THE INFLUENCE OF PERIODONTAL STATUS, IL-1β LEVEL, AND PMN PHAGOCYTOTIC FUNCTION AS RISK FACTORS ON TYPE 2 DIABETES MELLITUS PATIENTS

Oedijani-Santoso*, Prayitno SW**

*Department of Dental and Oral Diseases, Medical Faculty of the University of Diponegoro, Semarang
**Department of Periodontology, Faculty of Dentistry the University of Indonesia, Jakarta

Abstract
Periodontal status is a periodontum condition evaluated by using plaque index, calculus index, gingival index and pocket index. The main mediator of periodontum inflammation is IL-113 examined by ELISA method. There is an elevation of PMN s in periodontum inflammation, but the leucotoxin as well as the protease in turn lowers the PMN phagocytotic function. Phagocytotic function was measured by flowcytometry. The aim of the study was to evaluate the high risk factors of being type 2 DM. A diagnostic study was conducted by using cross-sectional design on 45 controlled DM (CDM) subjects, 45 uncontrolled DM (UCDM) subjects in the Metabolic Endocrinology Clinic Cipto Mangunkusumo Hospital Jakarta, as compared to 45 non-DM control subjects. The result of multivariate analysis showed that patients of older age (>54 years old), low periodontum status (periodontal index >1.80), high IL-113 level (>23.70 pg/mL), and low PMN phagocytotic function (<53.47%), were significantly at high risk of having DM compared to non-DM (p<0.05). Lower periodontum status showed an increase in IL-113 level, decrease PMN phagocytotic function, and consequently, an increase in the risk of being type 2 DM.

Key word: periodontal status; IL-1 13 level; PMN phagocytotic function; type 2 DM

Introduction
Periodontal inflammation is a response of periodontal tissue to bacterial plaque and its products as the local etiological factor. Although many literature sources state that the local factor is the main cause, periodontal injury is not free from the effects of systemic factors, such as genetic factors, diabetes mellitus (DM), hormonal imbalance, drugs, and bacterial, fungal or viral infections that influence the susceptibility and can speed the progression of periodontal disease. Socio-demographic factors such as age, gender, education, and socio-economic status can also affect the periodontal condition.1,2,3

Evaluation of periodontal inflammation using periodontal status used in the third National Health and Nutrition Examination Survey (NHANES III) comprises of evaluation of periodontal destruction, gum, calculus and plaque measured as the pocket index, gingival index, calculus index and plaque index.4

According to the Committee on Research, Science and Therapy, The American Academy of Periodontology, even relatively mild periodontal infection can affect the general health through the periodontal immune response.5,6 In vitro studies have showed that a low level of pro-inflammatory cytokines (IL-1, TNFα, IFNγ) have a cytostatic effect on pancreatic β cells, resulting in the inhibition of insulin synthesis and secreration.7 Periodontal immune response can be evaluated by the local cellular immune response or systemic immune response, such as polymorphonuclear leucocytic
(PMN) function, lymphocytic function, and cytokine level. The PMN phagocytic function is the primary immune mechanism in which the systemic immune system tries to eliminate extracellular microorganisms. PMN phagocytic function is clinically important to evaluate the function of phagocytic cells in their interaction with the microorganisms. This is related to the susceptibility of the body to infection, including periodontal infection. In periodontal infection, many PMNs are found and act as tissue defense against periodontal pathogens.

The main periodontal inflammation mediator is IL-1, especially IL-1B. Interleukin-1B increases the complementary regulation and Fc receptor in neutrophils and monocytic cells, which cause the formation of lymphoid cell receptor in extracellular matrix and stimulate the formation of osteoclasts, the cause of bone resorption.

In the field of dental and oral disease, periodontal abnormalities are often associated with DM. A study conducted by Fu et al. (2000) in China showed that controlling periodontal inflammation would improve the metabolic control of type 2 diabetic patients. A study conducted by Hernawan and Askandar (2002) in Surabaya showed that periodontal disease was more severe in uncontrolled (UCDM) than in controlled diabetes mellitus (CDM). However, these findings were different from those of Alpagot et al. (2001) that showed no relationship between periodontal clinical status and duration of disease and metabolic control of DM. Zielinski et al. (2002) stated that there were no differences in oral health parameters between DM and non DM subjects.

Studies in Indonesia on the periodontal status and immune response as risk factors for DM focusing on the roles of IL-1B and PMN phagocytic function have not been conducted before. Therefore, this study aimed to evaluate the related risk factors for type 2 DM.

Materials and Methods

Subjects

The subjects were 45 type 2 DM patients, ages between 40 and 60 years old, given the standard treatment of diet and oral hypoglycemic drugs (OHD) in the Metabolic-Endocrinology Clinic of Internal Medicine Department, Cipto Mangunkusumo Hospital for three consecutive months and determined to show UCDM and CDM. For comparison, 45 non-DM volunteers in good health history and with or without periodontal abnormalities were included for comparison.

Subjects with infection and inflammation, especially infection of the urinary tract and lungs, and subjects with neutrophil count of <50% or >70% times from normal leucocyte count (3000-7000/μL) were excluded from the study. Subjects using antibiotics, anti-inflammatory drugs, anticonvulsant drugs, drugs that can decrease immune response (steroids, aspirin), birth control pills, or pregnant, smoking or unwilling to participate in the study were also excluded.

Written form of informed consent was filled in by the DM and non-DM subjects after being explained about the study.

Periodontal clinical status of subjects: The cutoff point for high periodontal index was determined by using ROC (Receiver Operating Curve) on the sensitivity and specificity. The high categories were obtained for plaque index (>0.799), calculus index (>0.698), gingival index (>0.199), pocket index (0.199) and periodontal index (1.800). IL-1β level was evaluated using ELISA method. The cutoff point of high IL-1β level was determined using the ROC of the geometric mean IL-1β level. The resulting high category for IL-1β level was set to >23.6999 pg/mL.

PMN phagocytic function was evaluated using flow cytometry. The cutoff point of low phagocytic function was determined using the ROC of the geometric mean phagocytic function. The resulting low category for phagocytic function was set to <53.4744%.

Statistical analysis

Using Stata 7 program, all calculations applied significance value of p<0.05 and CI 95%. Multinomial logistic regression test was used to determine the factors that have the greatest role for type 2 DM.

Results

To determine the factors that affect UCDM and CDM, multinomial logistic regression bivariate analysis was used with non-DM subjects as reference.
Table 1. The Relative Risk Ratio in DM and Non-DM Subjects According to Demographic Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>non-DM RRR</th>
<th>non-DM RRR 95%CI</th>
<th>non-DM p</th>
<th>CDM RRR</th>
<th>CDM RRR 95%CI</th>
<th>CDM p</th>
<th>UCDM RRR</th>
<th>UCDM RRR 95%CI</th>
<th>UCDM p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Male</td>
<td>1</td>
<td>0.83</td>
<td>0.35 ; 1.94</td>
<td>0.664</td>
<td>0.74</td>
<td>0.32 ; 1.77</td>
<td>0.512</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>55-60 years old</td>
<td>1</td>
<td>9.85</td>
<td>3.72 ; 26.08</td>
<td>0.000</td>
<td>2.81</td>
<td>1.18 ; 6.72</td>
<td>0.020</td>
<td></td>
<td></td>
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<tr>
<td>Education</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Low</td>
<td>1</td>
<td>0.76</td>
<td>0.33 ; 1.75</td>
<td>0.526</td>
<td>0.84</td>
<td>0.37 ; 1.92</td>
<td>0.673</td>
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<tr>
<td>Occupation</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Unemployed</td>
<td>1</td>
<td>2.20</td>
<td>0.91 ; 5.33</td>
<td>0.081</td>
<td>2.20</td>
<td>0.91 ; 5.33</td>
<td>0.081</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: RRR (Relative Risk Ratio) and p value with non-DM subjects as reference based on multinomial logistic regression analysis.

Table 1 shows that subjects with age of 55-60 years have 9.85 times the risk of having CDM (p=0.000) and 2.81 times the risk of having UCDM (p=0.020) when compared to younger, 40-54 years old non-DM subjects.

Table 2 shows that when compared to non-DM subjects with low category index, the subjects with high calculus index (0.699 to maximum) have 1.74 times the risk of having CDM (p=0.027) and 2.74 times the risk of having UCDM (p=0.021).

The RRR value for subjects with high gingival index (0.200 to maximum) in CDM is 2.47 (p=0.037) and in UCDM is 2.99 (p=0.012). This means that compared to non-DM subjects with low gingival index, subjects with high gingival index have 2.47 times the risk of having CDM and 2.99 times the risk of having UCDM.

Table 2. Relative Risk Ratio of Periodontal Status and Immune Response of DM and Non-DM Subjects According to Oral Clinical Examination and Laboratory Tests

<table>
<thead>
<tr>
<th></th>
<th>Non-DM RRR</th>
<th>CDM RRR</th>
<th>CDM 95%CI</th>
<th>CDM p</th>
<th>UCDM RRR</th>
<th>UCDM 95%CI</th>
<th>UCDM p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plaque Index</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>0.800 – max</td>
<td>1</td>
<td>1.31</td>
<td>0.57 ; 2.99</td>
<td>0.527</td>
<td>2.07</td>
<td>0.89 ; 4.83</td>
<td>0.092</td>
</tr>
<tr>
<td>Calculus Index</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.699 – max</td>
<td>1</td>
<td>1.74</td>
<td>0.74 ; 3.94</td>
<td>0.207</td>
<td>2.74</td>
<td>1.16 ; 6.45</td>
<td>0.021</td>
</tr>
<tr>
<td>Gingival Index</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.200 – max</td>
<td>1</td>
<td>2.47</td>
<td>1.06 ; 5.77</td>
<td>0.037</td>
<td>2.99</td>
<td>1.27 ; 7.04</td>
<td>0.012</td>
</tr>
<tr>
<td>Pocket Index</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.200 – max</td>
<td>1</td>
<td>2.25</td>
<td>0.97 ; 5.24</td>
<td>0.059</td>
<td>2.74</td>
<td>1.16 ; 6.45</td>
<td>0.021</td>
</tr>
<tr>
<td>Periodontal Index</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.801 – max</td>
<td>1</td>
<td>1.88</td>
<td>0.81 ; 4.33</td>
<td>0.141</td>
<td>2.47</td>
<td>1.06 ; 5.77</td>
<td>0.037</td>
</tr>
<tr>
<td>IL-18 level</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23.6999 – max</td>
<td>1</td>
<td>16.78</td>
<td>5.24 ; 48.17</td>
<td>0.000</td>
<td>21.71</td>
<td>7.32 ; 64.45</td>
<td>0.000</td>
</tr>
<tr>
<td>Phagocytic function</td>
<td></td>
<td>41.3</td>
<td>1.69 ; 10.05</td>
<td>0.002</td>
<td>4.53</td>
<td>1.95 ; 11.08</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Note: RRR and p value with non-DM subjects as reference based on multinomial logistic regression analysis.

Subjects with high pocket index (0.200 to maximum) have 2.25 times the risk of having CDM (p=0.059) and 2.74 times the risk of having UCDM (p=0.021) compared to non-DM subjects with low pocket index. Subjects with high periodontal index (1.801 to maximum) have 1.88 times the risk of having CDM (p=0.141) and 2.47 times the risk of having UCDM (p=0.037) compared to non-DM subjects with low periodontal index.

Subjects with high IL-18 level (23.6999 g/mL to maximum) have 16.78 times the risk of having CDM (p=0.000) and 21.71 times the risk of having UCDM compared to non-DM subjects with low IL-18 level (minimum to 23.6999 pg/mL).

Compared to non-DM subjects with high phagocytic function (53.4744% to maximum), subjects with low phagocytic function have 4.13 times the risk of having CDM (p=0.002) and 4.53 times the risk of having UCDM (p=0.001).

The variables in bivariate analysis (Table 1 and Table 2) that had p value of <0.25 or p value >0.25 and had a substantial effect were tested with multivariate analysis to determine the risk factors for
controlled or uncontrolled DM, such as variables of age, occupation, periodontal status, IL-1β level, and phagocytic function. The results showed that only variables of age (55 to 60 years old), high IL-1β level, and low phagocytic function had p-values less than 0.05; therefore, multivariate analysis was conducted using those variables to determine the high risk factors for DM.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coef</th>
<th>RRR Rough</th>
<th>RRR Adjusted</th>
<th>p</th>
<th>Coef</th>
<th>RRR Rough</th>
<th>RRR Adjusted</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β level</td>
<td>2.40</td>
<td>16.78</td>
<td>10.97</td>
<td>0.000</td>
<td>2.95</td>
<td>26.00</td>
<td>19.08</td>
<td>0.000</td>
</tr>
<tr>
<td>Phagocyt function</td>
<td>1.37</td>
<td>4.13</td>
<td>3.93</td>
<td>0.013</td>
<td>1.45</td>
<td>4.52</td>
<td>4.25</td>
<td>0.008</td>
</tr>
<tr>
<td>Age</td>
<td>1.75</td>
<td>0.85</td>
<td>5.77</td>
<td>0.002</td>
<td>0.40</td>
<td>2.81</td>
<td>1.49</td>
<td>0.469</td>
</tr>
<tr>
<td>Constant</td>
<td>-2.61</td>
<td>0.000</td>
<td>-2.18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 3 shows high IL-1β level and low phagocytic function are the variables with p<0.05 in the multivariate analysis, indicating a high risk for type 2 DM.

### Discussion

Apparently, in bivariate analysis, the risk of having DM in males is lower than in females. This is possibly because women have certain conditions that are at risk for type 2 DM, such as pregnancy. Gestational diabetes occurs during pregnancy with a prevalence of 2-5% of the total cases of diabetes and the percentage is 2-2.6% of pregnant women.\(^{18,19}\)

This study showed that subjects more than 55 years old were at more risk of having DM than younger subjects. This finding is in accordance with Askandar's study (2002), which showed that the older the patient, the higher the oxidative stress and therefore, the higher the risk of having DM.\(^{20}\)

Determining category by using the intersection according to ROC for plaque index, calculus index, gingival index, pocket index and periodontal index resulted in an index score that was different from the previous study, because the categorization using ROC was determined by considering the sensitivity and specificity of the pure data obtained in the study population of Indonesian (Deutero Malay) subjects. The previous categorization was obtained by consensus, especially from the Western (Caucasian) countries, so that for example for the gingival index, the previous index score was 0.1-1.0 for mild, 1.1-2.0 for moderate, and 2.1-3.0 for severe level.\(^{21,22}\) If the cutoff point of the gingival index 1.0 was used, the ROC results suggested a sensitivity of 44.44% and specificity of 97.78%. However, using the cutoff point of the gingival index of 0.199 in this study resulted in sensitivity of 62.22% and specificity of 62.22%.

In the bivariate analysis using the non-DM group as reference, the results showed that those in high category of indexes were at more risk to have uncontrolled and controlled DM than those of non-DM group. The higher the periodontal index or the lower the periodontal status was, the higher the risk of having DM. In Table 2, the high periodontal index group has 1.88 times the risk of having CDM and 2.47 times the risk of having UCDM compared to non-DM subjects of low periodontal index. The explanation is that in subjects of high periodontal index or subjects of low periodontal status, there is stimulation of pro-inflammatory cytokine (IL-1β, TNFα) production. In vitro studies, these cytokines have been shown to stimulate iNOS (inducible nitric oxide synthase) expression and NOS (nitric oxide synthase) production of the pancreatic β cells, macrophages, and endothelial cells, resulting in dysfunction of the endothelia and β cells so that insulin secretion decreases and blood glucose level increases.\(^{23,24}\)

The subject group with high IL-1β level has 16.78 times the risk of having CDM and 21.71 times the risk of having UCDM compared to non-DM group. This occurred because in periodontal inflammation, the tissue gives an immune response to the periodontal pathogen infection by releasing pro-inflammatory cytokines, such as IL-1β, IL-6 and TNFα. A low concentration of the cytokine has a cytostatic effect on the pancreatic β cells and causes inhibition of insulin synthesis and secretion while a high concentration of cytokine has a cytotoxic effect on pancreatic β cells and causes a fall in insulin secretion. The cytokine also affects the function of tyrosine kinase receptor which is a catalyst for phosphorylation reaction, so that cytokine can affect the cellular response produced by the insulin receptor, such as glucose transport (GLUT4), glycolysis and glycogen synthesis.\(^{7,25}\)

Subjects of low phagocytotic function have
4.13 times the risk of having CDM and 4.53 times the risk of having UCDM compared to the non-DM group. Although PMN cell count increases in periodontal infection, leucotoxin produced by Actinobacillus actinomycetemcomitans and protease produced by Porphyromonas gingivalis will cause chemotactic and phagocytotic defects, and decrease PMN phagocytic function. Low PMN phagocytic function will exacerbate periodontal inflammation resulting in an increase of pro-inflammatory cytokine level which in turn will increase inhibition of insulin secretion and increase of blood glucose level.\textsuperscript{3,26}

Multivariate analysis showed that the statistically significant high risk factors for DM patients were high IL-1B level and low phagocytotic function in which a high IL-1B level and low phagocytic function were found in subjects with low periodontal status.

Conclusion

Low periodontal status will affect the immune response, which in turn results in increasing IL-1B level, decreasing PMN phagocytic function, and increasing risk of type 2 DM.

Recommendation

Especially patients suffering from DM should be asked to maintain their periodontal health by performing plaque and calculus control periodically, to reduce the risk factors affecting type 2 DM, similarly to what patients should do for controlling blood glucose level.

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