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by Dwi Murtiastutik

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Correlation between CD4 T lymphocyte and *Candida* Species Counts In Oral Candidiasis Patients with HIV / AIDS

Dwi Murtiastutik¹, Cita Rosita Sigit Prakoswa², Indah Setyawati Tantular³, Evy Ervianti¹, Afif Nurul Hidayati⁴, Muhammad Yulianto Listiawan⁴

¹Lecture, ²Professor, Department of Dermatology and Venereology, Faculty of Medicine, Universitas Airlangga-Dr. Soetomo General Hospital, Surabaya, Indonesia, ³Professor, Department of Parasitology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, ⁴Associate Professor, Department of Dermatology and Venereology, Faculty of Medicine, Universitas Airlangga-Dr. Soetomo General Hospital, Surabaya, Indonesia

Abstract

Background: *Candida sp.* is the most common opportunistic pathogen found during the development of Human immunodeficiency virus (HIV) & Acquired immune deficiency syndrome (AIDS) disease. The clinical severity of oral candidiasis and the prevalence profile of *Candida* species reflect immunological changes in HIV / AIDS patients. **Objective:** Evaluating the relationship between CD4 T lymphocyte cells counts and the number of *Candida* species. **Methods:** A cross-sectional analytical study was carried out at Dr. Soetomo, Surabaya, Indonesia. For identification of *Candida* species, culture was carried out on Chromagar media followed by culture with vitek 2. **Results:** There were 114 study subjects who were divided into three groups based on the number of CD4 T lymphocyte cells, with 158 isolates of *Candida* species growing in culture. The highest number of *Candida* species was *Candida albicans* with a total of 107 isolates (67.7%). *Candida non-albicans* were 51 isolates (32.3%). Statistical test results showed a significant correlation between the number of CD4 T lymphocyte cells and the number of *Candida* species ($p < 0.001$). **Conclusion:** The decrease in CD4 lymphocyte cell counts is influenced by various types of *Candida sp.* in oral candidiasis patients.

Keywords: *Candida sp.*, CD4 T lymphocytes, HIV / AIDS, oral candidiasis

Introduction

In the human oral cavity, there are various kinds of microorganisms which composition, metabolism activities and pathogenicity are influenced by external and internal factors. Among all fungi, *Candida* is a genus of yeast that is considered to be the most fungal species found in the oral cavity. *Candida* species are the main cause of mucocutaneous infections which are usually classified as oral, oesophageal and vulvovaginal candidiasis^(1,2).

Oral candidiasis (OC) is the most common mucocutaneous candidiasis found in patients with infection of Human Immunodeficiency Virus (HIV) / Acquired Immune Deficiency Syndrome (AIDS) worldwide. OC is an infection of the mucosa caused by *Candida* species. Nearly 90% of patients with HIV / AIDS have experienced OC in the course of their illness. High viral load and low CD4 T lymphocyte counts are found in OC patients^(1,2). Although oral candidiasis is caused by various genera of *Candida*, *Candida albicans* is the most common cause as stated by previous studies, but several studies found an increase in the cause of oral candidiasis due to *Candida non-albicans*^(3,4). This study aimed to analyse the relationship between CD4 cell counts and *Candida* species in oral candidiasis patients with HIV / AIDS.

Corresponding Author:

Dwi Murtiastutik

dwimurtiastutik@yahoo.co.id

Methods

Participants

Participants in this study were HIV / AIDS patients at Dr. Soetomo General Hospital Surabaya, Indonesia. Participant inclusion criteria included patients diagnosed with HIV / AIDS using a rapid test / HIV 3^(5,6), having a positive candidiasis diagnosis using 10-20% KOH^(7,8), and aged >18 years. Participant exclusion criteria included subjects taking anti-fungal drugs within 2 weeks before the study took place and no growth of colonies was found on culture examination. Participants in this study first filled out the consent form, in which the patient had received an explanation regarding the benefits and objectives of the study.

Design

This research was conducted at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, from May to September 2019. It was a cross-sectional study with consecutive admission sampling method. There were 114 participants, which were divided into 3 groups (38 participants in each group; Figure 1). This study was ethically approved by the ethics committee of Dr. Soetomo General Hospital (1125 / KEPK / IV / 2019). The research procedure consisted of identifying the type and amount of *Candida* fungus in the participant's oral cavity and calculating the participant's CD4 count.

Measurement of *Candida* Species

Participants were first told to not do oral hygiene before checking the type and amount of colonisation of *Candida* sp. Participants gargled using 25 ml of sterile water. The water was then put into sterile bottles that had been prepared. Water from the mouth rinse was immediately sent to Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. The water was shaken gently, and took $\pm 5 - 10$ ml and put in 15 ml sterile centrifuge tubes and rotate 2,500 revolutions per minute (rpm) for 5 minutes. Discard the supernatant and take the pellet using a sterile pipette, place it in an object glass and drop 10-20% KOH. *Candida* positive was planted on Sabouraud dextrose agar (SDA) at 37°C for 48 hours⁽⁹⁾. The SDA media used was CHROMagar *Candida* (CHROMagar *Candida*, France). Growing *Candida* specimens were identified using cornmeal agar and tween 80 incubated at 42-45°C^(10,11). *Candida*

specimens were also carbohydrate tested to identify *Candida* species⁽¹²⁾. *Candida* specimens that grew later were counted by specialists in the Department of Microbiology, Dr. Soetomo General Hospital, Surabaya, Indonesia, in a colony forming unit (CFU).

CD4 Measurement

The researchers took venous blood samples and put into K3EDTA-countaining tubes, stored at room temperature, immunophenotyping for 6 hours. CD4 count calculations used flow cytometry (BD FACSCountm System; San Jose, CA). Participants were divided into three groups: group I (participant with a CD4 level of $\leq 100 / \mu\text{L}$), group II (participant with a CD4 level of 101 - 200 / μL), and group III (participant with a CD4 level of $> 200 / \mu\text{L}$).

Lesion Area

The area of participant's candidiasis oral lesions was assessed using a score of 0 = no lesions, 1 = partial lesions, and 2 = all / all lesions. The locations assessed included the tongue, mucous membrane, and palate with a minimum score of 1 and a maximum score of 6. Calculation of the area of the lesion by adding up the three locations of the assessed lesion.

Statistical Analysis

The results of the study were presented in the form of mean \pm standard deviation (SD) or median (minimum - maximum) and percentage (%). Statistic analysis used IBM SPSS Statistics software version 23.0 (IBM Corp., Armonk, NY, USA). Analysis of the measurement data used the Chi Square test, Fisher exact test, the Spearman correlation test, or the Anova test with a significance level of $p < 0.05$.

Results

The study showed that 114 oral candidiasis patients with HIV / AIDS met the inclusion criteria as research subjects. The age characteristics of the study subjects had a standard deviation of 36.41 ± 9.825 years, with an age range of 18 years to 59 years (Table 1). Most research subjects worked as private employees (58.8%). As many as 48.2% of research subjects had a high school equivalent education level (Table 2).

The clinical condition of oral candidiasis in this study showed the main complaint in form of white patches in the oral cavity that was found in 100 subjects (87.7%). Most locations were found on the tongue in 54 people (47.4%). Most participants were not accompanied by swallowing pain (64 participants; 56.1%). The most common clinical condition of OC was pseudomembranous type found in 103 patients (90.4%), followed by acute cheilitis (10 participants; 8.8%) and acute atrophic (1 participant; 0.9%) (Table 2).

The identification of species from 114 patients found 158 isolates of *Candida* sp., consisting of *Candida albicans* (107 isolates; 67.7%) and 51 non-*albicans* isolates (32.3%). The highest number of *Candida non-albicans* species were *Candida krusei* (26; 16.5%),

Candida glabrata (13; 0.8%) and *Candida tropicalis* (7; 0.4%). This study also found rare *Candida* isolates, namely *Candida parapsilosis*, *Candida dubliniensis*, and *Candida lypholitica* (Figure 2). Spearman test obtained a significant relationship between the number of CD4 T lymphocyte cells with the number of species ($p < 0.001$), and a significant relationship between the number of species with lesion area ($p = 0.004$). However, the relationship was not significant between the number of species with duration of sickness ($p = 0.081$). Fisher Exact test showed no significant relationship between the number of CD4 T lymphocyte cells with clinical condition ($p = 0.016$), and a non-significant relationship between the number of species and clinical condition ($p = 0.284$; Table 3).

Table 1. Demographic data of research subjects

Characteristics	n (%)
Sex	
Male	83 (72.8)
Female	31 (21.2)
Occupation	
Unemployed	41 (36.0)
Public employee	6 (5.3)
Private employee	67 (58.8)
Education	
Not completed elementary school	1 (0.9)
Elementary school	20 (17.5)
Junior high school	21 (18.4)
Senior high school	55 (48.2)
University	17 (14.9)

Table 2. Clinical description of research subjects

Characteristics	n (%)
General complaint	
Red patches on the oral cavity, patches and sores on the corners of the lips	7 (6.1)
White and red patches on the oral cavity	4 (3.5)
White patches on the oral cavity	100 (87.7)
White patches on the oral cavity, patches and sores on the corners of the lips	3 (2.6)
Pain	
Yes	50 (43.9)
No	64 (56.1)
Location	
Mix	6 (5.3)
Tongue	54 (47.4)
Tongue, mix	2 (1.8)
Tongue, mucosa, mix	2 (1.8)
Tongue / mucosa	38 (33.3)
Tongue / mucosa / lips	11 (9.6)
Mucosa	1 (0.9)
Clinical condition	
Acute pseudomembrane	103 (90.4)
Acute atrophic	1 (0.9)
Cheilitis	10 (8.8)
Chronic hyperplastic	0 (0.0)

Table 3. Correlation between CD4 T lymphocyte counts and Candida species in oral candidiasis patients with HIV / AIDS

Variables	Number of Species			p
	I (%)	II (%)	III (%)	
CD 4 counts				
≤100	23 (60.5)	10 (26.3)	5 (13.2)	0.000**
101-200	23 (60.5)	15 (39.5)	0 (0.0)	
>200	37 (97.4)	1 (2.6)	0 (0.0)	
Lesion Area				
1-2	48 (57.8)	10 (38.5)	0 (0.0)	0.004*
3-4	29 (34.9)	15 (57.7)	1 (20.0)	
5-6	6 (7.2)	1 (3.8)	4 (80.0)	
Duration of sickness				
1-7 days	33 (39.8)	11 (42.3)	1 (20.0)	0.016*
8-30 days	30 (36.1)	12 (46.2)	3 (60.0)	
>30 days	20 (24.1)	3 (11.5)	1 (20.0)	
Clinical Condition				
Acute pseudomembrane	77 (92.8)	22 (84.6)	4 (80.0)	0.284
Acute atrophic	1 (1.2)	0 (0.0)	0 (0.0)	
Cheilitis	5 (6.0)	4 (15.4)	1 (20.0)	

Abbreviation: *significant p < 0.05; **significant p < 0.001

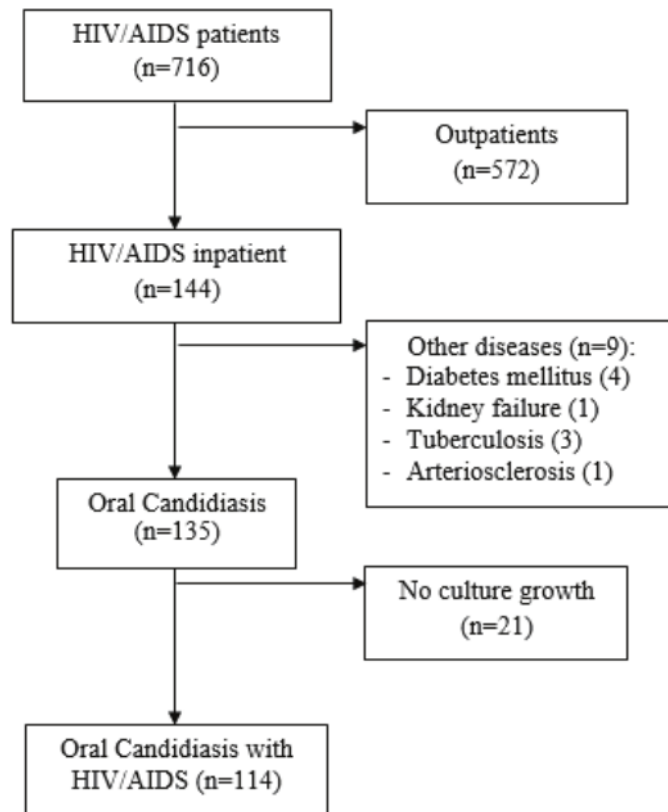


Figure 1. Flowchart Diagram of Participant

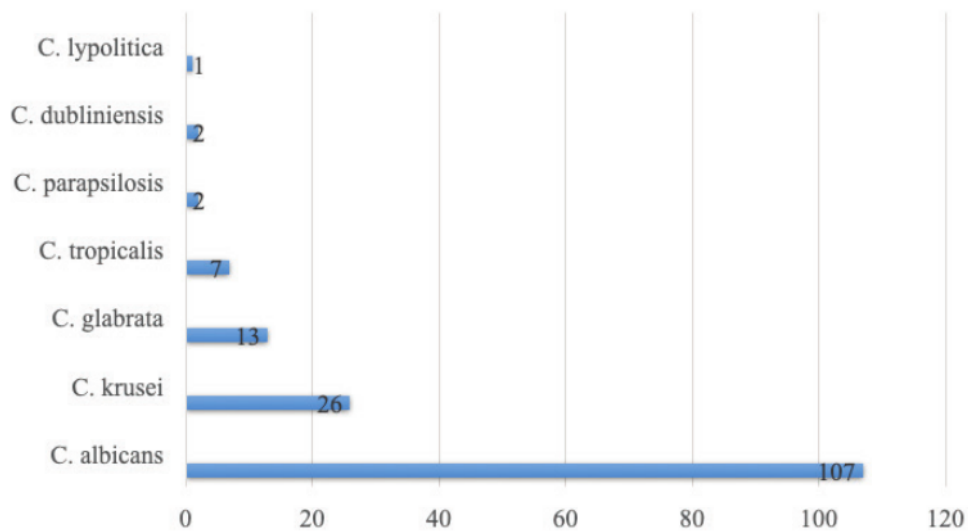


Figure 2. Prevalence of candida in Indonesian patient living with HIV / AIDS

Discussions

Based on gender, the distribution of subject was dominated by male patients (83; 72.8%) compared to female (31; 27.2%). This finding is consistent with data from the Indonesian Report of HIV Development Situation published by the Ministry of Health of the Republic of Indonesia in 2017, in which the pattern of HIV patients was more prevalent in male than female group⁽⁵⁾. In general, there is no difference in terms of disease occurrence based on gender because unlike vulvovaginal candidiasis, oral candidiasis is not influenced by hormonal factors. Characteristic of subject's age in this study had a standard deviation of 36.41 ± 9.82 years, with an age range of 17 years to 59 years. This age range is considered as productive age and sexually active, so that many may perform unprotected sexual behavior that is at risk of HIV transmission⁽⁴⁾. In terms of distribution of educational levels, there were still research subjects who did not complete elementary school (1; 0.9%), and 20 subjects (17.5%) had elementary school education. In this study, there were still HIV / AIDS patients with low educational background, so that they had lack of oral health knowledge and a very poor ability to maintain oral hygiene. Poor oral cleaning is one of the factors that could increase the incidence of oral diseases, including oral candidiasis^(5, 13). The distribution of occupational backgrounds showed that 41 (36%) patients did not work. Patients with HIV / AIDS in the course of their illness will experience opportunistic infectious diseases that will affect the quality of life, one of which is occupation^(2, 4, 13).

The description of the main complaints from OC patients with HIV / AIDS in this study had more than one type. The most common complaint was white patches found in the oral cavity (100 subjects; 87.7%), followed by white and red patches in the oral cavity (4 subjects; 3.5%). Complaints of red patches, patches or sores on the corners of the lips were found in 7 subjects (6.1%), while white patches on the oral cavity, patches and sores on the corners of the lips were found in 3 subjects (2.6%). Complaints of white patches in the oral cavity were found in the pseudomembranous OC (38.3%) and plaque hyperplasia (9.6%). The subjects were mostly not accompanied by complaints of swallowing pain (64; 56.1%). Patients with complaints of swallowing are clinical signs of lesions attacking the pharynx and

esophagus^(14, 15).

Infection of *Candida* sp. is always associated with OC in HIV / AIDS. *Candida* sp. is a commensal microorganism that develops into pathogens due to a decrease in the secretion of immunoglobulin A (sIgA) and a decrease in the number of T lymphocyte cells⁽⁸⁾. The pathogenesis of OC in HIV is closely related to proteins produced at the beginning of HIV virus replication cycle. Trans-Activating Transduction or Transcriptional Activator (TAT) is one of the proteins that regulates HIV virus replication. This protein can resemble the workings of extracellular matrix proteins (Integrin-like) in the regulation of cell life activities through activation of signal transduction pathways. The cell wall of *C. albicans* is thought to have receptors that resemble extracellular matrix (Integrin-like) which can bind to TAT. Interaction between HIV TAT protein with *C. albicans* cell wall allows the occurrence of specific gene transduction in candidiasis in HIV / AIDS with CD4 T cell lymphocyte counts <200 cells / mm^(16, 17).

In this study, identification of *Candida* species conducted on 114 subjects found 158 isolates of *Candida* species with different species variants. *Candida albicans* was found in 107 isolates (67.7%), whereas *Candida non-albicans* was found in 51 isolates (32.3%). The highest number of *Candida non-albicans* species were *Candida krusei* (26; 16.5%), *Candida glabrata* (13; 0.8%) and *Candida tropicalis* (7; 0.4%). This study also found rare *Candida* isolates, namely *Candida parapsilosis*, *Candida dubliniensis*, and *Candida lusitana*. This proves that in OC with HIV / AIDS, there has been a change in the spectrum of the causative species⁽¹²⁾. In addition, the high detection rate of *Candida non-albicans* in this study is due previously incorrect identification of species due to the similarity of the phenotype to *C. albicans* and is now increasingly recognized. The proportion of *Candida* infections caused by *C. albicans* in HIV / AIDS patients has shifted to *Candida non-albicans* species, corresponding to previous studies comparing *Candida* species causing OC in HIV-seropositive patients and HIV-seronegative patients. The study showed that colonies of *Candida albicans* species in HIV-seropositive was less than in HIV-seronegative. In HIV-seropositive patients, *Candida* species are also rarely found in immunocompetent patients, namely *Candida dubliniensis*, *Candida glabrata*, *Candida*

tropicalis ⁽¹⁸⁾. A meta-analysis and study conducted in Sub-Saharan Africa in 2005-2015 showed a prevalence of *Candida non-albicans* species as much as 33.5% in general, with dominant species namely *Candida glabrata*, *Candida krusei*, and *Candida tropicalis* ⁽¹⁹⁾. The results of this study also showed a mixed infection between *Candida albicans* and *Candida non-albicans*. Out of 114 subjects in this study, there were 31 subjects (27.2%) with more than one *Candida* species isolates, and 83 subjects (72.8%) with one type of *Candida* species isolate. A meta-analysis study conducted in Sub-Saharan Africa for 10 years also showed the incidence of mixed infections of *Candida albicans* and *Candida non-albicans* by 85.2% ^(19, 20).

³ Oral candidiasis in HIV / AIDS patients is often of longer duration, repeated, and has more severe clinical symptoms. In HIV / AIDS patients, viral replication has the potential to trigger OC, which directly increases the progressive rate of HIV / AIDS infection. The Long Repeat Terminal (TLR) at the end of the provirus has two sites that function as transcription factors that bind the host, NFkB and SP1. Infected T cells are activated and release cytokines such as Interleukine-2 (IL-2) and Tumor Necrosis Factor (TNF). IL-2 and TNF will induce HIV provirus to end the latent period and start replicating. T cells will facilitate the replication of the HIV / AIDS virus, so that CD4 T lymphocyte cell levels will continue to decrease ^(21, 22). This is supported by research on a strong correlation between the occurrence of OC in the number of CD4 T lymphocytes <200 cells / mm³ on arising clinical manifestations, including the number of species and lesion area ^(23, 24). Consistent results illustrated in ⁰ this study were a significant correlation between the number of CD4 T lymphocyte cells with the number of species and lesion area ⁽⁶⁻⁸⁾.

Conclusions

Participants with low levels of CD4 T lymphocytes allow the number of *Candida* species growing in the oral cavity to be more diverse with lesions found in the oral cavity. The reverse condition is also possible if the CD4 T lymphocyte levels are high then the possibility of the number of *Candida sp.* growing in the oral cavity and lesion area are getting smaller.

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⁵ **Conflict of Interest:** The authors declare that they have no conflict of interest.

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³⁴ **Ethical Approval:** This study was ethically approved by the ethics committee of Dr. Soetomo General Academic Hospital (1125 / KEPK / IV / 2019).

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