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Prevalence and Factors Associated with Intestinal Candidiasis among HIV Infected Clients Attending Anti-retroviral Therapy Clinic at Kisoro District Hospital, Western Uganda

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SB, MNM, JM, DAH, CA, BM and IMT designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BM, CA and IMT managed the analyses of the study. Authors MNM, SB, JM and DAH managed the literature searches. Author IMT critically reviewed the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To determine the prevalence and associated factors of intestinal candidiasis among people living with human immune deficiency virus (PLWHIV) attending Kisoro district Hospital in Western Uganda.
Study Design: This was a cross-sectional study.
Place and Duration of Study: This was conducted in the anti-retroviral therapy (ART) clinic at Kisoro District Hospital (KDH) from May 2016 to June, 2017.
Methodology: The study analyzed fresh stool and ethylene di-amine tetra acetic acid venous blood specimens from 148 HIV seropositive adult participants. Stool samples were microscopically

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examined as wet preparation and cultured on Sabouraud dextrose agar for isolation and identification of fungal pathogens. Blood was used to assay their CD_4+ cell counts. Data were analyzed, and presented as proportions.

Results: The prevalence of intestinal candidiasis was 62.84%. It was highest among participants with CD_4+ cells below 250/µL, and more among male participants 75% (36/48).

Keywords: Anti-retroviral therapy; CD_4+ cells; intestinal candidiasis; HIV; Uganda.

1. INTRODUCTION

Globally, the burden of candida colonization as a gastrointestinal tract (GIT) opportunistic fungal infection among people living with HIV (PLWHIV) is recognized [1,2]. The common *Candida* species affecting HIV patients include C. *albicans*, C. *glabrata*, C. *krusei*, C. *tropicalis*, C. *parapsilosi*s, *C. dubliniensis* and *C. guilliermond*. The *C. albicans* is common as it asymptomatically colonizes 25-50% of the affected individuals as normal flora [3]. At the same time, it is highly virulent, and it is associated with high risk of mortality among immune suppressed patients [4,5]. This is in part attributed to the reduced immune system [4,6], thereby allowing gastrointestinal colonization by opportunistic fungi. Varied pathogenesis has been described, such as genes expression, aggression and adhesion factors [6-8]. This, coupled with the ubiquitous nature of fungi augments the risk of opportunistic infections [7]. To avert this, people living with human immune deficiency virus (HIV) (PLWHIV) ought to be initiated on antiretroviral therapy (ART) to uphold their CD_4+ cell counts [7,8]. Also, whereas World Health Organization (WHO) [9] recommended prophylactic cotrimoxazole, its widespread use among PLWHIV clears bacteria normal flora and lets gastrointestinal opportunistic fungi to manifest [9-11]. This, in turn, leads to a gastric disturbance that is usually mistaken for bacterial infections [4]. In Uganda, there is a paucity of data on intestinal candidiasis; this study reports on the prevalence and associated factors of intestinal candidiasis among HIV clients attending ART clinic at Kisoro district Hospital, western Uganda.

2. MATERIALS AND METHODS

2.1 Study Site, Study Participants and Enrolment

The study was carried out at Kisoro district Hospital, a government hospital located in Kisoro district Council in western Uganda. The antiretroviral therapy (ART) clinic has 3,012 clients from within the district, neighbouring districts, parts of Democratic Republic of Congo (DRC) and Rwanda. It attends to approximately 250 clients daily. The study enrolled 148 HIV seropositive adult participants who presented with a history of diarrhoea. Study participants were purposefully enrolled, and sampled for laboratory analyses.

2.2 Sample

The study analyzed fresh stool and ethylene-di-amine tetra acetic acid (EDTA) blood samples from consented participants.

2.3 Laboratory Sample Analyses

Laboratory analyses done were a microscopic examination of stool using normal saline to identify the yeast cells. Also, Gram staining to identify the Gram-positive yeasts was done, and stool culture on Sabouraud dextrose agar (SDA) with chloramphenicol. The inoculated SDA plates were incubated for 48 hours at 37^{O}C. Further, about 2 grams of a stool sample from the respective participant was tested using a simple wet mount. Cluster of differentiation-4 (CD_4+) cell counts were determined using BD Facs Count (*Becton, Dickson and company 2008, United States of America*) following standard operating procedure (SOP) at Kisoro district Hospital.

2.3.1 Method for wet saline preparation

Placed a drop of 0.85% physiologic saline in the centre of a slide. Using an applicator stick, the laboratory personnel picked up a small portion (peanut size) of the stool specimen and mixed it with a drop of saline. Placed a cover slip on the preparation and examined the preparation microscopically with x10 objective and identification was done using x40 objective.

2.3.2 Gram staining technique

Using a loop, an appropriate portion of a sample was picked and spread evenly over an area of 2x1cm on a clean slide and left to air dry. The smear was heat fixed by slowly passing the slide

smear uppermost, three times through a flame and allowed to cool before staining. The smear was then flooded with Gentian violet stain for 60 seconds, washed off the stain with clean water, and tipped off excess water. It was then flooded with Gram's iodine for 60 seconds, washed the stain with clean water and tipped off excess water. Decolorisation was done using 50% Acetone alcohol over a few seconds to avoid over decolourisation and washed immediately with water. The smear was flooded with dilute Carbol fuchsin (1 in 20) stain for 3 minutes then washed off the stain with clean water, wiped the back of the slide clean and air dried. The smear was microscopically examined under the X100 objective for fungi shape, size and fungal arrangement.

2.3.3 Culture on sabouraud dextrose agar (SDA) with chloramphenicol

This was done by streaking the stool sample onto the surface of a Sabouraud Dextrose agar plate containing Chloramphenicol. The plate was then incubated at 37°C for overnight, and examined the plates for growth the next day for identification features like colour, size and texture.

2.3.4 Determination of CD4+ cell counts

Each reagent tube was labelled according to the patients' identification number and vortexed upside down for 6 seconds and upwards for 6 seconds to re-suspend the beads. The reagent was then opened with the coring station by sliding the reagent tubes upright into the coring station. Transferred the tubes from the coring station to the workstation, keeping the tubes upright. Inverted the EDTA vacutainer tubes five times to mix the blood uniformly, then pipetted out 50 µl of the blood into the reagent tube using reverse pipetting. Added 50 µl of fixative solution into the tube and recapped the reagent tube then vortexed upright for 6 seconds. The BD FACS Count machine (*BD 2008, USA*) then started, and the sample analyzed.

2.4 Quality Control

Sample analysis was done in strict adherence to the standard operating procedure (SOP). We tested for sterility of the culture media by incubating culture plate at 37°C for overnight, and then did fertility testing using standard strains of *Candida albicans* (ATCC 60193) as a positive control, *Staphylococcus aureus* (ATCC-25923) and *Escherichia coli* (ATCC-25922) as a negative control. Daily controls for the BD FACS count machine were done before running patient samples.

2.5 Data Analysis

Data were analyzed using STATA, and presented as a pie chart, whisk plot and table. The interpretation was done using the chi-square test statistic, and a *P*-values < 0.05was considered significant.

3. RESULTS

Of the 148 study participants, 67.57% (N=100) were females. The mean and median age of the study participants was 39 (SD±12.25) and 38 (IQR: 31-48) years, respectively. Their mean CD_4+ cell count was 538.03cells/µL (SD±308.6) with a median of 518 (IQR=334-689), as shown in Fig. 1.

The proportion of clients infected with intestinal candidiasis was 93/148 (62.84%, 95% CI=54.52 – 70.63). Assessment of the socio-demographic and immunologic factors associated with intestinal candidiasis revealed that the rate of infection was more among males (75%) (X^2= 4.50 *p. Value*=0.03). There was no difference among age categories (X^2=7.59 *p. Value*=0.06) and tribes (X^2=4.08 *p. Value*=0.54) as indicated in Table 1.

Association of intestinal candidiasis and CD_4+ cells indicated that the rate of infection was higher among clients with lower CD_4+ cell count below 250/µL (X^2=20.52 p. Value < 0.001), as given in Table 2, Fig. 2.

4. DISCUSSION

The prevalence of intestinal candidiasis was 62.84%. This is comparable to 64.1% reported in Malaysia [12], 65.6% in Nigeria [13] and 55.7% in India [14]. On the other hand, prevalence from this study was higher than the 46% reported from India [14] and 38.7% from Spain [15]. The higher incidence of candidiasis in our set up is ascribed to the clinical care management of PLWHIV whereby anti-fungal drugs are not prescribed [7].

Candidiasis was more among participants with CD_4+ cell counts less than 250cells/µL. This indicates that there is a correlation between CD_4+ cell counts and candida colonization. This relationship has been reported in other studies

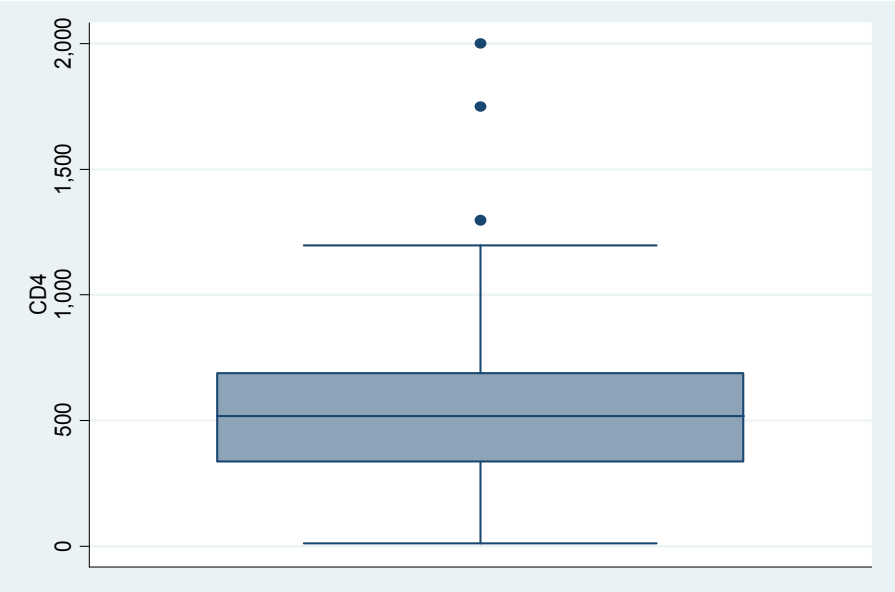

Fig. 1. CD4 distribution among the study participants

Table 1. Socio-demographic characteristics of PLWHIV at Kisoro District Hospital

Variable	Culture results		Total	X^2	*P value*
	Positive (%)	Negative (%)			
Age groups					
Children	2(40)	3(60)	5	7.59	0.06
Youth	38(73.08)	14(26.92)	52		
Adults	52(60.47)	34(39.53)	86		
Elderly	1(20)	4(80)	5		
Sex					
Male	36(75)	12(25)	48	4.50	0.03
Female	57(57)	43(43)	100		
Ethnicity					
Congolese	4 (50)	4	8	4.08	0.54
Mufumbira	58	38	96		
Muganda	1	0	1		
Mukiga	16	6	22		
Munyankole	6	1	7		
Rwandese	8	6	14		

Table 2. Association of CD4+ cell counts with intestinal candidiasis

Variable	Culture results		Total	X^2	*P value*
	Positive (%)	Negative (%)			
CD4 levels(cells/ul)					
10-250	21(91.30)	2(8.70)	23	20.52	<0.01
251-500	37(75.51)	12(24.49)	49		
501-2000	35(46.05)	41(53.95)	76		

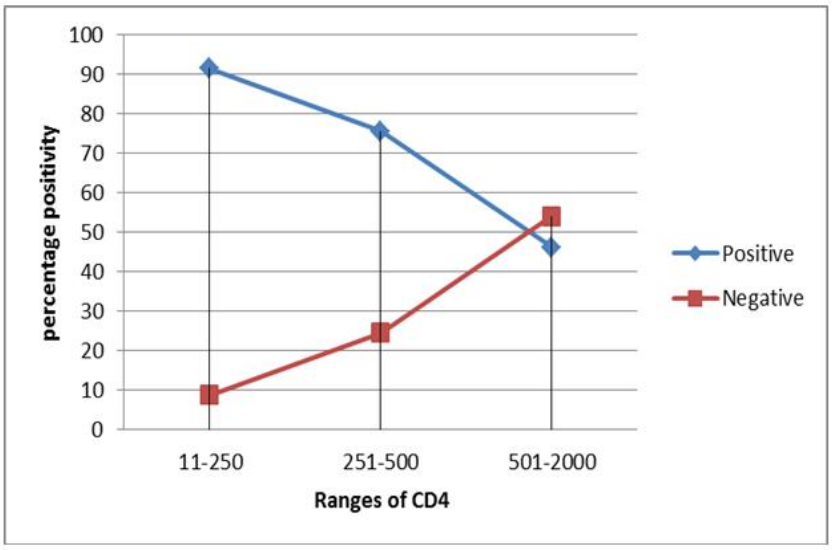

Fig. 2. Association between CD4 levels and the rate of infection with intestinal candidiasis

[15-17]. It is explained by two reasons; firstly, the fact that the body's CD_4+ cell counts below 200cells/µL risks one to opportunistic infections including intestinal candidiasis [14,16]. Secondly, all our study participants were not on anti-fungal treatment.

In this study, more males were infected with intestinal candidiasis compared to females. This is similar to findings from Brazil [7] and Nigeria [2]. This finding was also reported by Gangandhara and Ramesh [14], and it was explained by the fact that there are more HIV males practising homosexuality, but this does not explain our result since little is known about this illegal practice in our setup. Our findings are in agreement with a study done by Campo et al. [6] which indicated that there was no association between age and acquiring of intestinal candidiasis.

This study is limited by the fact that other opportunistic infections related to the gastrointestinal system were not investigated, and more sensitive diagnostic techniques like analytical profile index (API) and polymerase chain reaction (PCR) were not done.

5. CONCLUSION

The study findings revealed that intestinal candidiasis is highly prevalent (62.84%) among people living with HIV. In addition, isolation rates were higher in participants with CD_4+ cell counts below 250cells/µL and among male gender. Based on this, early diagnosis of intestinal candidiasis among PLWHIV is key to prompt management.

ETHICAL APPROVAL

Ethical approval was obtained from research and ethics committee of Clarke International University. We ensured strict confidentiality and high ethical standards. We obtained written informed consent from each participant. Results were only availed to the attending doctor for proper management. All authors at this moment declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed by the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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