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RESEARCH ARTICLE

Prevalence and identification of oral *candida* species in patients with type 2 diabetes in Yogyakarta

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ABSTRACT

Patients with diabetes are prone to recurring and even resistant Candidiasis, making treatment challenging. Many hypotheses proposed related to susceptibility of diabetic patients to *Candida*. The prevalence and species of *Candida* in a particular diabetic community might be different compared to other diabetic community. This study aimed to determine the prevalence of *Candida* colony and its species in the oral cavity of diabetic patients included in the CDM (Chronic Disease Management) program in Yogyakarta. One hundred patients with type 2 diabetes (n= 100) were recruited as the subjects of this study. The subjects were classified into controlled and uncontrolled Diabetes mellitus (DM). Samples of oral rinse solution were collected to determine the species of *Candida* and number of *Candida* colonies using CHROMagar *Candida* medium. There were 47 and 53 of subjects with controlled and uncontrolled DM, respectively. The mean number of *Candida* colony in the subjects with controlled diabetes (1003.13) was higher than that in the subjects with uncontrolled diabetes (478.43). The *Candida* colony most commonly identified in the subjects with controlled and uncontrolled diabetes were C. albicans and C. glabrata, respectively. Female patients had higher mean number of *Candida* colony (859.51) compared to male (299.21). The *Candida* colony most often identified in both genders was C. albicans. In addition, the subjects of this study consisted of 83 geriatric subjects and 17 non geriatric subjects, in which the mean number of *Candida* colony in the geriatric subjects (761.77) was higher than that in the non-geriatric subjects (545.71). The *Candida* colony most often identified in the geriatric subjects and non-geriatric subjects was C. albicans and C. glabrata, respectively. The Mann Whitney test demonstrated that there was a significant difference (p = 0.009) of the mean number of *Candida* colony between male and female. However, there was no significant difference of the mean number of *Candida* colony between ages (p = 0.060) and diabetic status (p=0.175). It can be concluded that the *Candida* species most commonly identified in all the subjects was *Candida albicans* with the mean colony number of 349.96, followed by C. glabrata (225.97), C. krusei (144.91), C. tropicalis (3.67), and other species (2.02).

Keywords: *Candida sp*; oral cavity; prevalence; type 2 DM

INTRODUCTION

Diabetes mellitus (DM) is a disease caused by a chronic metabolic disorder of carbohydrate, lipid, and protein due to absolute or relative reduction in insulin secretion or activation that is characterized by increasing blood glucose.[1] Indonesia is the fourth most populated country, with the seventh highest prevalence of diabetic patients in 2012 worldwide.[2] The Ministry of Health of the Republic of Indonesia reported in 2018 that around 1.5% (1.017.290) people of Indonesia suffered from diabetes.[3] There are two main types of DM, i.e. type 1 and type 2, and the majority of diabetic patients (±90%) had type 2 DM and the rest had type 1 DM.[2]

Many oral findings are attributed to uncontrolled blood glucose in diabetic patients such as xerostomia, hyposalivation, burning mouth sensation, periodontal disease, dysgeusia, and oral candidiasis. *Candida* itself is normal commensal of oral flora. However, if immune status of host is altered such as in patients with HIV-infection,

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malignant disease, nutritional deficiency, and metabolic disorder, *Candida* becomes pathogenic and manifests as oral candidiasis. There are around more than 150 species of *Candida*, but only ten species are more responsible for infection in human.[4] Out of the ten species, *Candida albicans* is the most common species isolated from the oral cavity. Other *Candida* species are *C. glabrata, C. tropicalis, C. krusei, C. dubliniensis, C. pseudotropicalis, C. lusitaniae, C. parapsilosis, C. stellatoidea,* and *C. gillieri-mondii*.[5-8] Principally, many hypotheses have been proposed related to the susceptibility of diabetic patients to *Candida,* i.e. (i) high salivary glucose; (ii) hyposalivation; (iii) chemotaxis disorder; (iv) phagocytosis defect especially due to polymorphonuclear leukocyte deficiency.[9,10] Several risk factors such as age, gender, nutrition, oral hygiene, smoking, denture usage, salivary pH, and xerostomia are contributed to the susceptibility of diabetic patients to oral candidiasis.[11,12] Clinically, oral candidiasis manifests as white lesion, erythematous lesion, or hyperplastic lesion accompanied by pain or burning sensation. *Candida* colonies could be isolated from patients with oral candidiasis through oral rinse, saliva, or swab technique. However, it is also possible that there is no clinical evidence of oral candidiasis although *Candida* colonies can be isolated from laboratory examination - it is called *Candida* carriage.[13] Previous studies showed that the prevalence of yeast carriage in uncontrolled diabetic patients reached up to 90%[10] and in controlled diabetic patients up to 63.3% of the subjects,[14] and *Candida albicans* was responsible for 51% of the isolates.[15]

In diabetic patients, oral candidiasis is difficult to cure, it is prone to be recurrent even resistant.[13] Pain and burning sensation in diabetic patients with oral candidiasis deteriorate oral functions which, in turn, will decrease oral health-related quality of life (OHRQoL). If the *candida* infection not adequately treated, it will extend to posterior region up to oropharynx and esophagus, finally causing dysphagia.[16-19]

The prevalence of *Candida* and its species in particular a diabetic community might be different compared to other diabetic communities, as the disease is closely related to diabetic conditions, systemic health, medications, oral microbiome and health, hyposalivation, lifestyles and habits, gender, ages, and denture usage. These factors might affect the way each species adapts and responds to those risk factors to survive.[20-22] Occurrence of oral candidiasis might also be affected by various diseases, such as HIV infection.[23] It was also reported that diet could also affect the disease progression, as fructose was found to be able to slow down *Candida* growth rate.[24] The prevalence, especially on chronic lesions, might also be affected by antifungal susceptibility profile of the dominating species and the antifungal drug prescribed.[25] Consequently, determination of treatment planning for diabetic patients with oral candidiasis varies, depending on the etiology, risk factors, and the nature of the dominant species in each patient.

The aim of this study was to determine the prevalence of *Candida* colony and its species in the oral cavity of Chronic Disease Management (CDM) diabetic patients in Yogyakarta. By knowing the prevalence and species of *Candida* in the oral cavity of diabetic patients, the results can be used as a reference to plan the adequate management for diabetic patients, since every *Candida* species has its own susceptibility to antifungal medication. By minimizing the oral discomfort faced by diabetic patients with oral candidiasis, it can finally increase the OHRQoL of diabetic patients.

MATERIALS AND METHODS

This was a laboratory observational community-based cross-sectional study. A total of 100 patients with type 2 diabetes in Yogyakarta (45-77 years), consisting of 24 males and 76 females, were recruited as the subjects of this study. The subjects came from CDM Program patients of Klinik Qta Minomartani Sleman Yogyakarta, of RSUD Wirosaban Yogyakarta, and of Puskesmas Mantrijeron Yogyakarta. Inclusion criteria of the subjects: being diabetic patients, being able to communicate, being able to perform activity

of daily living independently. Exclusion criteria of the subjects: being HIV-infected or AIDS patients, having a malignant disease, having a history of long antibiotic or immunosuppressive medication, undertaking antifungal medication in the last month. The subjects were classified into controlled DM if Fasting Blood Glucose (FBG) was < 126 mg/dL or 2 h Post Prandial Blood Glucose (2 pp BG) was < 200 mg/dL around the last few months. Meanwhile, the subjects were classified into uncontrolled DM if FBG was ≥126 mg/dL or 2 h Post Prandial Blood Glucose (2 pp BG) was ≥ 200 mg/dL around the last few months. The protocol of the study was approved by the Research Ethic Committee of Faculty of Dentistry, Universitas Gadjah Mada with approval number: 00140/KKEP/FKG-UGM/EC/2019.

The subjects were gathered and given explanation about the goal, significance, and course of the study. The subjects who were willing to participate in the study were asked to sign a written informed consent. The subjects were asked to fill in an identity form. Then, samples of oral rinse solution were collected in a sterile container by asking the subjects to rinse the mouth with 10 mL sterile saline (Otsuka™, Japan), which was held in the mouth for 15 seconds. The concentrated rinse solution was prepared by centrifuging it at 2000 x g for 10 min. After the supernatant was removed, the cell pellet was resuspended in 500 µL, which was then inoculated onto CHROMagar *Candida* medium (CHROMagar™, Paris, France) in 200 µL aliquots. *Candida* colonies were counted after incubation at 37 °C for 48 hours.

For the statistical analysis, the subjects were initially classified according to the diabetic status based on three variables i.e., age, gender, and presentation of *Candida* colony. Then, the mean number of *Candida* colony within the variable groups was counted based on four *Candida* species (*C. albicans, C. glabrata, C. krusei,* and *C. tropicalis*), others, and total *Candida*. Mann Whitney test for nonparametric data was conducted to know whether there was a significant difference in the mean number of *Candida* colony within the variable groups.

RESULTS

Type 2 DM was the only type of DM detected in this study (hereinafter referred to as DM). There were 47 and 53 of the subjects with controlled DM (M: 11, F: 36) and uncontrolled DM (M: 13, F: 40), respectively (Table 1). The subject age ranged from 45-89 years. Meanwhile, the duration of having DM in all the subjects was between <1 year up to 30 years.

Table 2 shows that the *Candida* colony most commonly identified in the subjects with controlled DM was *Candida albicans*, followed *by C. krusei, C. glabrata, C. tropicalis,* and other species of *Candida*, respectively. On the other hand, the *Candida* colony most often identified in the subjects with uncontrolled DM was *Candida glabrata*, followed by *Candida albicans, C. krusei, C. tropicalis,* and other species of *Candida*, respectively. The mean number of *Candida* colony in the subjects with controlled DM (1003.13) was higher than that in the subjects with uncontrolled DM (478.43). Females had higher mean number of *Candida* colony (859.51) compared to males (299.21). The *Candida* colony most commonly identified in both genders was *C. albicans***.** This study consisted of 83 geriatric subjects and 17 non geriatric subjects, in which the mean number of *Candida* colony in the geriatric subjects (761.77) was higher than that in the non-geriatric subjects (545.71). The *Candida* colonies most often identified in the geriatric subjects and non-geriatric subjects were *C. albicans* and *C. glabrata*, respectively. It can be said that the *Candida* species most frequently identified in all the subjects was *Candida albicans* with the mean number of *Candida* colony of 349.96, followed by *C. glabrata* (225.97), *C. krusei* (144.91), *C. tropicalis* (3.67), and other species (2.02).

The results from the Mann Whitney test (Table 3) demonstrated that there was a significant difference (p = 0.009) in the mean number of *Candida* colony between the male and female subjects, in which females had higher mean number of *Candida* colony than male. However, there was no significant difference in the mean

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Table 1. Diabetic status of subjects within variable groups

Variables	Diabetic status		Total
	Controlled	Uncontrolled	
Age			
Geriatric	43	40	83
Non-geriatric	4	13	17
Gender			
Male	11	36	24
Female	36	40	76
Candida			
Undetected	3	8	11
Detected	44	45	89

number of *Candida* colony between the other two variables (age and diabetes status).

DISCUSSION

In this study, the number of geriatric female subjects was higher than that of non-geriatric male subjects. This is understandable because in recent decades almost all countries in the world, including Indonesia, have experienced a rapid increase in the elderly population. Based on the population censuses in Indonesia in 1971, 1990 and 2010, there was an increase in the percentage of the elderly population by 4.5%, 6.3%, and 7.6%, respectively. The increase in the elderly population will continue, so it is estimated that by 2035 the number of elderly people in Indonesia will reach 15.8% of the total population.[26] This is in line with the increase in the proportion of the elderly population globally from 6.1% to 8.8% between 1990 and 2017, which is expected to be 16% by 2050.[27] The increase in the elderly population is partly due to an increase in the life expectancy of the population and improved health services. Based on the distribution of elderly population by province, Daerah Istimewa Yogyakarta had the highest percentage of elderly people in Indonesia.[28]

On the other hand, the proportion of women in Indonesia continues to increase in higher age groups. The proportion of women at the age of 60-64 years reaches >50%, and at higher ages the proportion of women becomes >65%. This indicates that elderly women have a higher life expectancy. Globally, the proportion of elderly women is also projected to increase, from 336 million in 2000 to more than one billion in 2050.[29]

The presence of *Candida* in the oral cavities of diabetic patients has been investigated in several places in the world, with quite diverse results. The prevalence of *Candida* in this study was 89%. Based on the results above, the number of colony in each *Candida* species between the controlled and uncontrolled DM groups varied. Previous studies reported their findings on the positive correlation and influence of blood glucose level on oral candidiasis and *Candida* colonization.[30,31] However, our study did not reveal statistically significant differences between the controlled and uncontrolled subjects. This might corroborate a

Table 2. Means of Candida colony number within variable groups

Variables	C. albicans	C. glabrata	C. krusei	C. tropicalis	Others	Total
Age						
Geriatric	373.33	210.83	172.84	3.99	1.78	761.77
Non-geriatric	240.76	299.88	0	1.88	3.18	545.71
Gender						
Male	274.33	18.96	2.67	0.79	2.46	299.21
Female	373.84	291.34	187.92	4.53	1.88	859.51
Diabetes status						
Controlled	516.36	212.62	271.09	0.98	2.09	1003.13
Uncontrolled	202.40	237.81	30.28	5.98	1.96	478.43

Table 3. Mann Whitney test of means of total Candida colony number within variable groups

Variables	Mean of total candida number	p value
Age		
Geriatric	761.77	p = 0.060
Non-geriatric	545.71	
Gender		
Male	299.21	p = 0.009*
Female	859.51	
Diabetes status		
Controlled	1003.13	p = 0.175
Uncontrolled	478.43	

* = significant (p < 0.05)

previous study that reported independency of *Candida* carriage prevalence from HbA1c level.[4] This shows that other risk factors might play bigger roles in the disease progression in diabetic patients.

Several risk factors such as age, gender, nutrition, level of oral hygiene, smoking habits, denture usage, salivary pH, and xerostomia are associated with the susceptibility of DM patients to Candidiasis.[12,17,32,33] However, in this study not all these factors were revealed. A study by Lynne et al. showed that *Candida* could be detected in 74.8% of 107 subjects with DM, namely 36 of 52 controlled subjects (69.23%) and 44 of 55 uncontrolled subjects (80%).[4] In this study, *Candida* was detected in 44 of 47 controlled subjects (93.61%) and 45 of 53 uncontrolled subjects (84.91%). A study by Mohammadi et al. showed a significant difference in *Candida* frequency among people with well-controlled, moderately-controlled, and poorly-controlled diabetes, as well as healthy people (p = 0.045), with the highest frequency found in people with poorly controlled diabetes.[12]

The results of this study indicated that the geriatric subjects had a higher mean number of *Candida* than the non-geriatric subjects. One of characteristics of elderly is a decline in immune system, so the elderly are more susceptible to diseases, especially chronic degenerative diseases (such as DM, malignant disease) and infections. *Candida* itself is a normal flora in the oral cavity. However, if the body's immune system decreases, *Candida* becomes pathogenic and can infect the oral cavity. There is a tendency that when someone is getting older, there is a higher possibility of *Candida* infection, especially related to deterioration of oral hygiene and use of dentures. In this study, there were only 6 subjects with a removable denture, so the possibility of association between *Candida* colonies and denture usage was less significant. The varying frequency of *Candida* in DM patients is due to the different abilities of fungi to attach to epithelial cells and genetic susceptibility of each patient with DM to infection. The increase in *Candida* colonies in diabetic subjects may be related to increased salivary glucose, oral micro-environmental changes such as reduced salivary flow or altered salivary composition, decreased immunity,[34] and disruption of oral microbial balance.[35] Another reason for the association between geriatric subjects and high number of *Candida* colony is related to polymedication and polypathology in older people. The elderly tend to suffer from some diseases, so they have to take more medications, such as antibiotics and immunosuppressive drugs which tend to make someone more easily develop Candidiasis in the oral cavity. Unfortunately, this study did not reveal anamnesis regarding longterm medication with antibiotics and immunosuppressive drugs.

Another result of this study also demonstrated that the female subjects had a higher mean number of *Candida* than the male subjects. In addition, the Mann Whitney test proved that there was a significant difference in the mean number of *Candida* between the female and male subjects. Several reasons can be put forward regarding this; one of which is because women have estrogen. Estrogen can increase the fungal virulence of *C. albicans,* so they are able to avoid the innate immune system.[36] Estrogen can also increase glycogen production in vaginal mucosa. Glycogen is a nutrient required for the growth of *C. albicans*.[36,37] Possibly, the same thing also takes place in the oral mucosa. The results of this study are supported by other findings, including Brazil's Center for Diagnosis of Oral Diseases,

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which revealed that women's risk of developing Chronic Atrophic Candidiasis is four times higher than that of men.[35] In addition, according to Loster et al., there was a significant relationship (p = 0.01) between intensity of fungal growth and gender, where infection-free individuals were more in males than females.[38]

Different works indicated *C. albicans* as isolated *Candida* with the highest frequency in the oral cavities of diabetic patients.[4,12,39,40] In this research, this species was the most prevalent and it was found more frequently in the controlled subjects, while *C. Glabrata* was found more frequently in the uncontrolled subjects. This is in line with previous findings that reported the same result, in which *C. glabrata* was found to be more prevalent in the uncontrolled diabetic patients compared to the controlled patients.[14] This is because of the nature of *C. glabrata* that is more aggressive and produces higher amount of biofilm than *C.* albicans. This result showed possible specific pathogenicity and colonization of each *Candida* species in different microenvironment. Uncontrolled blood glucose and microenvironment might promote the aggressivity and colonization of *C. glabrata* in the oral cavity.[11] The aggressivity will lead to enhanced pathogenicity and tissue destruction.

Diabetes mellitus could promote secreted aspartyl proteinases, thus leading to enhanced colonization, biofilm formation, and tissue invasion. Biofilm formation could impact antifungal treatment; each species has its own biofilm forming capacity. Biofilms formed by low biofilm former (LBF) were found to contain sparse yeast cells and pseudo hyphae, whereas high biofilm former (HBF) could produce denser hyphae. HBFs are known to need a specific consideration of drug choice and dose.[41] Treatment to each species might differ, as some species have been reported to be resistant to particular antifungal drugs, but still in the same principle of antifungal, etiological elimination and blood glucose control.[11,42] One of previous studies found that HBF needs antifungal dose of 8-fold higher than LBF.[43] Some studies reported that *C. parapsilosis* and *C. tropicalis* generate great heterogenic biomass, whereas *C. parapsilosis* and *C.glabrata* secret biofilm matrix containing more carbohydrates than *C.tropicalis*.[41] The specific nature of each species and its complexity in biofilm formation could affect treatment choice of patients with oral candidiasis because of potential resistance.

In other previous studies involving diabetic subjects, *C. albicans* was also the most commonly detected fungal species in the oral cavity.[12,34,44] *C. albicans* is known to predominantly exist in the oral cavity with its commensal nature[8,42] Abundant *C. albicans* on the mucosal surface might be caused by its pathogenicity and ability to adapt to oral microenvironment. *C. albicans* could infect diverse niches because it could change its morphological form, express adhesin and invasin, form biofilm to protect itself, and switch its phenotype. This led to rapid adaptation to microenvironment and pH to acquire nutrient and survive.[45] *C. albicans* were reported to produce lower amount of biofilm than non-albicans species, thus leading to consideration of emerging pathogens of non-albicans species. However, the lower capacity of biofilm formation was also reported to not particularly influence clinical manifestation of the infection.[41] This shows that various factors and co-infection might play roles in the disease progression.

The complexity of the relation between *Candida* spp. infection and DM causes the difficulty of concluding which species is the most prevalent in the oral cavity. We reported that the most prevalent species in the controlled patients was *C. albicans*, followed by *C. krusei, C. glabrata, C. Tropicalis,* and other *Candida* species. Meanwhile, the most prevalent species in the uncontrolled patients was *C. glabrata*, followed by *Candida albicans, C. krusei, C. Tropicalis,* and other *Candida* species. One of previous studies reported that the most prevalent species was *C. albicans,* followed by *C. parapsilosis, C.dubliniensis*, and *C. tropicalis*.[4] Meanwhile, another study reported that the most frequently identified *Candida* species were *C. albicans, C. krusei, C. glabrata, C. kefyr*, and *C.*

tropicalis.[12] Another study also reported the most prevalent species were *C. glabrata, C. albicans,* and *C. tropicalis* in both uncontrolled and controlled diabetic patients.[14] These differences are significantly affected by the complex and diverse risk factors that play roles in the disease progression; these could also be affected by environmental factors surrounding the subjects.

Many risk factors that might influence *Candida* colonization in the oral cavity in the subjects with DM such as xerostomia, smoking, and salivary pH were not revealed in this study. In principle, these risk factors will affect the ecology of the oral cavity, making it more susceptible to oral candidiasis. Therefore, future studies on these risk factors can be carried out more comprehensively, so preventive measures to minimize the occurrence of oral candidiasis in people with DM can be taken.

CONCLUSION

It can be concluded that the *Candida* species most commonly identified in all the subjects with type 2 DM in this study was *Candida albicans* with the mean of *Candida* colony number of 349.96, followed by *C. glabrata* (225.97), *C. krusei* (144.91), *C. tropicalis* (3.67) and other species (2.02).

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CONFLICT OF INTEREST

The authors declare no conflict of interest with the data contained in the manuscript.

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