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Publisher: Wolters Kluwer Health

ISSN: 1687-1537 E-ISSN: 2090-2565

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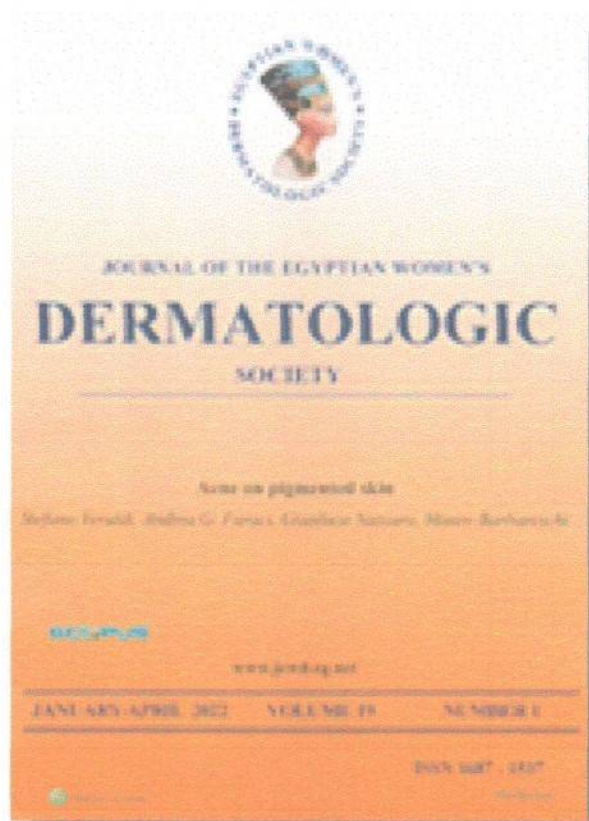
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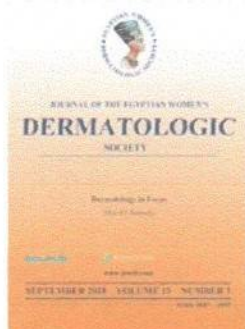
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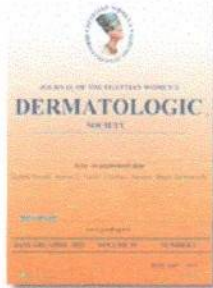
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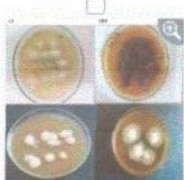


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Association between etiologic species with CD4 count and clinical features of oral candidiasis among HIV/AIDS patients

Dwi Murtiastutik^a, Cita R.S. Prakoeswa^a, Indah S. Tantular^{b,c}, Muhammad Yulianto Listiawan^a, Afif N. Hidayati^{a,d}, Evy Ervianti^a, Lunardi Bintanjojo^a

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Received: 10 December 2020

Revised: 15 August 2021

Accepted: 23 August 2021

Published: 2 January 2022

Journal of the Egyptian Women's Dermatologic Society 2022, 19:51–57

Background

Oral candidiasis (OC) is an oral mucosal disorder due to *Candida* genus. Its predisposing factor among patients with HIV/AIDS is mainly decreasing CD4 count. OC is commonly caused by *Candida albicans*. As CD4 decreases, the shift to *C. non-albicans* has been observed.

Objective

To evaluate the association of *Candida* species with CD4 count and clinical features in HIV/AIDS patients with OC.

Patients and methods

This is a cross-sectional study. A total of 114 oral rinse solution samples from HIV/AIDS patients with OC were collected. *Candida* species identification was done by culture in Chromagar followed by VITEK 2. The association of *Candida* species with CD4 count and clinical features was analyzed using Pearson's χ^2 and Kruskal–Wallis tests.

Results

There was growth of 149 isolates in culture from 114 patients. *C. albicans* was found in 104 (69.7%) isolates. *Candida non-albicans* were found in 45 (30.3%) isolates, namely *Candida krusei* in 22 (14.85%), *Candida glabrata* in 12 (8.1%), *Candida tropicalis* in six (4.05%), *Candida dubliniensis* in two (1.3%), *Candida parapsilosis* in two (1.3%), and *Candida lipolytica* in one (0.7%) isolate. *Candida* species was significantly associated with clinical types, episode types, pain on swallowing, CD4 count, and antiretroviral (ARV) use among all patients.

Conclusion

Among HIV/AIDS patients with OC, growth of *C. albicans* only was more common in higher CD4 count, while mixed growth of *C. albicans* and *C. non-albicans* was more common in lower CD4 count. Clinical features associated with growth of *C. albicans* only were pseudomembranous type, recurrent OC, absence of pain on swallowing, and patients on ARV, whereas those associated with mixed growth of *C. albicans* and *C. non-albicans* were cheilitis type, first-episode OC, presence of pain on swallowing, and ARV-naïve patients.

Keywords:

AIDS, *Candida albicans*, candidiasis, CD4 counts, HIV

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Introduction

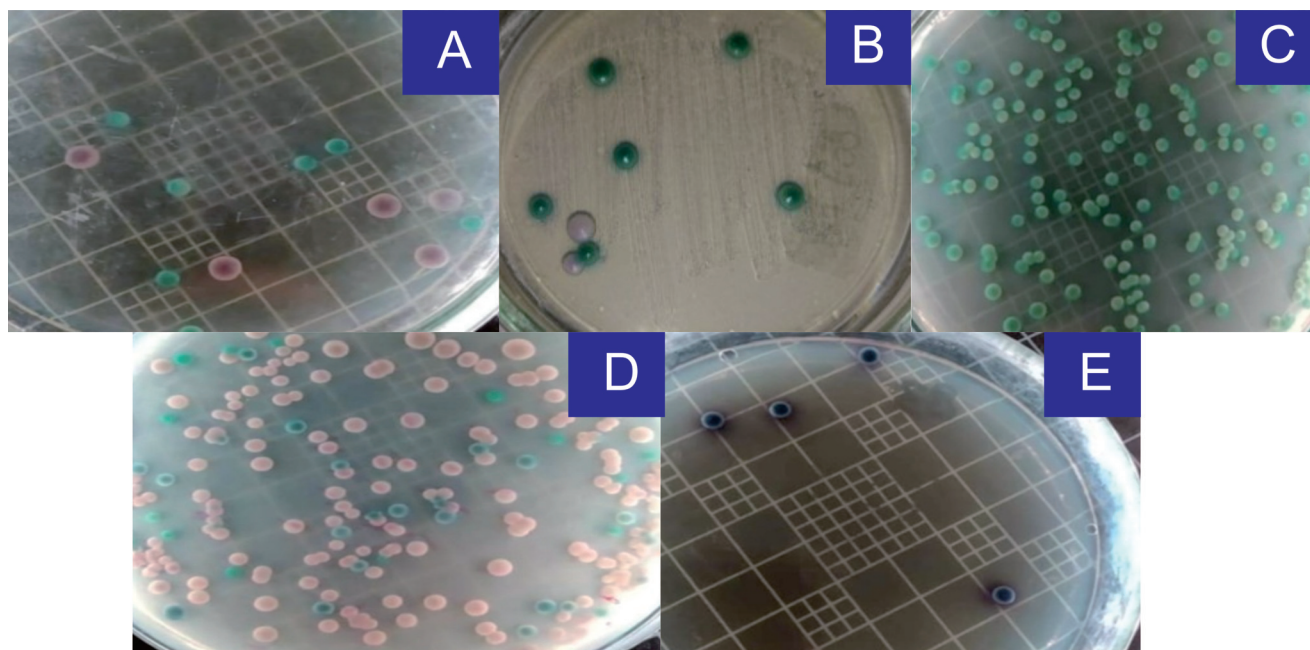
Oral candidiasis (OC) is an oral mucosal disorder due to *Candida* genus [1], and an independent predictor of immunodeficiency in AIDS patients [2]. The predisposing factor for OC among HIV/AIDS patients is mainly decreasing CD4 count [1]. OC is commonly caused by *Candida albicans*. As CD4 decreases, the shift to *C. non-albicans* like *Candida tropicalis*, *Candida glabrata*, and *Candida krusei* has been observed recently [2]. *Candida* species has been associated also with other features such as antiretroviral (ARV) use among HIV/AIDS patients with OC [3,4]. This study evaluates the association of *Candida* species with CD4 count and clinical features among HIV/AIDS patients with OC.

Patients and methods

This was an observational analytical cross-sectional study. The participants in this study were HIV/AIDS patients who visited our institution and had OC opportunistic infections. Diagnosis of HIV/AIDS was done by rapid test/HIV three methods using Fokus (PT Fokus Diagnostic Indonesia, Jakarta Selatan, Indonesia), InTec (InTec Product Inc., Xiamen, China), and VIKIA (Biomérieux, SA, Marcy l'Etoile, France). Diagnosis of OC was based on

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Figure 1



Candida colonies in Chromagar: (a) *Candida albicans* (green) and *Candida glabrata* (baby purple), (b) *C. albicans* (green) and *Candida parapsilosis* (white), (c) *C. albicans* (green), (d) *C. albicans* (green) and *Candida krusei* (pink), (E) *Candida tropicalis* (prussian blue).

clinical examination and 10–20% KOH examinations, followed by culture in Chromagar media (Becton Dickinson and Company, Franklin Lakes, New Jersey, USA) with subsequent culture in VITEK 2 (Biomérieux). CD4 T-lymphocyte count was evaluated with BD FACSCalibur device (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA).

The inclusion criteria were male or female patients and above 18 years of age. Patients who have or have not taken ARV were included in this study. The exclusion criteria were patients with no growth of fungal colony in the culture, history of other immunosuppressive diseases, consumption of antifungal medications within 3 months before the study, immunosuppressive medications, prolonged antibiotics or contraceptives, and dental caries. Patients who agreed to take part in the study received explanation about the study and gave informed consents. Ethical clearance has been given by the Health Research Ethics Committee of our institution with number of 1129/KEPK/IV/2019, dated April 20, 2019.

The patients were grouped based on CD4 T-lymphocyte level into three groups: patients with CD4 T-lymphocyte level of 1–100 cells/ μ l, CD4 T-lymphocyte level of 101–200 cells/ μ l, and CD4 T-lymphocyte level more than 200 cells/ μ l. Each group had 38 patients and the total sample was 114 patients.

Samples were taken by consecutive sampling from patients fulfilling the inclusion criteria, until the required number of samples for each group was fulfilled.

The study commenced on May 2019 until 114 patients were obtained. Samples were taken from oral rinse solutions collected by asking patients to gargle using 25 ml of sterile aquadest, which were then stored in a sterile container and sealed, labeled with the identity of the patient, date and time of collection, and sent to the laboratory. Fungal culture is done in Chromagar media (Fig. 1). In order to identify the species of *Candida*, subsequent culture was done in VITEK 2.

Statistical analysis

The results were analyzed with SPSS, version 17 (IBM, Armonk, New York, USA). Pearson's χ^2 test was used to analyze the association between *Candida* species and sex, clinical types, episode types, pain on swallowing, CD4 count, and ARV use. Kruskal–Wallis test were used to analyze the association between *Candida* species and age. A *P* value of <0.05 was considered statistically significant.

Results

This study described 114 patients with OC and HIV/AIDS who fulfilled the inclusion criteria as study participants. The age characteristic of the study

Figure 2



Clinical types of OC: (a) pseudomembranous, (b) cheilitis, (c) acute atrophic.

Table 1 Clinical features of the study patients

Characteristics	n (%)
Sex	
Male	83 (72.8)
Female	31 (27.2)
Age (years)	
Range	18–59
Mean±SD	36.4±9.8
Chief complaint	
Whitish patches in oral cavity	100 (87.7)
Reddish patches in oral cavity, patches and sores on corners of lips	7 (6.2)
Whitish and reddish patches in oral cavity	4 (3.5)
Whitish patches in oral cavity, patches and sores on corners of lips	3 (2.6)
Location	
Tongue	54 (47.3)
Tongue and mucosa	49 (43.0)
Tongue, mucosa and lips	10 (8.8)
Mucosa	1 (0.9)
Clinical types	
Acute pseudomembranous	103 (90.3)
Cheilitis	10 (8.8)
Acute atrophic	1 (0.9)
Chronic hyperplastic	0
Episode types	
First time	33 (28.9)
Recurrent	81 (71.1)
Pain on swallowing	
Yes	50 (43.9)
No	64 (56.1)
Total	114 (100.0)

patients was mean±SD of 36.4±9.8 years old with range from 18 to 59 years old. There were more male (72.8%) than female patients (27.2%). This study showed chief complaint of whitish patches in

the oral cavity in 100 (87.7%) patients, reddish patches in the oral cavity with patches and sores on the corners of the lips in seven (6.2%) patients, whitish and reddish patches in the oral cavity in four (3.5%) patients, and whitish patches in the oral cavity with patches and sores on the corners of the lips in three (2.6%) patients. The most common location was on the tongue in 54 (47.4%) patients, tongue and buccal and palatal mucosa in 49 (43.0%) patients, tongue, buccal, and palatal mucosa and lips in 10 (8.8%) patients, and buccal and palatal mucosa only in one (0.9%) patient. The clinical types of OC were mostly pseudomembranous type (Fig. 2a), followed by cheilitis type (Fig. 2b) and acute atrophic type (Fig. 2c), in 103 (90.4%) patients, 10 (8.8%) patients, and one (0.9%) patient, respectively. Pain on swallowing was absent in 64 (56.1%) patients and present in 50 (43.9%) patients. Most patients had recurrent OC (88 patients, 71.1%), while others had first episode of OC (33 patients, 28.9%) (Table 1).

In this study, all patients grew fungal colonies in culture. There were 149 isolates of *Candida* species from 114 patients. The predominant species in this study was *C. albicans* in 104 (69.7%) isolates. *Candida* non-*albicans* were found in 45 (30.3%) isolates, namely *C. krusei* in 22 (14.85%), *C. glabrata* in 12 (8.1%), *C. tropicalis* in six (4.05%), *Candida dubliniensis* in two (1.3%), *Candida parapsilosis* in two (1.3%), and *Candida lipolytica* in one (0.7%) of the isolates. Growth of *C. albicans* only was found in 69 patients and *C. non-albicans* only were found in 10 patients. Mixed growth of *C. albicans* and *C. non-albicans* was found in 35 patients. *C. krusei* was found growing alone in nine patients and mixed with *C. albicans* in 13

patients. *C. tropicalis* was found growing alone in one patient and mixed with *C. albicans* in five patients. Other *C. non-albicans* species were found in mixed growth with *C. albicans*, namely *C. glabrata* in 12 patients, *C. dubliniensis* in two patients, *C. parapsilosis* in two patients, and *C. lipolytica* in one patient.

Candida species was significantly associated with clinical types ($P=0.011$), episode types ($P=0.003$), pain on swallowing ($P=0.002$), CD4 count ($P=0.000$), and ARV use ($P=0.004$), but was not significantly associated with sex ($P=0.747$) and age ($P=0.188$) among all patients. *Candida* species was associated with pain on swallowing and CD4 count, regardless of ARV use. *Candida* species was also significantly associated with clinical types among patients on ARV, and with sex among ARV-naive patients (Table 2). In general, growth of *C. albicans* only was more common in patients with higher CD4 count, pseudomembranous type and recurrent OC, without pain on swallowing, and patients on ARV, while mixed growth of *C. albicans* and *C. non-albicans* was more common in patients with lower CD4 count, cheilitis type and first episode of OC, presence of pain on swallowing, and ARV-naive patients (Table 2).

Discussion

OC is the most common opportunistic fungal infection among individuals infected with HIV/AIDS [2]. OC can affect various age groups and sex, from infants to the elderly. Marak and Dhanashree [5] showed that the most affected age group is 51–60-year-old group, which may be due to the low immunity and immunosuppressive diseases. In this study, there were more male than female patients. This is concordant with data from the Health Ministry of the Republic of Indonesia in 2017, which showed that HIV patients were more common in males than females [6]. Ambe *et al.* [7] showed that the prevalence of OC was not significantly associated with sex. In general, there is no difference of the prevalence of OC based on sex, because OC is different from vulvovaginal candidiasis that is influenced by hormonal factor [8,9]. The age of the patients had mean±SD of 36.41±9.825 years old with range of 17–59 years old. This result may be due to that adults are productive and sexually active, thus engaging in many unsafe sexual practices risky for HIV transmission [10].

OC may be the first sign or symptom of HIV/AIDS disease, and may sometimes be the

presenting complaint for patients [11]. *Candida* can spread extensively, directly from the oral cavity to other organs such as the digestive tract and many more [12]. The most common symptom was whitish patches in the oral cavity and the most common locations were tongue and also mucosa, which correspond to the most common clinical type of OC, pseudomembranous OC [2,9]. There was no associated pain on swallowing in the majority of patients. Pain on swallowing is a clinical sign that the lesion affects the pharynx and esophagus [9].

In this study, *C. albicans* was the predominant species growing in 104 isolates (69.7%), while *C. non-albicans* were found in 45 (30.3%) isolates, namely *C. krusei*, *C. glabrata*, *C. tropicalis*, *C. dubliniensis*, *C. parapsilosis*, and *C. lipolytica*. This proved that *C. albicans* was still the leading cause of OC, but *Candida non-albicans* began to be identified. Ambe *et al.* [7] showed similar result in which there were more *C. albicans* isolates (60.2%) than *C. non-albicans* (39.8%), and there were similar isolated *Candida non-albicans* species such as *C. glabrata*, *C. krusei*, *C. tropicalis*, and *C. parapsilosis* from HIV patients with OC in Cameroon. A meta-analysis study in sub-Saharan Africa from 2005 to 2015 also showed a prevalence of 33.5% for *C. non-albicans*, the most common among which was *C. glabrata*, followed by *C. krusei* and *C. tropicalis* [13]. Nelwan *et al.* [14] found *C. albicans* in 56.2% and *C. glabrata* in 15.3% of OC in Indonesian HIV/AIDS patients.

Predominance of *C. albicans* in OC among HIV/AIDS patients may be related to the pathogenicity of this species by production of hydrolytic enzymes, presence of multiple-adhesion factor, and ability to convert from yeast form to hyphal form that is crucial for tissue invasion [7]. *C. albicans* also synergistically aided colonization and infection by *C. glabrata*, while it was suppressed by *C. krusei*, which may explain the mixed growth of *C. glabrata* with *C. albicans*, and the growth of *C. krusei* only in some patients of this study [15]. *Candida non-albicans* are considered less virulent than *C. albicans* because of their lower adherence ability to mucosal and endothelial surface, less production of proteinase enzymes, and lack or lower ability to form hyphae [16]. However, increasing prevalence of *Candida non-albicans* is of importance because these species are inherently resistant to widely available fluconazole, resulting in a public health problem among HIV/AIDS population in sub-Saharan Africa [13,17].

Table 2 *Candida* species and associated clinical and laboratory features

Clinical and laboratory features	All patients				Patients on ARV				ARV-naïve patients			
	<i>Candida albicans</i> only	<i>Candida non-albicans</i> only	Mixed#	P value	<i>Candida albicans</i> only	<i>Candida non-albicans</i> only	Mixed#	P value	<i>Candida albicans</i> only	<i>Candida non-albicans</i> only	Mixed#	P value
Sex												
Male	52	7	24	0.747	38	6	13	0.951	14	1	11	0.038*
Female	17	3	11		17	3	5		0	0	6	
Age (mean±SD)	35.3±8.7	40.9±9.3	37.5 ±11.3	0.188	35.9±9.4	39.8±9.1	38.8 ±10.1	0.339	33.1±5.1	51	36.2 ±12.6	0.353
Clinical types												
Pseudomembranous	66	10	27	0.011*	53	9	14	0.021*	13	1	13	0.278
Cheilitis	2	0	8		2	0	4		0	0	4	
Acute atrophic	1	0	0		0	0	0		1	0	0	
Episode types												
First episode	16	0	17	0.003*	8	0	4	0.305	8	0	13	0.198
Recurrent	53	10	18		47	9	14		6	1	4	
Pain on swallowing												
Yes	23	3	24	0.002*	20	2	12	0.036*	3	1	12	0.015*
No	46	7	11		35	7	6		11	0	5	
CD4 (cells/μl)												
1–100	18	0	20	0.000*	18	0	7	0.002*	0	0	13	0.000*
101–200	18	6	14		11	5	10		7	1	4	
>200	33	4	1		26	4	1		7	0	0	
ARV use												
Yes	55	9	18	0.004*	55	9	18	-	0	0	0	-
No	14	1	17		0	0	0		14	1	17	
Total	69	10	35		55	9	18		14	1	17	

ARV, antiretroviral. *Growth of *Candida albicans* and *Candida non-albicans* in one patient. *P-value <0.05 is considered statistically significant.

In this study, growth of *C. albicans* only was more common in patients with higher CD4 count, pseudomembranous type and recurrent OC, without pain on swallowing, and patients on ARV, while mixed growth of *C. albicans* and *C. non-albicans* was more common in patients with lower CD4 count, cheilitis type and first episode of OC, presence of pain on swallowing, and ARV-naïve patients. Mushi *et al.* [3] found that *C. non-albicans* was increased in patients with low CD4. Substantial immunosuppression in HIV as reflected by low CD4 count may promote the growth of the less-pathogenic *C. non-albicans* and cause OC [3,13]. Lam-Ubol *et al.* [4] showed that *C. non-albicans* was more common in highly active ARV therapy (HAART)-naïve patients, and use of HAART was associated with the decrease of these species. HAART may act directly by inhibition of secretory aspartyl proteinase enzymes needed for growth of Candidal hyphae, and indirectly by improving CD4 levels to reduce *Candida* colonization [18,19]. Nair and Shetti [20] showed the association between pseudomembranous OC and *C. albicans*. Ribeiro *et al.* [21] found that *C. albicans* was more common in recurrent OC, while *C. non-albicans* was found in primary OC. Although *C. albicans* was the most common cause of esophageal candidiasis [9], Redding *et al.* [22] found that the presence of *C. non-albicans* produced more severe pain on swallowing, as also seen in this study. Previous antifungal treatment increased the prevalence of *C. non-albicans* by exerting positive-selection pressure to these species that were less sensitive to these drugs [15]. Antibiotics use has also been associated with mixed infection by *C. non-albicans* and *C. albicans* [17]. However, intake of those medications has been excluded in this study.

This study has some limitations such as the relatively smaller number of ARV-naïve patients and lack of details of ARV therapy. Future studies with larger samples of both patients on ARV and ARV-naïve patients, and analysis of the association of duration and types of ARV to *Candida* species, are recommended.

In conclusion, *Candida* species was significantly associated with CD4 count, ARV use, clinical and episode types, and pain on swallowing among HIV/AIDS patients with OC. *C. albicans* was the predominant species, but growth of *C. non-albicans* alone or mixed with *C. albicans* was identified. Mixed growth was more common in lower CD4 count, cheilitis type, first-episode OC, pain on swallowing, and ARV-naïve patients.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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