Biomedical Engineering’s Recent Progress in Biomaterials, Drugs Development, and Medical Devices
Proceedings of the First International Symposium of Biomedical Engineering (ISBE 2016)

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Yudan Whulanza, Sugeng Supriadi, Muhamad Sahlan, and Basari

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Levels of Interleukin-1β in Gingival Crevicular Fluid in Patients with Coronary Heart Disease and Its Relationship to Periodontal Status

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Abstract. Periodontitis is a risk factor for coronary heart disease (CHD). Both diseases are an inflammatory diseases and have the same potential pathogenic mechanisms. Interleukin-1β as a pro-inflammatory main cytokine, can be found in this both diseases. Gingival crevicular fluid (GCF) derived from the serum of gingival alveolus, affected by inflammatory mechanism and the amount of this fluid will increase in that situation. Objective: To analyze the relationship of Interleukin-1β levels in gingival crevicular fluid (GCF) of CHD and non-CHD patients with periodontal status. Methods: Oral clinical examination (plaque index, bleeding on probing, pocket depth and clinical attachment loss) for 25 subjects with CHD and 35 non CHD were checked, laboratory test to measure the levels of Interleukin-1β was checked with enzyme-linked immunosorbent assay (ELISA). Results: There was no significant differences between interleukin-1β levels in CHD and non-CHD patients (p<0.05); there was no significant difference between the level of interleukin-1β with periodontal status in CHD and control (non CHD) patients (p>0.05). Conclusions: levels of Interleukin-1β in CHD patients do not have a relationships with plaque index, pocket depth and clinical attachment loss, but has a relationships with bleeding on probing.

Keywords: interleukin-1β, coronary artery disease, periodontal status, gingival crevicular fluid

INTRODUCTION

Cardiovascular disease is the second highest disease after the infectious disease that causes mortality in worldwide. One of cardiovascular disease is coronary heart disease (CHD), a major cause of premature death among men in industrialized countries, and its pathological basis is atherosclerosis [1]. Coronary heart disease (CHD) is a disease caused by narrowing of the coronary arteries as a result of atherosclerosis, spasm or a combination of both [2]. Early in the formation of atherosclerotic plaques, circulating monocytes adhere to the vascular endothelium. This adherence is mediated through several adhesion molecules on the endothelial cell surface, including intercellular adhesion molecule-1 (ICAM-1), endothelial leukocyte adhesion molecule-1 (ELAM-1), and vascular cell adhesion molecule-1 (VCAM-1). These adhesion molecules are upregulated by a number of factors, including bacterial LPS, proinflammatory cytokines, such as IL-1, tumor necrosis factor alpha (TNF-α) and prostaglandin E2 (PGE2). These cytokines then propagate the atherosclerotic lesion [3].

Chronic periodontitis is an infectious disease resulting in inflammation within the supporting tissues of the teeth leading to progressive attachment and bone loss. It is also characterized by pocket formation, bleeding on probing.
and gingival recession [4]. Periodontitis may affect the onset or progression of atherosclerosis and CHD through both direct and indirect pathways [3]. There is growing evidence that poor oral health increases the risk of coronary heart disease [1]. Recent observational studies showed a mild but significant increased risk of cardiovascular disease among people with periodontitis [11, 5]. Periodontitis and atherosclerosis have common etiological factors, and have the same potential pathogenic mechanisms. Therefore, it is important to know the relationship between periodontal disease and atherosclerosis. Many studies showed that periodontal disease is not only caused by plaque bacteria, but host response against bacterial plaque also has a considerable effect on periodontal tissue destruction [6].

Gingival crevicular fluid (GCF) is an altered inflammatory exudate that flows into the oral cavity from gingival crevice. GCF flow increases during inflammation and thus may be considered as a way of throwing out the toxin and bacteria from gingival sulcus. It also contains inflammatory immune cells that contribute to local immune regulation through many mediators, including prostaglandin, leukotrienes, IL-1, IL-6, TNF and IL-8 and hormonal antigenic activity [7]. Interleukin-1 (IL-1) is a key mediator in the process of immune response by acting as an inducer of prostaglandin synthesis, and affects the proliferation of fibroblasts and keratinocytes. Dietrich et al. in 2013 suggests that the incidence of cardiovascular disease is higher in patients with poor periodontal status [8]. Interleukin-1α as a mediator of inflammatory immune system has been linked to coronary heart disease and periodontal disease. Many studies show the relationship between CHD and periodontal status. Some research give conflicting results [9]-[12]. Those studies also do not give the explanation about levels of interleukin-1α in GCF can show the relationship between CHD and periodontitis, because there were much factor can caused and influenced both diseases. This study purposes are to compare the levels of Interleukin-1α in GCF in CHD and non CHD patients, and to find the relationship between Interleukin-1α in GCF with periodontal status of the CHD and non CHD patients.

The study consists of male and female patients, 40 - 70 years old. The test group were CHD patients with diagnosed stable angina and will undergo coronary artery bypass graft (CABG surgery) at Harapan Kita Cardiovascular Hospital. The control group were the patients at clinic of Periodontology, Dental Hospital of Faculty of Dentistry, Universitas Indonesia without CHD and confirmed with negative treadmill test and normal EKG by the cardiovascular doctor at Prodia Clinic. Exclusion criteria of this study were edentulous patients, pregnant and another systemic diseases.

This study has been conducted between November 2015 until February 2016. Laboratory test for levels of Interleukin-1α performed in the biomolecular laboratory Diagnostik Cancer Hospital. This study has ethical clearance from ethical committee of Faculty of Dentistry Universitas Indonesia, Number: 103/CN2.F2.KEPKG/OTL 06.01 Surat Peangan/2015 on August 3rd 2015, and also from ethical committee of Harapan Kita, Cardiovascular Hospital Number: 2015.B.02.01/VI/003/KEP.015/2015 on October 6th 2016.

MATERIALS AND METHODS

This study is a clinical and laboratory observational study, using the gingival crevicular fluid from CHD and non-CHD patients. Patients from both groups were interviewed about their medical status and common habits. Data about antibiotics that are being consumed, habit of cleaning the oral cavity and smoking status were recorded.

Measurement of periodontal status CHD and non CHD patients were clinically examined after the patients signed their informed consent to participate in the research. All teeth excluding third molar were studied and clinical data were recorded regarding: plaque index (Silness and Löe), bleeding on probing index (Saxer and Muñizan), probing depth (distance between the gingival margin and base of the pocket) in millimeter, and clinical attachment loss (distance between the cemento-enamel junction and base of the pocket) in millimeter. Probing depth were checked at 6 (six) sites per tooth (mesial buccal, distal buccal, mesial lingual, distal lingual, and distolingual).

The GCF were taken with three pieces of paper point, then inserted into the sulcus as deep as 1 mm for 30 minutes on the mesial or distal side of one posterior teeth with pockets 4-6 mm. Paper point was then inserted into the appendage tube containing 250 mL of phosphate buffer saline (PBS) and stored at -80 °C until laboratory tests would be conducted. GCF extract was obtained from centrifugation, then its concentration was calculated with nine standard of concentration of 2000 pg/mL, 1500 pg/mL, 1000 pg/mL, 750 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 25 pg/mL, using the Bradford method. Level of Interleukin-1α was measured by an enzyme-linked immunosorbent assay (ELISA) using commercially available Kombiotech for human IL-1α (South Korea) according to
manufacturer's instructions. The absorbance of each well was read in a microplate spectrophotometer at 450 nanometers, and GCF concentration of IL-1β was calculated from the standard curve included with each assessment kit. Data was analyzed by Shapiro-Wilk test to determine normal distribution of data. According to the distribution, data was summarized by mean values and standard deviations. The groups were compared using t-test independent for the parameters with normal distribution and Mann-Whitney test for the parameters that were not normal. The correlation between clinical parameters and level of IL-1β in CHD and non CHD patients were analyzed using Spearman's correlation test. Statistical significant was defined at p<0.05.

RESULTS AND DISCUSSION

Table 1 shows the descriptive statistical results of clinical parameter, and found that the mean of plaque index of control group was higher (1.44 (0.60)) than CHD group (1.38 (0.65)). Also the mean values of bleeding on probing, pocket depth and level IL-1β of control group is higher than CHD group, but the clinical attachment loss of control group (5.54 (1.01) mm) is lower than CHD group (5.9) (1.54 pg/ml).

<table>
<thead>
<tr>
<th>Variable</th>
<th>CHD (n=35)</th>
<th>Non-CHD (n=35)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Min – Max</td>
</tr>
<tr>
<td>Plaque index</td>
<td>1.38 (0.65)</td>
<td>0.3–3</td>
</tr>
<tr>
<td>Bleeding on probing</td>
<td>0.80 (0.62)</td>
<td>0.2–3.8</td>
</tr>
<tr>
<td>Pocket depth (mm)</td>
<td>4.74 (0.78)</td>
<td>4–6</td>
</tr>
<tr>
<td>Clinical attachment loss (mm)</td>
<td>5.91 (1.54)</td>
<td>4–11</td>
</tr>
<tr>
<td>Level of Interleukin-1β (pg/ml)</td>
<td>2.92 (4.51)</td>
<td>0.11–21.34</td>
</tr>
</tbody>
</table>

The results of non-parametric Mann-Whitney test in table 2 showed that there was no significant difference between the levels of Interleukin-1β CHD and non-CHD patients (p = 0.312; p > 0.05). Also the result for the clinical parameters; there was no significant difference between the bleeding on probing CHD patients and non CHD (p=0.09; p>0.05). Pocket depth in CHD patients also did not have a relationship with non CHD patients (p>0.05; p<0.05). Moreover there was no significant difference between the clinical attachment loss of CHD and non-CHD patients (p = 0.493; p>0.05).

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Median (Min – Max)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level of Interleukin-1β (pg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHD</td>
<td>15</td>
<td>1.83 (0.11-21.34)</td>
<td>0.312</td>
</tr>
<tr>
<td>Non CHD</td>
<td>15</td>
<td>0.89 (0.18-29.67)</td>
<td></td>
</tr>
<tr>
<td>Bleeding on Probing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHD</td>
<td>35</td>
<td>0.58 (0-2.38)</td>
<td>0.09</td>
</tr>
<tr>
<td>Non CHD</td>
<td>35</td>
<td>1.04 (0-3.14)</td>
<td></td>
</tr>
<tr>
<td>Pocket Depth (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHD</td>
<td>15</td>
<td>5 (4-6)</td>
<td>0.06</td>
</tr>
<tr>
<td>Non CHD</td>
<td>15</td>
<td>5 (4-6)</td>
<td></td>
</tr>
<tr>
<td>Clinical Attachment Loss (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHD</td>
<td>35</td>
<td>6 (4-11)</td>
<td>0.493</td>
</tr>
<tr>
<td>Non CHD</td>
<td>35</td>
<td>6 (4-8)</td>
<td></td>
</tr>
</tbody>
</table>
Comparative analysis using T-Test independent for plaque index between CHD and non CHD patients showed that there was no significant difference between plaque index CHD with non-CHD patients (p=0.676; p<0.05). We can show it on table 3.

TABLE 3. Comparative Analysis of plaque index in CHD and Non CHD Patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Mean (SD)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plaque index</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHD</td>
<td>35</td>
<td>1.38 (0.65)</td>
<td>0.676</td>
</tr>
<tr>
<td>Non-CHD</td>
<td>35</td>
<td>1.44 (0.60)</td>
<td></td>
</tr>
</tbody>
</table>

Spearman test for analyzing the correlation between the levels of IL-1β with periodontal status in CHD patients showed in table 4. The results show that there are no significant correlation (p>0.05) between levels of IL-1β and the periodontal status, consist of plaque index, pocket depth and clinical attachment loss. There was a significant correlation between levels of IL-1β and bleeding on probing in CHD patients.

TABLE 4. Correlation between Levels of Interleukin-1β with Periodontal status (plaque index, bleeding on probing, pocket depth and clinical attachment loss) in CHD patients

<table>
<thead>
<tr>
<th>CHD (n=35)</th>
<th>Plaque Index</th>
<th>Bleeding on Probing</th>
<th>Pocket Depth</th>
<th>Clinical Attachment Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β (pg/ml)</td>
<td>0.171</td>
<td>0.371</td>
<td>0.301</td>
<td>0.281</td>
</tr>
</tbody>
</table>

Spearman test; p < 0.05 significant correlation

The correlation between the levels of IL-1β with periodontal status in non CHD patients is shown in table 5. The results show that there are no significant correlation (p>0.05) between levels of IL-1β and the periodontal status (plaque index, bleeding on probing, pocket depth and clinical attachment loss) in non CHD patients.

TABLE 5. Correlation between levels of Interleukin-1β with Periodontal status (plaque index, bleeding on probing, pocket depth and clinical attachment loss) in non CHD patients

<table>
<thead>
<tr>
<th>Non CHD (n=35)</th>
<th>Plaque Index</th>
<th>Bleeding on Probing</th>
<th>Pocket Depth</th>
<th>Clinical Attachment Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β (pg/ml)</td>
<td>0.010</td>
<td>0.091</td>
<td>0.336</td>
<td>0.093</td>
</tr>
</tbody>
</table>

Spearman test; p < 0.05 significant correlation

Interleukin-1β (IL-1β) is a major pro-inflammatory cytokine, produced by monocytes, macrophages and dendritic cells. Interleukin-1β is a multifunctional cytokine that responds to infection, stress conditions, tissue injury and other pathogenicity (11). Increased levels of IL-1β is associated with inflammatory-related disorders such as heart disease and periodontal disease were examined in this study. Subjects in this study had an age range of 40-70 years with a mean age of subjects of CAD 60.37 (6.93) years and 52.63 (6.60) years for control subjects or non-CHD. This shows that the mean age of the control subjects is younger than CHD subjects. The highest levels of IL-1β is 21.34 pg/ml for the subjects of CHD and 29.67 pg/ml for the control subjects or non-CHD. The mean (SD) level of IL-1β in control subjects was also higher than CHD subjects, namely 3.52 (6.50) pg/ml for control subjects and 2.92 (6.51) pg/ml for CHD subjects. Mann-Whitney test results show lower IL-1β levels in CHD patients than non CHD. These results are consistent with research Shabir et al., which suggests that there is a negative correlation between age and levels of IL-1β. Levels of IL-1β subject of CHD was significantly lower than the control group. Levels of IL-1β affected by cholesterol-lowering drugs, age and ethnicity. Age is a secondary factor that determines the levels of IL-1β. Statin treatment is shown to inhibit the expression of IL-1β at both mRNA and protein levels. Atherosclerosis in patients with CHD who routinely receive statin therapy proved that statins lower levels of IL-1β through biochemical mechanisms of change luid [14]. Periodontal disease contribute to the development of cardiovascular disease. Porphyromonas gingivalis is Gram-negative bacteria which causes periodontitis will release products such as lipopolysaccharide derived from cell walls
thereby triggering the host response to produce and release pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α. The role of bacteria in the formation of atherosclerotic plaques proves that periodontitis cause a buildup of fat in the heart. Chronic periodontitis alters biochemical profile as well as the number of leukocytes (20% higher) [15]. Interleukin-1β is a pro-inflammatory cytokine in an immunological response of the body against the two diseases, periodontal disease and cardiovascular disease. Levels of IL-1β is influenced by many factors so that this cytokine relationship with multifactorial diseases such as periodontal disease and cardiovascular disease in general and in particular coronary heart disease remains to be investigated further.

CONCLUSION

Levels of IL-1β in the GCF patients CHD lower than non-CHD. There is no correlation between the levels of IL-1β in GCF and accumulation of plaque, accumulation of calculus, oral hygiene, pocket depth, clinical attachment loss in patients with CHD and non CHD. There is correlation between the levels of IL-1β and bleeding on probing in patients with CHD, but not in patients non CHD.

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