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DIABETIC ORAL CANDIDIASIS : PREVALENCE DETERMINATION BASED ON A1C VALUE AT HAJI HOSPITAL, SURABAYA

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ABSTRACT : Hyperglycemia due to insulin resistance, lack of insulin secretion or both, in long-term condition can lead to metabolic disease known as diabetes mellitus. A1c which refers to glycated hemoglobin can be used in diabetes diagnosis that can indicate long-term blood sugar level and blood sugar control. Along with other habitual predisposing factor, uncontrolled diabetes is the main predisposing factors of oral candidiasis. A descriptive observational study with a cross-sectional method was conducted. Inclusive criteria were patients with A1c value > 6.5%, aged ≤65 years old and outpatients in Haji Hospital Surabaya. Exclusive criteria were smokers, denture wearers, patients who consumed medications other than ones for diabetes, and unable to open mouth. Oral swab was done in participants with and without oral candidiasis lesion for fungal examination using direct KOH and *Candida* culture to identify the species. Out of 38 diabetic patients, 18 suffered from oral candidiasis in which 6 of them were categorized as controlled diabetes (A1c > 6.5-8%) while the rest 12 patients included in uncontrolled diabetes category (A1c > 8%). Statistical analysis showed no significant relation between oral candidiasis and A1c value (p=0.4373; p>0.05). This may conclude that the occurrence of oral candidiasis was not fully affected by A1c value, it might be affected by other oral candidiasis predisposing factors.

Key words : Diabetes mellitus, A1c, oral candidiasis.

INTRODUCTION

Hyperglycemia due to insulin resistance, lack of insulin secretion or both, in long-term condition can lead to metabolic disease known as diabetes mellitus (Suciadi *et al*, 2019). Compared to several types of diabetes, type-1 and type-2 diabetes are the most frequent with higher prevalence in type-2 (Al-Maskari *et al*, 2011; Narmada *et al*, 2019).

Diabetes is one of the predisposing factors of oral candidiasis. Along with other predisposing factor such as smoking, denture wearing, medications (steroid and broad-spectrum antibiotics), and poor glycemic control, diabetes trigger opportunistic infection of *Candida* spp (Poradzka *et al*, 2013; Al-Maskari *et al*, 2011). Main species of *Candida* spp. namely *C. albicans* often found in oral, genital and skin infection besides other pathogenic non-albicans species such as *C. dubliniensis*, *C. glabrata*, *C. guilliermondii*, *C. krusei*, *C. lusitaniae*, *C. parapsilosis, C. pseudotropicalis* and *C. tropicalis* (Anaissie *et al*, 2009; Nugraha *et al*, 2018a).

Patients with poor glycemic control have uncontrolled glucose levels, and salivary glucose may increase which affects the loss of homeostasis and increases the susceptibility of infections in the oral cavity and wound healing (Humairo and Apriasari, 2014; Rezkita *et al*, 2020). High concentrations of glucose in blood and saliva may stimulate the growth and amplify adherence of yeast to epithelial cell surfaces. This condition also weakened the functions of polymorphonuclear (PMN) leukocytes leading to reduced phagocytosis, intracellular killing and chemotaxis which may grant to the increased colonization of *Candida* and susceptibility to oral candidiasis (Nugraha *et al,* 2018b).

A1c refers to glycated hemoglobin which can be used in diabetes diagnosis. In every â-chain of hemoglobin A (HbA), glucose attached to N-terminal valine of amino acid, therefore A1c can indicate long-term blood sugar level in diabetic conditions (Welsh *et al*, 2016; Florkowski, 2013). According to American Diabetes Association, blood sugar control considered good for non-pregnant adults if A1c level < 7%, and poor if A1c level found to exceed 8% (Cosson *et al*, 2018; Omar *et al*, 2018). A study

conducted by Jabber and Adi, showed that A1c values above 12% were significantly correlated with an oral yeast infection, which suggested that fungal infections on mucous membranes probably only happen if diabetic patients have had hyperglycemia for a quite long time (Jabber and Adi, 2011).

MATERIALS AND METHODS

A descriptive observational study with a cross-sectional method was conducted with a total sampling of 38 diabetic patients, who agreed to participate in this study. These patients, male or female, have A1c value > 6,5%, aged ≤ 65 years old and they are outpatients in Haji Public Hospital Surabaya. Smokers, denture wearers, patients who consumed medications other than ones for diabetes therapy and unable to open their mouths were taken off the study.

A1c level of all participants were collected from medical records. Interview and oral examination also performed to all participants, including oral swab in participants with and without oral candidiasis lesion for fungal examination using direct KOH and Candida culture.

Ethical clearance number 073/44/KOM.ETIK/2017 was issued by Research Ethics Committee of the Haji Public Hospital of Surabaya. Research was explained in detail to all participants and consent form had signed in advance of the research.

RESULTS

Total participant in this study was 38 patients, 6 were males (15.78%) and 32 were females (84.21%). Based on clinical and laboratory examinations, 6 controlled diabetic patients (15.8%) had oral candidiasis and 12 uncontrolled diabetic patients (31.6%) had oral candidiasis (Table 1). The clinical feature of oral candidiasis in the patient as shown in Fig. 1. Statistic test of Mann Whitney Test was next applied because the data was not normally distributed. It turned out that based on the statistical analysis, there were no significant difference between the findings ($p = 0.4373$; $p>0.05$).

Based on laboratory examination in patients with oral candidiasis, direct KOH revealed yeast cell and pseudohyphae (Fig. 2). There were mixed infections found

Table 1 : Oral candidiasis prevalence in diabetic patients according to A1c value.

A1c Value	Oral Candidiasis	
	+	-
> 6.5-8%	6 patients (15.8%)	12 patients (31.6%)
> 8%	12 patients (31.6%)	8 patients (21%)
Total	**18 patients (47.4%)**	**20 patients (52.6%)**

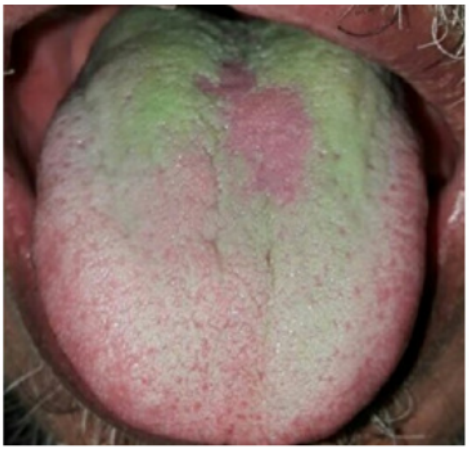

Fig. 1 : Pseudomembranous candidiasis in uncontrolled diabetic patient.

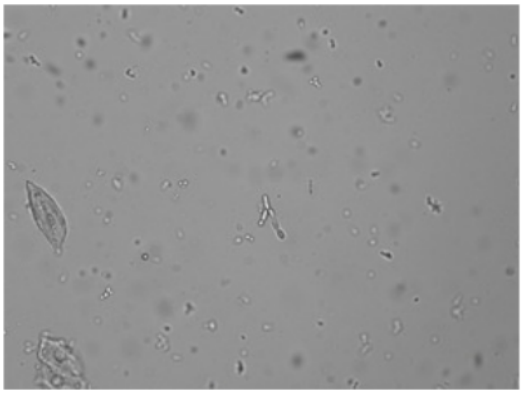

Fig. 2 : Direct KOH examination (yeast cell and pseudohyphae are found).

Table 2 : Identification of *Candida* species in diabetic patients with oral candidiasis.

Candida spp.		A1c value	
		> 6.5-8%	> 8%
Single infection	*C. albicans*	3 patients	6 patients
	C. glabrata	-	1 patient
Mixed infection	*C. albicans* and *C. glabrata*	3 patients	3 patients
	C. albicans and *C. tropicalis*	-	1 patient
	C. albicans and *C. krusei*	-	1 patient

in those patients. Multiple-species colonization occurred in 8 patients with combination of *C. albicans* along with -non-*albicans* species (Fig. 3), which are *C. glabrata* (6 patients), *C. tropicalis* (1 patient) and *C. krusei* (1 patient) (Table 2).

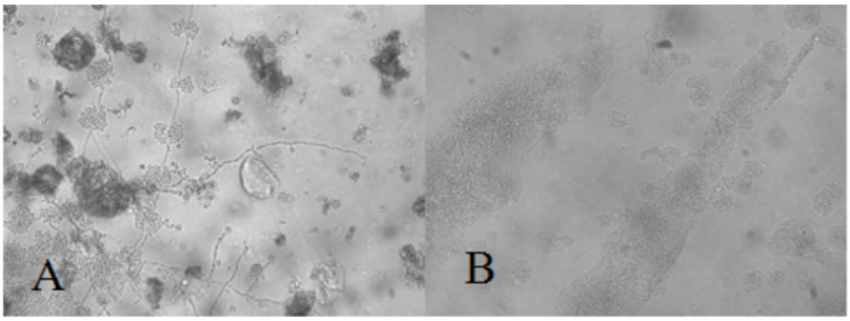

Fig. 3 : Microscopic view of *Candida* spp. **A.** *C. albicans*; **B.** *C. glabrata*.

DISCUSSION

Hyperglycemia in diabetes is linked with long-term damage, dysfunction and failure of different organs, this includes impaired innate and adaptive immune system. The impaired innate immune system affects the ability of phagocytosis and neutrophil function meanwhile neutrophil has protection function against invasive candidiasis (Anaissie *et al*, 2009). Furthermore, the cell maturation will be inhibited, which disrupts the process of desquamation of epithelial cells, while the desquamation of keratinized cells plays an important role in the clearance of adhered fungi (Fidel and Huffnagle, 2005). In an adaptive immune system, there is an alteration in lymphocytes T response, while both $CD4^+$ (T-helper) cells and $CD8^+$ (T-cytotoxic) cells have been shown to play a role in antifungal immunity and their activation is controlled by the dendritic cell. Th1 and Th17 are T helper subset, which is considered important to successful immune defense against *Candida* infections (Casqueiro *et al*, 2012; Richardson and Moyes, 2015; Nugraha *et al*, 2019a).

Diabetic patients also experience salivary gland dysfunction. The etiology of diabetic salivary gland dysfunction may be associated to multiple problems, including polyuria, poor hydration, and salivary gland pathology due to alterations in the basement membranes of the glands because of AGEs (*Advanced Glycation End Products*). Poor glycemic control, autonomic nervous system dysfunction, and related medications may also account for salivary dysfunction (Glick, 2015). Salivary gland dysfunction causes hyposalivation which manifests as dry mouth or xerostomia. Decreased salivary flow rate leads to decreasing self-cleansing activity which allows *Candida* to remain attached to oral epithelial cells. In addition to the decreasing salivary quantity, the salivary quality will also decrease (Nugraha *et al*, 2019b). Decreased salivary quality leads to decreasing salivary antimicrobial activity and components, such as lactoferrin, lysozyme and sIgA (Moyes and Naglik, 2011).

Saliva was produced from plasma, so that the high level of glucose in saliva indicate high glucose level of plasma. Resembling diabetic pancreas condition, lymphocytic infiltration of salivary parenchyma in diabetes-induced neuropathic changes caused salivary gland impairment (Lasisi an Fasanmade, 2012). Diabetic neuropathy occurred as the result of AGEs aggregation in peripheral nerves causing structural and functional impairment of protein. Indirectly, AGEs aggregation activates its receptor so the bond between them causes endoneurial-vascular dysfunction (Singh *et al*, 2014).

The ability to maintain good oral hygiene also affects the presence of oral candidiasis. But, it is considered important that both blood sugar control and oral hygiene maintenance can suppress oral lesion progression, so that education to patients is needed especially in dietary aspect. Furthermore, medication of dry mouth and routine dental examination should be programmed, because studies stated that oral hygiene improvement support better blood glucose control in uncontrolled diabetic patients (Yuen *et al*, 2009).

Micronutrient deficiency also affects the occurrence of oral candidiasis. Iron deficiency causes function impairment of transferrin and other iron-dependent enzymes, which have fungistatic effect to prevent formation of *Candida* colony. In addition, deficiency of essential fatty acids, folic acid, vitamins A and B6, magnesium, selenium and zinc also related to oral candidiasis. Also, deficiency of vitamin B12 affects *Candida* overgrowth because in this condition, the number of red blood cell decreases and this impact on the immune system, allowing the body to become more susceptible to infection. Diabetic patients may also have magnesium and vitamin B_{12} deficiency. The deficiency of vitamin B_{12} is caused by medication to treat diabetes, such as metformin (Patil, 2015; Sona and Hena, 2011; Walker, 2007).

Candida spp. play an important role in oral candidiasis occurrence through its virulence factor, namely defense mechanism against cleansing action of saliva (Prabajati et al, 2017). Specific adhesin molecules were produced by *Candida* spp. able to strengthen attachment on oral mucosal surface. Other virulence factors are the capability to withstand human immune system through phenotypic and morphological alteration, along with the secretion of hydrolytic enzyme (*e.g.* aspartyl proteinase and phospholipase) to destruct host cells (Raju and Rajappa, 2011).

Absence of oral candidiasis in uncontrolled diabetes with A1c value > 8% probably due to good maintenance of oral hygiene so the risk of oral candidiasis becomes lower. In controlled diabetes with A1c value > 6,5-8%, there were 6 patients with oral candidiasis, this could happen because of several things. First, false-lowered A1c value. There are several cases like blood loss, hemolytic anemia and splenomegaly are known to shorten life cycle and accelerate cell turnover of erythrocytes. Both conditions reduce glucose exposure to the cell, so that A1c value read as false-lowered (Radin, 2013). Second, patients weren't maintaining their oral hygiene therefore there was an accumulation of microorganisms that lead to oral disease. Third, A1c captures only chronic hyperglycemia, but it will miss acute hyperglycemia (Bonora and Tuomilehto, 2011). Meanwhile, that time, the value of 2-hour plasma glucose of 5 patients are 278 mg/dL, 315 mg/dL, 260 mg/dL, 327 mg/dL and 219 mg/dL, these acute conditions might be the cause of oral candidiasis. Fourth, the impact of medication used to treat diabetes which is metformin. Metformin can increase the risk of vitamin B12 deficiency that leads to oral candidiasis. This could happen in 1 patient who was not included in the 5 patients above. The value of the 2-hour plasma glucose of this patient was 138 mg/dL and the medications used to treat diabetes were metformin and insulin.

The result of the oral candidiasis culture could be improved by modifying the isolation method. This study used a swab method to detect yeast organisms. Swab method is relatively simple, but it's critical to select which location to be swabbed. Meanwhile this study examined all patients with or without apparent oral candidiasis lesion, thus probably could benefit from the oral rinse method. This method is done by instructing participants to rinse for 60 seconds with phosphate-buffered saline at pH 7.2, 0.1 M. Put back after-rinse solution to container, followed by centrifugation and prepared for culture. Oral rinse method is simple, has better results if there are more than 50 CFU/cm^2 and it is suggested for surveillance cultures when no focal lesion was found (Glick, 2015). *Candida* colonization that consist of multiple species is frequent, proved by several studies in healthy people, elderly, patients with diabetes mellitus, HIV, nasopharyngeal cancer and hematologic malignancy, which are resulting in approximately 44% incidence rate of multiple species for *Candida* colonization (Anaissie *et al*, 2009).

Two patients with mixed infection between *C. albicans* and *C. glabrata* had the colony forming unit of *C. glabrata* > 100/µL and *C. albicans* 18/µL and 31/µL. A study was conducted to observe the interaction of biofilm formation by *C. albicans* and non-*albicans* species and resulting a phenomenon where *C. albicans* biofilm was significantly diminished by the presence of non-*albicans* species. Nutrients and adhesion sites competition believed to be the main cause of those results. *C. krusei*, the first species to be found in early colonization, produce specific signaling molecules that impede *C. albicans* growth up to 85%. Compared to *C. krusei,* suppression rate of *C. glabrata* is lower, however the inhibitory activity against *C. albicans* colony forming still show a significant rate in those 2 patients. So it must be taken as serious consideration that multiple-species colonization of *Candida spp.* is one risk factor which leads to more severe infections (Santos *et al*, 2016).

A1c value was not considered as main factor in diabetic oral candidiasis, other factor such as immune dysfunction, salivary gland dysfunction, oral hygiene, micronutrients deficiency, and pathogenicity of *Candida* spp. also predispose the incidence of diabetic oral candidiasis.

CONCLUSION

Glycemic control monitored by A1c value could lead to false results because the blood glucose level does not describe the actual condition and it is affected by several factors. Oral candidiasis has many predisposing factors thus oral candidiasis may not occur in uncontrolled diabetes with fewer factors.

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