

The Significance of CD4 to the Number of Grown Candida Colonies in Oral Candidiasis Patients with HIV / AIDS

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Abstract

Introduction: Oral candidiasis is a common symptom in HIV/AIDS patients and in some cases have low CD4 levels. **Objectives:** Analyzing the correlation of CD4 counts with Candida colonization in oral candidiasis patients with HIV/AIDS. **Methods:** The research procedure consisted of identifying the type and number of Candida species grown in the participant's oral cavity, and calculating the CD4 count. The statistical tests used Chi Square, Fisher exact, Spearman correlation test, or Anova test (95% CI; $p < 0.05$). **Results:** Candida colonization found in group I was 1 – 10 (71.05%), group II was 11-100 (60.53%), and group III was >100 (50.00%; $p < 0.001$). The patient's lesion area in group I was 3-4 (47.37%), group II was 3-4 (65.79%), and group III was 1-2 (94.74%; $p < 0.001$). Most patients had acute pseudomembranous type, consisting of 81.58% (group I), 92.10% (group II), and 97.37% (group III; $p < 0.001$). **Conclusion:** This study found a significant correlation between CD4 counts and Candida colonization in oral candidiasis patients with HIV/AIDS. The lower the CD4 counts, the higher the number of Candida colonization in the oral cavity.

Keywords: CD4 count, HIV/AIDS, candida species, oral candidiasis

Introduction

Human Immunodeficiency Virus (HIV) and Acquired Immunodeficiency Syndrome (AIDS) infections are still major problems throughout the world and most of the clinical manifestations are oral candidiasis. Candida can spread widely, directly from the oral cavity throughout the body depending on the target organ experiencing immune deficiency. In general, HIV/AIDS patients likely have immune deficiency, and cause individual vulnerability to be detected. CD4 counts in peripheral blood are important when evaluating immune status.^{1,2}

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Common oral candidiasis is estimatedly found in 4.2% of HIV patients and 11.6% of AIDS patients. Some literature stated that CD4 counts <200 per mm^3 are found in HIV/AIDS patients.³ The pathogenesis of HIV infection is largely due to a decrease in the number of T-cells (certain types of lymphocytes) that contain CD4 cell surface receptors. The immune status of HIV/AIDS patients can be assessed by measuring the absolute number (per mm^3) or percentage of CD4 cells. This is considered a way to assess the severity of immunodeficiency associated with HIV/AIDS. The decreasing number of T cells (CD4) progressively marks the prognosis of HIV/AIDS decreases and increases the chance of other infections. HIV will infect and eliminate Th17 cells, resulting in a reduction in IL-17 secretion which then results in a reduction in b-defensin induction, a downregulation of CXC chemokine expression, a decrease in the production of antifungal cytokines, and the result is a shape transition. Candida albicans forms a hyphae which is pathogenic. This causes Candida, which was initially commensal to turn pathogenic and

causes a fungal infection in the oral cavity mucosa, so it is called oral candidiasis.^{4,5}

Dr. Soetomo General Hospital, Surabaya, Indonesia, is the highest referral hospital in East Java, Indonesia. The number of HIV/AIDS patients has increased every year. In 2017, 1891 patients were reported for treatment, 1997 patients in 2018, and 1671 patients in September 2019. The number of oral candidiasis patients with HIV/AIDS in 2017 was 30%, 32% in 2018, and 33% by September 2019. Based on the description above, researchers were interested in conducting research aimed at analyzing the correlation of CD4 counts with Candida colonization in oral candidiasis patients with HIV/AIDS.

Methods and Material

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^{6,7}, having a positive candidiasis diagnosis using 10- 20% KOH^{8,9}, and aged > 18 years. Participant exclusion criteria included subjects taking antifungal drugs within 2 weeks before the study and no colonies 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

The study was conducted at Dr. Soetomo General Hospital, Surabaya, Indonesia from May 2019 to September 2019. This research was a cross-sectional study with consecutive admission sampling method. The number of participants was 114 participants, which were divided into 3 groups (each group = 38; Figure 1). We have conducted an ethics test at the ethics committee Dr. Soetomo General Hospital (1125 / KEPK / IV / 2019). The research procedure consisted of identifying the type and number of candida fungi in the participant's oral cavity and calculating the participant's CD4 count. The patient will take anamnesis, physical examination, laboratory examination, namely KOH and culture with material from the patient's mouth rinse water, and blood

as much as 3 cc for CD4 examination.

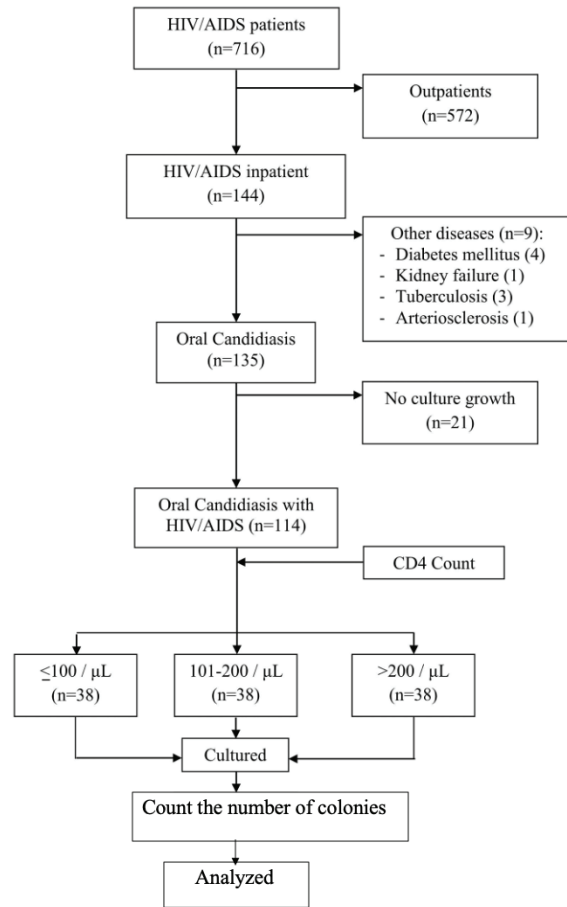


Figure 1. Flowchart Diagram of Participant Candida Species Measurement

Before checking the type and amount of Candida colonization, patients were told not to do oral hygiene. Participants gargled using 25 ml of sterile water, then the water was put into a sterile bottle. Water from the mouth rinse was immediately sent to Dr. Soetomo General Hospital, Surabaya, Indonesia. The water was shakened slowly, then taken ± 5 - 10 ml and put in 15 ml sterile centrifuge tubes and rotated 2,500 revolutions per minute (rpm) for 5 minutes. Eliminate the supernatant and took the pellet using a sterile pipette, placed it in a glass object and added 10-20% KOH. Candida positive was planted on Sabouraud dextrose agar (SDA) at 37°C for 48 hours.¹⁰ The SDA media used was CHROMagar Candida (CHROMagar Candida, France).Candida specimens were also carbohydrate tested to identify Candida species.¹¹ 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

Patient's venous blood samples were taken, then put into K3EDTA-containing tubes, stored at room temperature, and followed by immunophenotyping for 6 hours. CD4 count calculations used flow cytometry (BD FACSCounttm 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 (%). Static analysis used IBM SPSS Statistic software version 23.0 (IBM Corp., Armonk, NY, USA). Analysis of measurement data used

Chi Square test, Fisher exact test, Spearman correlation test, or Anova test with 95% CI and significance level of $p < 0.05$.

Results

Characteristics of Participant

There were 114 patients who were divided into 3 groups based on CD4 count, with each group consisting of 38 participants. The average age of participants in group I was 43.15 ± 3.67 years, group II was 40.02 ± 10.23 years, and group III was 51.02 ± 3.28 years ($p = 0.812$). They were divided into several age groups, where most participants were in the age range of 36-45 years (37 participants; 32.46%), followed by the age group of 46 - 55 years (28 participants; 24.56%), and the age group of 26 - 35 years (26 participants; 21.05%). Most participants attended senior high school (55 participants; 48.24%), consisting of 42.10% in group I, 52.63% in group II, and 50.00% in group III ($p = 0.015$). Most participants were Madurese (60 participants; 52.63%) and followed by Javanese (41 participants; 35.96%). Most of the participants in group I were Madurese (63.16%) and most in groups II and III are Madurese too (47.37%; $p = 0.002$). Most of the participants were private employees (67 participants; 58.77%), consisting of 52.63% in group I, 76.31% group II, and 47.37% in group III ($p = 0.025$; table 1).

Table 1. Demographic Characteristics of Participant

Variable	Group			p
	I (n=38)	II (n=38)	III (n=38)	
Age (mean \pm SD)	43.15 \pm 3.67	40.02 \pm 10.23	51.02 \pm 3.28	0.812
Education (%)				0.015*
Not attending school	0 (0.00)	0 (0.00)	1 (2.63)	
Elementary school	12 (31.58)	4 (10.52)	4 (10.52)	
Junior high school	7 (18.42)	9 (23.68)	5 (13.16)	
Senior high school	16 (42.10)	20 (52.63)	19 (50.00)	
Undergraduate/Diploma	3 (7.89)	5 (13.16)	9 (23.68)	

Cont... Table 1. Demographic Characteristics of Participant

Ethnic (%)				
Java	6 (15.79)	17 (44.74)	18 (47.37)	0.002*
Madura	24 (63.16)	18 (47.37)	18 (47.37)	
Other	8 (21.05)	3 (7.89)	2 (5.26)	
Profession				
Nothing	17 (44.74)	8 (21.05)	16 (42.10)	0.025*
Government employees	1 (2.63)	1 (2.63)	4 (10.53)	
General employees	20 (52.63)	29 (76.31)	18 (47.37)	

SD=standard deviation; *significant <0.05

Patient's Clinical Description

Most participants complained about the appearance of white patches on the oral cavity (100 participants; 87.72%), followed by participants who complained of red patches and sores on the oral cavity and/or the corners of the lips (7 participants; 6.14%), participants with complained of white and red patches on the cavity mouth (4 participants; 3.51%), and patients with white patches and sores in the oral cavity and/or the corners of the lips (3 participants; 2.63%). Most participants had no pain complaint (64 patients; 56.14%), while

the rest (50 patients) complained of pain with an average pain scale of 4.13 ± 0.62 . The pain included swallowing pain or accompanied by a burning sensation in the throat. Most patients had lesion location on the tongue (54 participants; 47.37%), followed by lesions on the tongue and mucous membrane (38 participants; 33.33%), and on the tongue, mucous membrane, and lips (11 participants; 9.65%), palate (6 participants; 5.26%), tongue and palate (2 participants; 1.75%), tongue, mucous membrane, and palate (2 participants; 1.75%), and mucous membrane (1 participant; 0.88%). The patient's physical condition can



Figure 2. Clinical Conditions of Oral Candidiasis 1 Patients while Under Treatment (A, B, C, D)