Cytokine Profile Analysis of IL - 1β and TNF-α in Diabetic Patients Infected by *C.albicans*

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Abstract: Background: The prevalence of Diabetes Mellitus (DM) in Indonesia in 2020 is expected to affect more than 7 million people. The disease would lead to complication and oral manifestations. One of the oral manifestations of DM is an oral infection caused by *Candida albicans.* The patients are often in an immunocompromised state with an aberrant immune response. It was reported that diabetic patients have a higher level of pro-inflammatory cytokines. Objectives: This study aims to analyze the cytokine profile of diabetic patients infected with *C.albicans*. Methods: The subjects consist of 33 people with oral candidiasis, who were grouped into regulated DM, unregulated DM, and non-DM individuals. HbA1C was measured to classify the patients into the regulated DM or unregulated DM groups. Diagnosis of oral candidiasis was based on the clinical appearance of oral mucosa and Papanicolaou staining. There was 7cc of peripheral blood that was obtained from all subjects to analyze IL-1β and TNF-α levels, using an indirect ELISA technique. The data were statistically analyzed using one-way ANOVA. Results: The level of IL-1β and TNF-α were significantly different ($\alpha = 0.05$) between each group. On the contrary, there was no significant difference in IL-1β and TNF-α level between the regulated DM group and the control group. Conclusion: The oral candidosis plays a role in altering pro-inflammatory cytokines levels in individuals with DM, in both regulated and unregulated groups.

1 INTRODUCTION

Diabetes Mellitus (DM) is indicated with chronic hyperglycemia due to impaired insulin secretion and function (Bigna et al., 2018). As well as in Indonesia, the global prevalence of DM is continuously increasing each year. There were approximately 415 million people suffering from DM according to the International Diabetes Foundation (Tankeu et al., 2016). It is estimated that the world prevalence of DM will increase to 7.7% (439 million) by 2030. An epidemiological study of diabetic patients in Indonesia showed that the prevalence of DM is 5.7% (Mihardja et al., 2014). There were 8.5 million of adults with DM in Indonesia in 2013, while in 2035 it is estimated there will be 14.1 million of adults suffering from DM based on the International Diabetes Foundation (Forouhi and Wareham, 2014). The disease may progressively develop complications with increased morbidity, disability, and mortality that threatens life (Papatheodorou et al., 2016).

The mechanism that underlies complications is Advanced Glycation End (AGE) products. The substance targets extracellular proteins to undergo damaging crosslinks (Nass et al., 2007). The accelerated AGE formation occurs in people with DM as an effect of abundant concentration of glucose, AGE precursors, and oxidative stress. AGEs are known for their damaging effect of inducing cell death, reducing cell adhesion and migration, and interfering protein function (Nowotny et al., 2015). Moreover, AGE deposition causes complications and oral manifestations in people with DM (Nass et al., 2007).

One of the oral manifestations in diabetic patients is a candida infection. The frequency of oral infection with candida is higher in diabetic patients due to a high concentration of salivary glucose and low salivary secretion that facilitates the adherence of yeast in epithelial cells (Obradović et al., 2011). Diabetic patients are prone to infection because of

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their impaired immune system. Chronic hyperglycemia provides a disadvantageous environment that compromises the immune system, which is known as the immunocompromised state. The impaired immune system is characterized by suppressed neutrophil function, antioxidant system, cellular and humoral immunity (Casqueiro et al., 2012). Diabetic patients have a 21% higher risk of infection than non-diabetic people, and it affects all organs and systems, as well as oral cavities (Duka et al., 2017). DM is reported to elevate production and function of Interleukin 1-beta (IL-1β) and Tumor necrosis factor-alpha (TNF-α) to facilitate systemic and tissue inflammation, as well as contribute to insulin resistance (Cardoso et al., 2017; Mohammadi et al., 2017; Peiró et al., 2017). Based on the pivotal roles of IL-1β and TNF-α in DM and oral candidiasis, the present study aims to analyze the cytokine profile of IL-1β and TNF-α in people diagnosed with DM and Oral candidiasis.

2 MATERIALS AND METHODS

The subjects consist of 33 people with oral candidiasis who were grouped into regulated DM, unregulated DM, and non-diabetic. The measurement of HbA1C was performed to classify the patients into regulated DM or unregulated DM groups. Subjects with HbA1C higher than eight were considered as unregulated DM, subjects with HbA1C ranges from 6.5 to 8 were considered as regulated DM, while subjects with HbA1C lower than 6.5 were considered as a non-diabetic population or control. The subjects were diagnosed from suffering oral candidiasis based on the clinical appearance of oral mucosa and laboratory examination. There were 7 ccs of peripheral blood obtained from all subjects to analyze the level of cytokines IL-1β and TNF-α using an indirect ELISA technique. Scrubbing of oral mucosa was performed to obtain *C.albicans* and was cultured in Saboroud Dextrose Agar medium (Difco) afterwards to conduct a gram staining and carbohydrate fermentation test. The obtained *C.albicans* were also stained using Papanicolaou staining. The data were statistically analyzed using a one-way ANOVA test.

3 RESULTS

Indirect Enzyme Linked Immuno Assay (ELISA) was performed to analyze IL-1β and TNF-α level from the blood serum of all subjects. The ELISA is an effective method to identify the immunocompromised status of subjects with regulated DM, unregulated DM, and non-diabetic control affected with oral candidiasis. According to the one-way ANOVA analysis, it was shown that $p < 0,012$ ($\alpha = 0,05$). In accordance with IL-1β, the result for TNF-α is $p < 0,007$ ($\alpha = 0,05$), then it was followed by the Tukey HSD test.

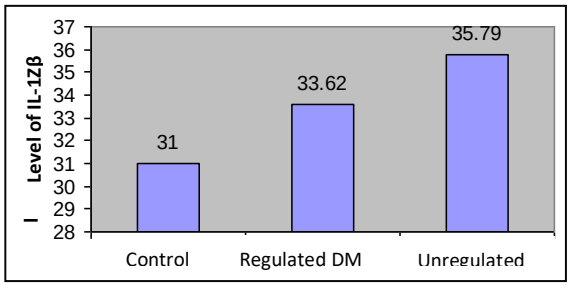
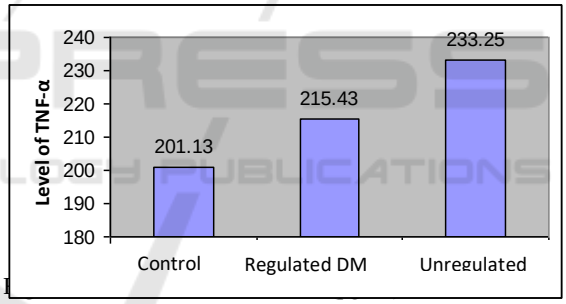


Figure 1 : The level of IL-1β (pg/ml) in non-diabetic subjects, subjects with DM, and subjects with unregulated DM.



subjects, subjects with DM, and subjects with unregulated DM.

Statistical analysis showed there were significant differences ($\alpha = 0,05$) for IL-1β and TNF-α analysis between unregulated diabetic patients groups and non-diabetic control populations infected with *C.albicans.* The level of IL-1β in unregulated diabetic patients showed no insignificant difference compared to non-diabetic group (Figure 1). There was a significant difference in TNF-α level between unregulated diabetic patients compared to the non-diabetic group, while the level of TNF-α in regulated diabetic patients group and the non-diabetic group was not significant (Figure 2). The aberrant cytokine profile obtained from the present study was due to the impaired immune response in unregulated diabetic patients that induced AGEs product to stimulate a higher level of IL-1β and TNF-α.

4 DISCUSSION

An indirect ELISA (ELISA Bendermed system Kit) was used in the present study to analyze the immunocompromised state with oral candidiasis in unregulated diabetic patients, regulated diabetic patients, and non-diabetic control populations. Oral infection by *C.albicans* stimulated pro-inflammatory cytokines. IL-1β and TNF-α level were investigated in the present study as they are major cytokines involved in inflammation both in diabetic and non-diabetic populations (Peiró et al., 2017).

The level of IL-1β and TNF-α in the present study was not measured in local tissue, but systemically through plasma obtained from the whole blood. This consideration is due to the fact that there is no significant difference in the oral mucosal immune response in *C.albicans* infection between the regulated diabetic, unregulated diabetic, and non-diabetic populations. According to statistical analysis, there was a significant difference ($\alpha = 0,05$) of IL-1β level in the unregulated diabetic group compared to the IL-1β level in the regulated diabetic and non-diabetic populations (Figure 1). The level of TNF-α in regulated diabetic and non-diabetic groups was significant ($\alpha = 0,05$; Figure 2). On the contrary, the level of IL-1β and TNF-α in the regulated diabetic group were not significantly different to their level in a non-diabetic population (Figures 1 and 2). The data obtained from the study reveals that the immune response in regulated diabetic patients is relatively impaired and could generate an immune response against *C.albicans.* It is confirmed through ELISA analysis that pro-inflammatory cytokine levels in regulated diabetic patients were not significantly different from the non-diabetic population. Whereas the pro-inflammatory cytokine levels were exacerbated in unregulated diabetic patients, represented by cytokine IL-1β and TNF-α.

Prolonged hyperglycemia initiates a non-enzymatic glycosylation process that alters the structure and function of proteins and biologic molecules (Negre-Salvayre et al., 2009). AGEs were formed through prolonged glycation induced by chronic hyperglycemia. AGEs stimulate macrophages to continuously release pro-inflammatory cytokines, particularly IL-1β and TNF-α (Byun et al., 2017). The literature is coherent to results in the present study, which stated that the levels of IL-1β and TNF-α in unregulated diabetics were significantly higher ($\alpha = 0,012$ for IL-1β and $\alpha = 0,05$ for TNF-α) than in unregulated diabetics and the control group. The underlying mechanism that makes unregulated diabetic patients prone to infection is the high concentration of pro-inflammatory cytokines detected in unregulated diabetic patients' impaired immune response, in addition to AGE (Cardoso et al., 2017; Mohammadi et al., 2017; Peiró et al., 2017).

The immunocompromised condition that commonly affects unregulated diabetic patients suppresses antigen recognition activity, interferes with phagocytosis, and intracellular killing that would lead to immune defects (Gordon, 2016). Moreover, reduced cytokines levels also occur in diabetic patients due to the impaired function of polymorphonuclear (PMN) and macrophages. T lymphocyte deficiency and neutrophil dysfunction contribute to the pathogenesis of oral candidiasis. Cytokines mainly regulate the activity of PMN. However, the antifungal activity of PMN is initiated by Large Granular Lymphocytes (LGLs) that secrete interferon-γ (IFN-γ), interferon-α (IFN-α), TNF-α, and IL-1. Thus, lymphocyte deficiency may reduce cytokine levels secreted by Th1 and Th2 (Samaranayake et al., 1990).

5 CONCLUSION

The oral candidosis plays a role in altering pro-inflammatory cytokine levels in individuals with DM, in both the regulated and unregulated groups. Unregulated diabetic patients in this study had significantly greater IL-1β and TNF-α levels compared to regulated diabetic patients and non-diabetic controls. Moreover, the levels of pro-inflammatory cytokines could be used to determine the severity of diabetic patients and their risk of suffering from oral candidiasis.

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