The Effect of the Application of HA-TCP Bone Graft towards Gingival Crevicular Fluid Osteocalsin in the Treatment of Periodontal Infrabony Defects

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Abstract

HA-TCP bone graft is a material used to stimulate alveolar growth. During the process of bone formation, several biomarkers are identified in the gingival sulcus fluid, such as Osteocalcin.

This research aims to analyze the effect of regenerative periodontal surgery using HA-TCP bone graft towards the levels of osteocalcin of gingival crevicular fluid in the treatment of infrabony defects.

A total of 32 respondents, consisting of 12 men and 19 women, were divided into test (HA-TCP Bone graft and membrane) and control group (membrane). The research adopted a randomized controlled trial method with a single blind-design and the gingival crevicular fluid was collected on day 0 (H0), 14 (H14), and 21 (H21). Meanwhile, the measurement of osteocalsin was examined with the ELISA method, and data were analyzed statistically using t-test ($p < 0.05$).

The result showed the mean osteocalcin levels in the test group on day 0 (0.33 µg / ml), 14 (1.34 µg / ml), and 21 (3.42 µg / ml) while the control group was 0.26, 0.56, and 145 µg / ml, respectively. Furthermore, the comparison of the mean osteocalcin levels in the two groups had a significant difference (p-value <0.000), with the application of HA-TCP Bone Graft showed higher osteocalsin levels.

The use of HA-TCP bone graft in regenerative periodontal surgery increases osteocalcin levels of gingival crevicular fluid in the treatment of infrabony defects of periodontal disease.

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Introduction

Bone grafts are widely used to aid regeneration and also to create an osteoinductive and/or osteogenic environment.[1] An ideal bone graft has low toxicity and morbidity level, available in large quantities, and at a relatively low price.[2] Currently, the materials of alloplast bone graft is widely used because of its advantages compared to others. It has no risk of transmitting infectious diseases from donors, no additional surgical procedures, and restrictions due to religious reasons, such as ingredients containing pork elements.[3]

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Furthermore, hydroxyapatite is used as an alloplast material, which has the molecular formula $[CA_{10}(PO_4)_6(OH)_2]$ and included in a calcium phosphate derivative.[4] Meanwhile, tricalcium phosphate is made up of tertiary calcium, which is also known as bone ash $[Ca_3(PO_4)_2]$.[5] This material is often used as a combination of hydroxyapatite, since it is very biocompatible and has similar properties to hydroxy apatite, however, it is absorbed by the body faster due to its smaller size and low crystallization.[6] It is widely used because of its excellent biocompatibility with hard materials, reconstructs damaged bone tissue, has a low degradation rate, high osteoconduction, non-toxic, and does not results in inflammation[7,8].

Osteocalcin (OC) is a proteinous non-collagen calcium ion binder, which is also known as γ -carboxy -glutamic acid protein (bone-Gla-protein)[9,10]. It is produced by both osteoblasts and odontoblasts and is measured by

immunoassay.[10] Several investigations into osteocalcin levels in the gingival crevicular fluid (GCF) of patients with periodontitis were conducted and the result showed that the degree of osteocalcin in GCF may reflect inflammation. Also, there is recent interest in osteocalcin as a potential marker of bone turnover in periodontal disease.[11,12] However, its role in periodontal disease progression and the outcome of periodontal treatment is unclear.[12]

In previous studies, bone turnover markers were extensively examined in the GCF samples of patients of various periodontal diseases and associated with its progression. Several investigations provide contradictory evidence regarding the role of osteocalcin in the GCF of patients with periodontitis.[11,13] However, no studies were revealed on the level of osteocalsin after the HA-TCP Bone graft application. Therefore, this research aims to measure the osteocalsin level of gingival crevicular fluid (GCF) after flap surgery with the application of HA-TCP bone graft in the treatment of the infrabony defects of periodontal disease.

Materials and methods

This study was approved by the Research Ethics Committee (No. 1168 / UN6.KEP / EC / 2019), and the subjects were given an explanation regarding the procedure and were further requested to sign an informed consent. However, some respondents were not willing, then there was no coercion and their rights were respected not to be included in this study. Furthermore, a randomized control trial method was adopted and the test group comprises respondents that were given a regenerative periodontal surgical treatment of HA-TCP bone graft application (BPPT/ Agency for the Assessment and Application of Technology, Indonesia) with pericardium membrane (BATAN, Indonesia). Meanwhile, the control group comprises of the subjects that were given regenerative periodontal surgery with pericardium membrane only.

The criteria for research subjects include:

1) Inclusion Criteria
   (1) Patients with a periodontal pocket of ≥ 6 mm and also had vertical bone defects involving at least 1 tooth in one jaw
   (2) Patients indicated for regenerative periodontal surgical therapy using bone graft
   (3) Male or female patients with age ≥ 30 years
   (4) No carious lesions
   (5) Patients who have gone through the initial phase with a plaque score of ≤ 10%

2) Exclusion Criteria
   (1) Smoke
   (2) Using orthodontic appliances
   (3) Taking antibiotics, anti-inflammatory, and immunosuppressant for the last 6 months (use of Host Modulation Therapy)
   (4) Pregnant and breastfeeding women
   (5) Have uncontrolled systemic disease (hypertension, diabetes, osteoporosis)

The Collection of GCF samples

The sampling of gingival crevicular fluid, with the isolated dental procedures was carried out using a cotton roll and dried. Subsequently, periopaper strips (Oraflow Inc., Plainview, NY, USA) were inserted into the gingival sulcus under light pressure for 30 seconds and it was then placed into a 1.5 ml Eppendorf tube containing 0.5 ml phosphate buffer saline (PBS). Furthermore, the levels of osteocalsin were measured using the USCN ELISA Kit (Cloude-Cloune) from the USA.

Surgical procedure

Sides or spots were randomly selected to determine the experimental or control site. Furthermore, after adequate local anesthesia, a crevicular incision was made in the gum margin of the involved tooth with a full-thickness flap cut, which was then reflected and debrided to remove the granulation tissue. This is prior to the incision and it was then followed by root planing and irrigation with normal saline. The bone defect in the test group was filled with HA-TCP bone-graft to cover the coronal crest or remaining wall, and then coated with the pericardial membrane. Meanwhile, in the control group, it was only closed with the pericardium membrane. Afterward, the surgical site was then sutured with 4-0 silk thread and a periodontal dressing was applied (Coe-Pack, GCAmerica, Inc., Chicago, IL). Furthermore, all study subjects were prescribed with Diclofenac analgesic sodium 50 mg and Amoxicillin 500 mg, twice and three times daily, respectively, for five days. One week after surgery, the periodontal dressing and sutures were removed, and the surgical area was irrigated with normal saline. The healing was

quite good, and there were no complaints or allergic reactions from all subjects. After a month, the patients were asked to return for the collection of gingival crevicular fluid collection as well as to measure the level of osteocalsin, and at each visit, oral hygiene instructions were given.

Statistical analysis

The data normality test was performed using Saphiro Wilk, before the statistical experiment was carried out. Furthermore, the levels of Osteocalcin in gingival crevicular fluid in both groups were analysed using the statistical t-test, and are considered significant when the p-value <0.05.

Results

The research subjects comprise a total of 32 participants, however, 29 were fully involved in the research, and 3 dropped out because they were unable to follow the research until the final stage. Furthermore, the characteristics of the subjects ranged from 30-59 years old with the majority in the age range of 50-59 years. Also, most of the respondents were female with a percentage of 58.62%. as shown in Table 1 and the average levels of Osteocalcin in both groups are shown in table 2.

Characteristics	Test Group (with bone graft)		Control Group (without bone graft)		Total	
	(n)	(%)	(n)	(%)	(n)	(%)
Gender						
Male	9	56.25	3	23.08	12	41.38
Female	7	43.75	10	76.92	17	58.62
Total	16	100.00	13	100.00	29	100.00
Age (year)						
30-39	6	37.50	3	23.08	9	31.03
40-49	4	25.00	4	30.77	8	27.59
50-59	6	37.50	6	46.15	12	41.38
Total	16	100.00	13	100.00	29	100.00

Table 1. Patient Characteristics.

Groups	Conditions	Mean ± SD
Test	H_0	0.33 ± 0.24
	H_{14}	1.34 ± 0.22
	H_{21}	3.42 ± 0.68
Control	H_0	0.26 ± 0.16
	H_{14}	0.56 ± 0.26
	H_{21}	1.45 ± 0.86

Table 2. The mean Osteocalcin levels in the Test and Control Groups.

The result showed that the respondent in the test group increase in mean Osteocalcin levels after the periodontal regenerative surgical treatment. The increase on day 0 to 14 was 1.01 ± 0.21, 14 to 21 was 2.08 ± 0.75, and 0 to 21 was 3.09 ± 0.66. Similarly, the control group increased after periodontal regenerative surgical treatment, these include 0.29 ± 0.18 on day 0 to 14, 0.90 ± 0.84 on 14 to 21, and by 1.19 ± 0.88 on 0 to 21 (table 3). The statistical test results showed there were significant differences in osteocalsin levels before and after treatment in both groups. However, osteocalsin levels in the test group had a higher increase than the control. Furthermore, there is a significant difference in the level of osteocalcin in the test group compared to the other, both on day 14 and day 21 (Table 4).

Groups	Conditions	Δ Mean ± SD	T Count	P Value
Test	$\Delta H_0 - H_{14}$	1.01 ± 0.21	19.46	0.000*
	$\Delta H_{14} - H_{21}$	2.08 ± 0.75	18.63	0.000*
	$\Delta H_0 - H_{21}$	3.09 ± 0.66	11.07	0.000*
Control	$\Delta H_0 - H_{14}$	0.29 ± 0.18	5.77	0.000*
	$\Delta H_{14} - H_{21}$	0.90 ± 0.84	4.88	0.000*
	$\Delta H_0 - H_{21}$	1.19 ± 0.88	3.86	0.001*

Description: *) Significant (p-value <0.05), paired t test

Table 3. Difference in mean osteocalcin levels (µg / mL) before and after regenerative periodontal surgery treatment in the test and the control groups.

	Difference in the Mean *Osteocalcin* Levels		P - Value
	Control group	Test group	
$H_0 - H_{14}$	0.26 ± 0.16	1.01 ± 0.21	0.000*
$H_0 - H_{21}$	0.56 ± 0.26	3.09 ± 0.66	0.000*
$H_{14} - H_{21}$	1.45 ± 0.86	2.08 ± 0.75	0.002*

Description: *) Significant (p-value <0.05), one tailed t-test

Table 4. The Difference of Mean Osteocalcin Levels After Treatment Day 0 (H_0), Day 14 (H_{14}), and Day 21 (H_{21}) in the Control and the Test Groups.

Discussion

The results of this study that GCF osteocalsin levels were higher in the group with HA-TCP bone graft application compared to the other. Several studies have shown the clinical success of HA-TCP bone grafts with bone

formation. Bayani, et al. (2017) reported that the regenerative periodontal surgical treatment for infrabony defects using bone graft with hydroxyapatite (HA) content had a higher success rate for stimulating bone formation.[14] Singh, et al (2012) further stated that the treatment of infrabony defects using a combination of HA and membrane bone grafts provides more clinically and radiologically significant results when compared to others with only pocket elimination surgery.[15] Furthermore, Lee et al. (2013) stated that the use of bone grafts with a combination of HA (60%) and TCP (40%) components on defects indicates the occurrence of bone formation and is considered more effective than that of Bio-oss.[16] Meanwhile, the hydroxyapatite content in bone graft material has similar physical and chemical structures to human bone tissue minerals and has a high-affinity level. Therefore, it binds to bone and stimulates osteoblasts in the early stages of repairing periodontal tissue defects.[17]

Additionally, bone metabolism is a dynamic process that involves its formation and resorption. Osteoblasts and osreoclasts play a role in bone apposition and resorption, respectively, this constant reconstruction of tissue is known as remodeling.[17] There are various biochemical markers used during the bone metabolism process, in which the enzymes and proteins are released into the circulation during osteoblast and osteoclastic activity reflecting existing changes. Afterward, the changes in the concentration of the metabolic is used to assess bone remodeling.[18,19]

This study measured the level of osteocalsin as an indicator of bone turnover. Meanwhile, osteocalcin is a biomarker produced by osteoblasts during the bone formation process and a type of non-collagen protein commonly found in the human bones.[17] Its distribution is closely related to the value of osteocalcin, in which an increase is associated with improved formation processes and turnover, whereas a decrease is associated with reduced activity of cortical bone remodeling. Therefore, osteocalcin is considered as an important and sensitive marker of bone turnover, as the body stops producing osteoclasts and replaces them with osteoblasts for bone formation.[20,21]

A previous studies conducted by Bullon et al. (2007) on salivary osteocalsin, serum, and GCF levels after periodontal treatment) showed that low levels of osteocalsin were associated with decreased probing depth and Clinical Attachment Loss (CAL). Consequently, 11 Osteocalcin periodontitis were suggested to be a marker of bone formation in cases where resorption is greater than formation.[12] However, there is still controversy about GCF osteocalcin levels in periodontal disease. Also, Kunimatsu et al. found no osteocalcin in the GCF of patients with gingivitis, whereas in periodontitis, it was positively correlated with clinical parameters.[22] Additionally, Wilson et al. detect no osteocalcin in the GCF of untreated periodontitis patients.[11] However, Nakashima et al. stated that its total number in GCF related to periodontitis was higher than in healthy and gingivitis sites.[23]

Furthermore, this research found no publications regarding the analysis of osteocalsin levels in gingival sulcus fluid after bone graft application treatment. The high GCF osteocalsin level after surgical treatment with HA-TCP bone graft is probably influenced by the material which induces better formation. In some experimental animal models, it is possible to verify that serum osateocalcin levels are a determinant of bone formation and have been shown "in vitro" to have a role in resorption.[24,25] However, it should be noted that osteocalcin is produced by osteoblasts and only a small proportion is secreted directly in circulation.[26]

Additionally, osteocalsin levels on days 14 and 21 after surgery showed an increase in both the test and control groups. A higher increase occurred on days 14 to 21 after surgery, which is probably due to osteoblast cells starting to be present in the bone remodeling process, therefore, the osteocalsin level in GCF is higher. Also, the use of HA-TCP bone graft showed a higher GCF osteocalsin level than the side without bone graft application. It is, therefore assumed that HA-TCP bone graft increases osteoblast cell count and causes better bone formation.

This research uses biological parameters, such as the levels of osteocalsin in gingival sulcus fluid. Furthermore, there are limited studies that assess the treatment success through the analysis of the levels of osteocalsin. Further research with positive control, namely the comparison between the HA-TCP bone graft from BPPT with other types, which have the same and different material components is required to determine the comparison, and

longitudinal studies with longer evaluation times and need to assess the clinical and radiological success of treatment.

Conclusions

Conclusively, the application of HA-TCP bone graft in regenerative periodontal surgery increases the levels of osteocalcin in gingival crevicular fluid.

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Declaration of Interest

The authors report no conflict of interest.

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