of 10.5% in this study is low compared to 22.3% and 32.4% that reported among women in Kigali University Teaching Hospital, Rwanda and Lagos Teaching Hospital, Nigeria.20,22. This reduction of prevalence could be attributed to the fact of availability of antiretrovirals to the patients, strict adherence to their antiretroviral regimen and sensitive sampling method (vaginal swabs) used.

Considering the prevalence among the study populations; *C. albicans* in HIV positive women was high 13.7% compared to 12.5% and 9.7% of the seropositive population in Nigeria.23,24. This could be due to the fact that they included both HIV positive male and female who were on antiretroviral therapy with the exclusion of the associated risk factors. Contrary, it was low compared to 22.3% and 50% reported among seropositive patients in Kigali and India respectively.13,22.

As observed in the present study, the prevalence of *Candida albicans* among the study population in relation to pregnant status was similar to 34.3% reported among pregnant women in a federal medical centre, Nigeria.20. This was supported by the facts that, pregnancy could be a risk factor which increases the possibility of getting candida infections due to the increases of the sensitivity of vagina to infection and increase of reproductive hormones that elevate the amount of glycogen in the vagina which can suppress the normal vaginal environment.20,25. The 21-30 age groups were shown to be more susceptible to candida infection compared to other groups (Table 1). This work agreed with that reported in the previous studies which showed that 20-29 age groups had a higher prevalence of candida colonizers with predominant aetiology of *C. albicans* to cause vaginal candidiasis.26. This could be attributed to the fact of the active age group with the highest risk of pregnancies, indulgence in family planning pills and immunosuppression due to HIV/AIDS. Contrary, the population aged 46 years or above have reached menopause and have shown to develop resistance to candida infection due to the fact that, they are less or not sexually active, not using contraceptive drugs to prevent pregnancy and they have increased vaginal immunity as they have reduced the level of estrogen and corticoid.21.

With the regards to the antibiotics usage facts, the prevalence of *C. albicans* among antibiotics users living with HIV was 36.8% and 10.4% for antibiotics non-users while the prevalence of *C. albicans* among antibiotics users and non-users who were HIV negative was 25% and 4.6% respectively. These results disagreed with that of reported among women in primary health care centres of Jos, Nigeria that, the broad-spectrum antibiotics users accounted for 16% and 33.6% respectively as the risk for vaginal candidiasis.20,26. The fact of using antibiotics can be a predisposing factor that leads to candida colonization but the absence of this factor does not necessarily defend against Candida colonization.25.

CONCLUSION

Low prevalence of *Candida albicans* infection was significantly observed compared to that conducted in Rwanda in 1995. Pregnancy and antibiotics usage was revealed to significantly contribute to candida infection. Thus, regular and early medical checkup and a regular public education on the importance of personal hygiene and proper use of antibiotic should reduce disease burden. Further research should be conducted to explore more about *C. albicans* infection and burden of the fungal etiological agent in women by using different sampling method and improved diagnostic test.

Limitation

This study was limited by small sample size and less sensitive samples used which might have interfered with the accuracy of the results.
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