

Prevalence of Candida Species in Oral Candidiasis and Correlation with CD4+ Count in HIV/AIDS Patients at Surabaya, Indonesia

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Abstract

Candida is most common opportunistic infection during Human Immunodeficiency Virus (HIV) infection. The clinical severity of candidiasis and Candida species prevalence may be a reflection of decreased CD4+ counts in HIV seropositive patients.

To investigate the changing pattern of Candida species prevalence and its correlation with CD4+ count in HIV/AIDS patients at UPIPI RSUD Dr. Soetomo 2014.

The study was analytical observational research with cross-sectional and total sampling method. The samples consisted of 88 HIV/AIDS seropositive were being followed up at the service treated in UPIPI RSUD Dr. Soetomo Surabaya from July-August 2014. HIV/AIDS Patients were recruited for Candida microbial screening and examined by Oral Medicine Specialist (n=5). Clinical specimens including oral swabs were collected to determine the clinical, immunological, and microbiological Candida species prevalence in HIV/AIDS seropositive cases. CD4+ count obtained from patient's medical record.

There were 68 cases of Oral Candidiasis (OC), 50 cases (73,53%) Pseudomembranous Candidiasis(PC), 9 cases (13,24%) Erythematous Candidiasis (EC), 1 case (1,47%) Denture Stomatitis (DS), 8 cases (11,76%) Chronic Hyperplastic Candidiasis (CHC). Candida species were identified. *C. albicans* (51%) was the most common species isolated followed by *C. glabrata* (19%), *C. Dubliniensis* (11.5%), *C. Krusei* (10.5%), *C. tropicalis* (8%). OC was found to be significantly correlated with decreased CD4+ counts <200 cells/mm³ ($p < 0.05$).

PC was the highest prevalence and *C. albicans* (50%) was the most common species isolated. OC was found to be significantly correlated with decreased CD4 cell counts.

Clinical article (J Int Dent Med Res 2018; 11(1): pp. 81-85)

Keywords: Oral Candidiasis, Candida Species, Candida Albicans, HIV/AIDS, CD4+ cells counts.

Received date: 09 October 2017

Accept date: 26 October 2017

Introduction

Acquired Immunodeficiency Syndrome (AIDS) is an infectious disease caused by Human Immunodeficiency Virus (HIV).¹ Indonesia was 1st ranked country in the South East Asia (SEA) with high prevalence of

HIV/AIDS cases in 2013 around 610.000 patients.² East Java province was 2nd ranked with the highest HIV-infected with 19.249 patients and 8,976 AIDS patients in 2014.³

Owing to a weakened immune system, the HIV/AIDS infected person placed at an increased risk of a wide variety of opportunistic infections.⁴ HIV related Opportunistic Fungal Infections (OFIs) cause of morbidity and mortality and compromise the Quality of Life (QoL) of such individuals.⁵ It has been estimated that 60% to 90% of people with HIV disease will present with at least one oral manifestation. The importance of oral lesions as clinical biomarker indicator of HIV infection and predictor of progression of HIV to AIDS. Oral manifestations are among the

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earliest and most important indicator of HIV infection. Oral Candidiasis (OC) is the one of seven oral manifestation which is strongly associated with HIV Infection. OC have been identified and internationally calibrated. It has been seen that almost all HIV infected people are colonized with *Candida* and up to 90–95% develop clinical lesions as the viral disease progresses.⁶ It has also been observed that low CD4 counts and high plasma HIV RNA, viral load (VL) significantly correlate with candida carriage as well as with OC in HIV patients.⁷ Clinical appearance of OC types which is could be seen in HIV/AIDS patient are Pseudomembranous Candidiasis (PC), Erythematous Candidiasis (EC), Denture Stomatitis (DS) and Chronic Hyperplastic Candidiasis (CHC).⁸

Though *Candida albicans* is the most frequently isolated species as a colonizer and pathogen of the oral mucosa, other *Candida* species, such as *C. tropicalis*, *C. krusei*, *C. glabrata*, *C. dubliniensis*, *C. guilliermondii*, *C. parapsilosis*, *C. kefyr*, and *C. pelliculosa*, has became a significant cause of infection in patients with HIV/AIDS.⁸ The clinical importance of these non-*albicans* *Candida* species lies in the fact that they are usually less susceptible to the more commonly used azole antifungal drugs, a factor that poses significant difficulties in effective treatment. Thus the modern Mycology Laboratory has an important role to play in several aspects relating to these organisms, including detection, identification, epidemiological analysis, and therapy in an attempt to better understand these pathogens and provide an effective cure.⁹ The study of oral manifestation in some regions of the world play an important role to detect the infection, prediction of viral infection progress and progression to AIDS, to know in depth the descriptions and analyzing of the HIV/AIDS epidemic that can be the reference for the future research.¹⁰

The aim of this study was to investigate the changing pattern of *Candida* species prevalence with its correlation with CD4+ count in HIV/AIDS patients at UPIPI RSUD Dr. Soetomo Surabaya a tertiary care hospital in East Java Indonesia.

Materials and methods

This is an analytical observational research with cross-sectional and total sampling method.

All patients have been given informed consent and this study was approved by the institutional ethics committee, RSUD Dr. Soetomo Surabaya (301/Panke. KKE/VI/2014 20 June 2014).

The samples consisted of 88 HIV/AIDS seropositive who were being followed up at the service and treated in UPIPI RSUD Dr. Soetomo Surabaya from July-August 2014. Diagnosis of HIV infection has been done at Internal Medicine Department Tropic Disease Division and Clinical Pathology RSUD Dr. Soetomo Surabaya by following the standard protocol at Integrated Counselling and Testing Centre that employs pretest and posttest counselling and filled informed consent before HIV testing. Serial HIV test was performed such as rapid tests were used to detect HIV-1 and HIV-2 antibodies (CombAids (Span Diagnostics Ltd.), ELISA, and Western Blot. CD4⁺ counts obtained from patient's medical record. All the patients were recruited for *Candida* microbial screening. Clinical specimens including oral swabs were collected. Diagnosed of OC by fungal test and clinical appearance, the oral cavity of research subjects examined by dentists specialized in Oral Medicine (n=5) from Division of Dental and Oral Health, RSUD. Dr. Soetomo Surabaya and Department of Oral Medicine, Faculty of Dental Medicine, Universitas Airlangga Surabaya.

The samples were subjected to direct microscopy Potassium Hydroxide (KOH) preparation. In KOH-Calcofluor fluorescent-stain method fungal characteristics like hyphae, yeast cells, and other fungal elements will fluoresce. Fungal culture was done on Sabouraud Dextrose Agar (SDA) with and without chloramphenicol (16 µg/mL) and with cycloheximide (0.3 µg/mL) plus chloramphenicol (16 µg/mL). Specimens were streaked and incubated at 37°C and they were examined every other day for growth up to 4–6 weeks before discarding as negative. Fungal growth was identified by colony morphology. Identification and speciation of yeast isolates were done on the basis of germ tube production and also identification based on carbohydrate test.

Correlation OC and CD4+ counts was determined using Pearson's test with $p < 0.05$. Statistical analysis was done using Statistical Package for the Social Sciences (SPSS) 17.0 software for windows 8.1 by SPSS Inc, Chicago, United State.

Results

In this study, from 88 HIV/AIDS patient there were 68 cases of Oral Candidiasis (OC). Clinical appearance of OC in this study can be seen at figure 1, the most common was Pseudomembranous Candidiasis with 50 cases (73,53%) that can be seen in table 1. *C. albicans* (50%) was the most common candida species isolated in this study (table 3.).

Age (year)	CD4+ < 200 cell/mm ³	CD4+ 200-500 cell/mm ³	total
16-25	10	2	12 (17,65%)
26-35	23	3	26 (38,24%)
36-45	13	1	14 (20,59%)
46-55	9	1	10 (14,71%)
>55	6	0	6 (8,23%)
Total	61 (89.71%)	7 (10.29%)	68 (100%)

Table 1. Distribution of Age and CD4⁺ Counts in HIV/AIDS patient.

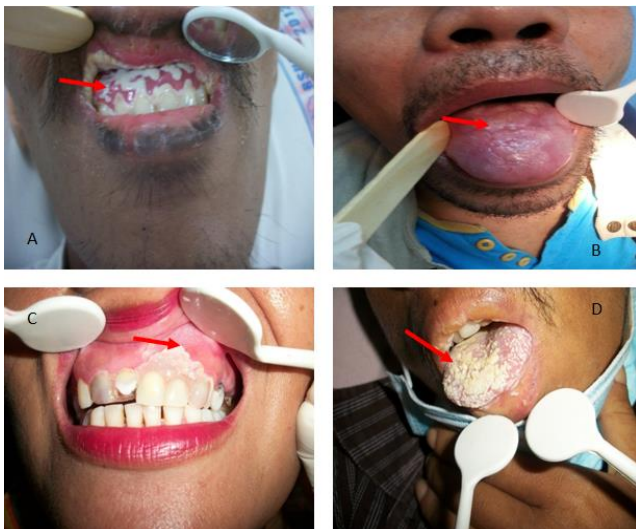


Figure 1. Clinical appearances of Oral Candidiasis in HIV/AIDS Patients. (a) Acute Pseudomembrane Candidiasis; (b) Acute Atrophic Erythematous Candidiasis; (c) Chronic Atrophic Erythematous Candidiasis / Denture Stomatitis. (d) Chronic Hyperplastic Candidiasis / Candida Leukoplakia.

Most of OC cases can be found 26-35 years old patients group (38,24%) and most of them are male (67,64%) (Table 1.). In this study, OC was found in HIV/AIDS patients with decreased CD4⁺ counts < 200 cell/mm³ (89.71%). Pearson's Correlation Test, showed that there is a correlation between the OC and decreased CD4⁺ counts on a significant level of -0,004

(p < 0.05).

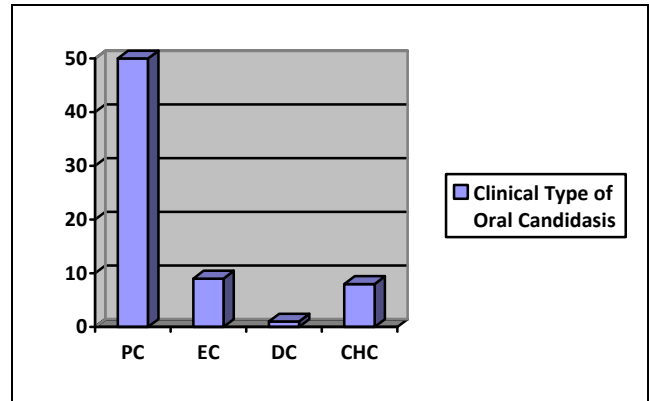


Figure 2. Clinical Type of Oral Candidiasis.

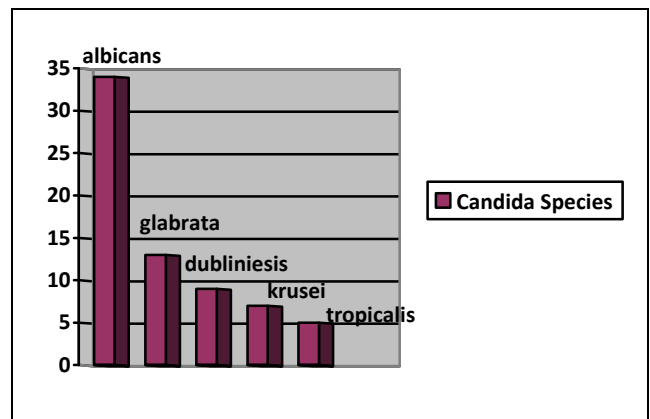


Figure 3. Prevalence of Candida Species in HIV/AIDS Patients.

Discussion

Candida species are ubiquitous fungal causing severe disease in immunocompromised individuals such as HIV/AIDS patients with extremely varied clinical manifestations like OC. The OC cases in this study are greater than the number that showed in previous study at UPIPI in 2011 found 31 cases, study at *Persatuan Waria Kota Surabaya* (PERWAKOS) found 22 cases and study at injecting drug users at Orbit Foundation Surabaya found 15 cases.^{11,12,13}

The occurrence of Candida infection always associated with OC in HIV/AIDS. Candida actually comensal, but the decrease of secretory Immunoglobulin A (sIgA) and also the decrease of lymphocytes T cell become pathogen.¹³ Patobiogenesis OC closely related to HIV/AIDS. Trans-Activating Transduction or Transcriptional Activator (TAT) is one of six HIV regulatory proteins (TAT, Rev, Vif, VPR, Vpu and Nef) were

produced at the beginning of the virus replication cycle. TAT is able to mimic the effect of extracellular matrix proteins in the regulation of cell life activity through the activation of signal transduction pathways. *C. albicans* cell wall thought to have receptors such as the extracellular matrix (integrin-like) that is capable of binding TAT. The interaction between the HIV TAT protein to the cell wall of *C. albicans* is expected to enable specific gene transduction pathway formation of hyphae Hypal Specific Genes (HSG).^{14,15,16}

In this study, high isolation rate of *C. albicans* species in HIV/AIDS patients was consistent with many previous published reports from India and Nigeria.^{16,17} Most common non-*albicans* Candida species identified in our study population were *C. glabrata* (19.12%), *C. dubliniensis* (13.24%), *C. Krusei* (10.3%), *C. tropicalis* (7.34%). The higher detection rate of non-*albicans* Candida species in our study may be due to the fact that this species was previously misidentified due to its phenotypic resemblance to *C. albicans* and it is now being increasingly recognized. The proportion of Candida infections caused by *C. albicans* in HIV/AIDS patient has shifted towards the non-*albicans* Candida species. Non-candida species tend to be less susceptible to antifungal agents and this has accounted for their emergence as a significant pathogen. The use of accurate and reliable diagnostic methods which readily identify the non-*albicans* species could assist the clinicians in making the right therapeutic choices and check the emergence of antifungal resistant strains.^{19,20}

In HIV/AIDS patients, viral replication could potentially trigger OC thus directly increasing the progresivity speed of HIV/AIDS infection. Terminal Long Repeat (TLR) at the end of the provirus has two sites for transcription factors bind to the host, ie NFkB and SP1. Infected T cells respond normally to activator polyclonal such as cytokines Interleukine-2 (IL-2) and Tumor Necrosis Factor (TNF). IL-2 and TNF interaction actually support the HIV proviral end the latent period and (begin replicating. T-cells are forced facilitate replication of HIV/AIDS will death through various mechanisms, consequences of CD4+ levels decreased continuously.^{21,22} This observation has been well supported by other investigators who have reported a strong correlation of the occurrence of

candidiasis in HIV/AIDS CD4+ counts < 200 cell/mm³ (normal 600 – 1200 cell/mm³).²³

This also reinforces the World Health Organization (WHO) recommendations to initiate treatment in adults living with HIV when their CD4⁺ cell counts decreased to 500 cells/mm³ or less in order to maximize the drug benefits and prevent Candida related morbidity in People Live with HIV/AIDS (PLWHA).²⁴

Conclusions

PC was the highest prevalence and *C. albicans* (50%) was the most common species isolated. OC was found to be significantly correlated with decreased CD4 cell counts. This study suggested strong clinical suspicion along with optimal sampling of an accurate diagnosis of Candida species involved would go a long way in decreasing the morbidity associated with non-*albicans* Candida species.

Acknowledgements

The authors would like to thank the Universitas Airlangga (UNAIR), Faculty of Medicine and Faculty Dental Medicine, RSUD Dr. Soetomo Surabaya doctor, dentist, all paramedic and patients of UPIPI RSUD Dr. Soetomo who participated in data collection.

Declaration of Interest

The authors report no conflict of interest and the article is not funded or supported by any research grant.

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