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RESEARCH ARTICLE

Effectiveness of MIST with hydroxyapatite and β-tricalcium phosphate in alveolar bone density and osteocalcin level improvement during treatment of infrabony pockets

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ABSTRACT

The minimally invasive surgical technique (MIST) is a method for periodontal regenerative treatment by minimizing trauma so it can preserve vascular supply of the interdental papillae. This study aims to determine the effectiveness of the MIST method with the addition of a combination of hydroxyapatite and β-tricalcium phosphate (HA + β-TCP) in treatment of infrabony pockets in terms of alveolar bone density and osteocalcin levels of gingival crevicular fluid. The study sample was taken from 20 teeth with infrabony pockets (n = 20), which were divided into two groups: 10 teeth treated with MIST and the other group with open flap debridement (OFD). Both groups received combination of HA + β-TCP. The osteocalcin levels of gingival crevicular fluid were checked on day-0 prior to the flap surgery, day-7 and day-14 after flap surgery using the Human Osteocalcin Elisa Kit. Radiological evaluation of alveolar bone density at day-0 and day-90 was done using cone beam computed tomography. Data of osteocalcin levels were analyzed using two-way ANOVA and continued with LSD Post Hoc test, while data of alveolar bone density reduction were analyzed using the Independent t-test parametric test. The results showed that there was no significant difference ($p > 0.05$) in the values of alveolar bone density between the MIST and OFD groups on day-0 and day-90, while the osteocalcin levels in both groups showed an increase from day-0 to day-7 and a decrease from day-7 to day-14. There was a significant difference ($p < 0.05$) on day-0 and 7 and day-7 and 14 between MIST and OFD groups. The MIST method with HA + β-TCP was effective and further increases alveolar bone density and osteocalcin levels of gingival crevicular fluid.

Keywords: alveolar bone density; infrabony pocket; MIST; osteocalcin

INTRODUCTION

Periodontitis is an irreversible chronic inflammatory disease with structural loss of tooth supporting tissue, such as gingiva, periodontal ligament, and alveolar bone.[1] Periodontitis accompanied by alveolar bone loss requires treatment to stimulate periodontal tissue regeneration using bone graft material.[2] The function of bone graft includes osteoconduction, osteoinduction and osteogenesis to induce bone formation and regenerate periodontal tissue through a new attachment process.[3] Based on the origin, there are four types of bone graft, namely autograft, allograft, xenograft and alloplastic.[4] One of the alloplastic material that can be used are hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP).[5] The healing process of bone loss occurs one month after bone grafting and after three months, it will be clearly visible on the radiograph.[6]

Periodontal surgery can be conducted to achieve a good access to the root surface so that debridement can be performed.[7] Currently, there are alternative methods to access bone damage with infrabony pockets using an invasive approach known as the minimally invasive surgical technique (MIST).[8] The MIST method is carried out by minimizing the trauma to the soft tissue due to the vascular supply of the interdental papillae, which is maintained to remain intact and remove the granulation tissue from periodontal damage with a smaller incision than using an open flap procedure.[9] The MIST method aims to maintain the tissue in order to achieve primary healing and provide space for the formation and

maturation of the blood clots needed during the healing process.[10]

Improvement in diagnosis establishment of periodontal disease and treatment continues to progress towards methods that can be assessed and identified through measurement using biomarkers.[11] One of the biomarkers that can be used is osteocalcin. Osteocalcin is a non-collagen bone matrix protein that is synthesized by osteoblasts, and thus it can demonstrate activity of osteoblast.[12] This study aims to examine the effectiveness of the minimally invasive surgical technique and open flap method with the addition of a combination of hydroxyapatite and β-tricalcium phosphate on osteocalcin levels of gingival crevicular fluid and alveolar bone density in pocket treatment.

MATERIALS AND METHODS

This study was designed as a quasi-experimental study. The subjects of this study were patients with periodontitis who came to the Clinic of Periodontia, RSGM Prof. Soedomo, Faculty of Dentistry, Universitas Gadjah Mada. Ethical approval has been obtained from the Faculty of Dentistry Ethics Commission, Universitas Gadjah Mada, Yogyakarta No. 00289 / KKEP / FKG-UGM / EC / 2019.

Patients who met the following criteria were included: 1) periodontitis patients with infrabony pockets of 5-8 mm deep, 2) patient aged 35 - 55 years, 3) patients having no history of systemic disease, 4) Patients having O'Leary's plaque index of ≤ 10%. Patients who were not cooperative and unwilling to take part in the study, consumed drugs and patients who were pregnant or undergoing estrogen therapy did not meet the inclusion criteria for study subjects. The research samples were determined using the following formula to compare between the two groups using quantitative data:[13]

$$\text{Sample size} = n_1 = n_2 = \left[\frac{\left(Z_\alpha + Z_\beta\right) \text{x } S}{\left(x_1 - x_2\right)} \right] 2$$

$$n_1 = n_2 = \left[\frac{\left(1.96 + 1.28\right) \text{x } 0.316}{\left(0.33\right)} \right] 2$$

$$n_1 = n_2 = 9.61 \left(\approx 10\right)$$

Explanation :

$n_1 = n_2$= number of samples per group

Z_α = alpha standard derivative (a = 0.05) $\rightarrow$ 1.96

Z_β = beta standard derivative $\rightarrow$ 1.28

S= standard deviation of the difference in values between groups

x_1-x_2= minimum difference between the mean which is considered significant

The sample size was rounded to 10 samples of teeth with infrabony pockets in each group. The samples were divided into two groups, namely MIST with the addition of a combination of HA + β-TCP group and open flap debridement method with the addition of bone graft combinations of HA + β-TCP group.

Osteocalcin Sampling, gingival crevicular fluid was collected before MIST and open flap debridement procedures with the addition of bone graft using sterile No. 30 paper point. On the mesial and distal aspect of each tooth with an infrabony pocket, a sterile No. 30 paper point was inserted for 60 seconds with a depth of 2 mm in the gingival sulcus, before the paper point was moved into the eppendorf tube which contained 500 µl of phosphate buffer solution (PBS). The eppendorf tube was centrifuged with 2000 xg for 5 minutes, closed, and given a tape, then stored at -80 °C. Osteocalcin levels were analyzed using the Human OT/BGP Enzyme Linked Immunosorbent Assay (ELISA) Kit CUSABIO E9047Hu 96 test technique. Osteocalcin analysis of gingival sulcus fluid were performed on day-0, 7 and 14. The cone beam computed tomography (CBCT) radiographic examination was conducted to record the alveolar bone density as the data baseline that would be measured using *OnDemand* software. Radiographic data were collected on day-0 and 90.

MIST, disinfection of the gingival and teeth area that would be treated was carried out and then the surgical area was locally anesthetized with 2.5% Pehacaine infiltration. The width of the interdental papilla was measured to determine the papilla preservation technique using a simplified papilla preservation flap (SPPF). The working end of probe was positioned perpendicular to the tooth

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axis in the interdental area to be dissected. Incision was carried out with a microblade at a height of 1 mm in the coronal of alveolar crest until 1 tooth on mesial and distal from the infrabony defect. The blade position was 45° in coronal direction. Sulcular incision was performed as minimum as possible in the involved tooth area with limited mesio-distal extension, followed by the making of a full thickness mucoperiosteal flap and elevation of the flap with a micro periosteal elevator (Figure 1). The interdental papillae was elevated on the flap lingual. The granulation tissue was removed and the root surface of the tooth was smoothed with a mini curette then irrigated with saline, while the root surface was conditioned using EDTA gel for 2 minutes and rinsed with saline solution. HA + β-TCP (*Osteon™ 3 Collagen*, Dentium) combination bone graft material was hydrated using saline solution for 3 minutes and applied to the area of infrabony defect up to the alveolar crest border. The flaps on the buccal, lingual, and interdental papillae were repositioned and they were sutured using internal mattress suture and interrupted sutures and covered with periodontal dressings.

The gingiva and teeth of the open flap debridement (OFD) that would be treated were disinfected and the surgical area was locally anesthetized with 2.5% Pehacaine infiltration. Afterwards, the area was incised with a #15 blade followed by the making of a full thickness mucoperiosteal flap with a triangular incision. Granulation tissue was removed and smoothed with Gracey curette, irrigated with saline, then the root surface was conditioned using EDTA gel for 2 minutes before subsequently rinsed using saline solution. HA + β-TCP (*Osteon™ 3 Collagen*, Dentium) combination bone graft material was hydrated using saline solution for 3 minutes and applied to the area of infrabony defect up to the alveolar crest border. The flap was repositioned with a coronally advanced flap followed by suturing, then covered with a periodontal dressing.

Post-operative patients were instructed not to brush their teeth on the treated area for the first 1 month. Cleaning was done by gargling and wiping it with a gauze moistened with mouthwash at least 2 times a day. Oral hygiene and wound healing control were carried out once a week for the first 1 month. The patients were prescribed with antibiotic of 500 mg amoxicillin for every 8 hours for 5 days and analgesics. All samples were controlled on day-7 for the periodontal dressing removal and gingival crevicular fluid collection. Until the third month, plaque control was carried out once a month.

The data were analyzed using IBM SPSS version 22.0 software. The normality test of data was carried out using the Saphiro-Wilk test and the data homogeneity test was conducted by means of the levene test. The analysis of data reduction between alveolar bone density groups was performed using the Independent t-test parametric test if the data were homogeneous. The analysis for osteocalcin levels was carried out using two-way ANOVA with repeated measures.

RESULTS

The alveolar bone density (Figure 2) and (Figure 3) were calculated by measuring the gray scale value of each sample on CBCT radiographs with *OnDemand* software using *an Instrumentarium OP300* machine. Table 1 showed that the mean of alveolar bone density on the day-90 between the two groups was almost the same with the mean and standard deviation in the MIST + BG group of 344.32 ± 151.32 and the OFD + BG group of 344.33 ± 127.47. The increase in bone density of the MIST group was 166.65 ± 1.77 and the OFD + BG group was 107.55 ± 26.84.

The significance level of the non-parametric Wilxocon Signed Ranks Test obtained p value < 0.05, which indicated that there was a significant difference in alveolar bone density between observation times in each group, namely the MIST and OFD groups. The Independent t-test on alveolar bone density reduction between day-0 and day-90 in Table 3 showed a significance level of p > 0.05, which means that there was no statistically significant difference between MIST + BG and OFD + BG during the observation

period. Data obtained from this study provided the numbers of the reading outcome of microplate reader using a spectrophotometer technique at a wavelength of 450 nm in the form of optical density (OD). The OD value that was read was then converted into a standard curve to obtain the value of osteocalcin levels.

Table 4 and Figure 4 showed the lowest osteocalcin levels of gingival crevicular fluid in the MIST group on day-0 with mean and standard deviation of 295.55 ± 75.41. The highest osteocalcin level in the gingival crevicular fluid was in the MIST group on day-7 with the mean and standard deviation of 530.81 ± 97.03. The data in Table 4 showed an increase in osteocalcin levels on day-7 and day-14 compared to that in day-0, both in the MIST and OFD groups. Osteocalcin levels on day-14 were lower than that on day-7, meaning that there was a decrease in osteocalcin levels in both the MIST and OFD groups from day-7 to day-14. The results of the Shapiro-Wilk normality test in Table 5 indicated that the data were normally distributed.

Data in Table 6 showed that the interaction of the type of treatment group had no effect on osteocalcin levels ($p > 0.05$), but there was an effect of observation time on osteocalcin levels ($p < 0.05$), while the interaction of the type of method and time of observation had no effect on osteocalcin levels ($p > 0.05$). Further tests were carried out to determine the effect of time and the interaction of the type of method and time of observation on the osteocalcin levels of the gingival crevicular fluid.

The results of Post Hoc LSD test in Table 7 showed that there was no significant difference ($p < 0.05$) on day-0 between the MIST + BG and OFD + BG groups. This showed the similar osteocalcin levels between the two treatment groups before the procedure. There was a significant difference on day-0 and 7 and day-7 and 14 between the MIST + BG and OFD + BG groups, whereas there was no significant difference in osteocalcin levels between the MIST + BG and OFD + BG groups on day-0, day-7 and day-14 with a significance level of $p > 0.05$.

DISCUSSION

One of the utilizations of radiographic equipment in the field of dentistry is the evaluation of treatment outcomes. Three-dimensional (3D) radiographic images can be used to evaluate bone tissue characteristics such as depth, width, density and tissue structure.[14] Diagnosis of density changes in radiographic techniques is based on the dark and brightness of an image expressed in the gray scale value on CBCT.[15] The results of the mean between day-0 and day-90 in each group showed a significant difference, revealing the bone repair over time, as marked by an increase in the gray scale value on the radiographic image in the area of bone defect that has undergone periodontal regenerative treatment. Singh et al showed that an increase in bone height and density can be obtained using bone graft material in the bone defect area.[16]

There was no significant difference in the increase in alveolar bone density in the MIST and OFD groups, possibly because both groups used same bone graft material. The bone graft material must have bioabsorbable properties that allowed new bone growth in the host. The combination of hydroxyapatite and β-tricalcium phosphate is osteoconductive in nature, which allows new bone growth in the area of bone defect. The combination of these materials has the ability to create a strong direct bond to the bone in the host, resulting in a strong interface bond between the bone graft material and the surrounding bone. Histologically, it was also found that this combination of materials could integrate the spongious bone, which would result in a complete bone formation and unification in the area of bone damage.[17] The maturation process of the bone graft material in the area of bone defect in the laminar bone requires mineralization time, which varies from 3 to 6 months depending on several factors such as age, extent of bone defect, and patient habits. This bone formation process is also supported by surgical techniques that maintain a good vascular supply to achieve the maximum healing process.[18,19]

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Gingival crevicular fluid can be a non-traumatic identification method for providing information on the condition of the periodontal tissue. The components of the gingival crevicular fluid can be a prognostic marker for evaluation of post-regenerative periodontitis treatment, one of which is the osteocalcin marker as a marker for bone formation.[20] Osteocalcin levels of gingival crevicular fluid will increase after treatment for periodontitis conditions.[21] The mean of osteocalcin levels of gingival crevicular fluid increased to its peak on day-7 and decreased on day-14 both in the MIST + BG group and the OFD + BG group. Bizelli-Silveira et al stated that the expression of osteocalcin levels would increase at the beginning of mineralization on day-7 to day-14 and the peak of osteocalcin expression occurred on day-7.[12] The increase that was observed in osteocalcin levels indicated that mineralization was taking place in the formation of woven bone because osteocalcin was secreted by mature osteoblasts, which directly affected the bone mineralization process and decreased mineralization to normal values on day-14 along with the appearance of osteoclasts, which started to play a role in modeling and remodeling processes.[7] However, the mechanism of osteocalcin in the bone formation process as the results of periodontal treatment was still not clearly known.[21]

The mean of osteocalcin levels of gingival crevicular fluid in the MIST + BG group were higher than the OFD + BG group both on day-0, day-7 and day-14, which could be related to the use of the MIST method as a method with minimal trauma. As a result, vascular supply support of the interdental papilla remained in a good condition and was capable of increasing wound stability. Subsequently, it triggered the primary healing, as compared to the OFD method, which required a wider tissue incision and caused a greater trauma.[22,23]

Vascularization has an important role on the success of bone regeneration, including the support of the bone graft material used.[24] A good vascular supply from the interdental papilla can be a source of nutrition for bone-forming cells through tissue fluid passing through the bone canaliculi.[23] Vascular supply can be obtained through vascular anastomosis in the alveolar crest area that originate from the periodontal ligamentum arteries, supraperiosteal arteries and intra-alveolar arteries.[25] The blood clot and tissue stability on the scaffold that is formed supports the ability of good bone graft material affinity to bone protein, including osteocalcin.[26]

Osteocalcin levels of gingival crevicular fluid between the MIST + BG and OFD + BG groups showed no significant difference at any time of observation. This was related to the use of same bone graft material in both groups, which was HA + β-TCP combination. HA + β-TCP combination has osteoconductive ability during bone regeneration, which plays an important role in scaffold formation. The combination of these two would trigger osteoclast cell maturation, which would release cytokines and affect osteoblasts in the process of bone formation. Content of HA + β-TCP as a resorbable ceramic material can bond directly to bone and is able to release calcium and phosphate ions to trigger the bone regeneration process.[27,28]

HA + β-TCP combination, according to Annibali et al, on histological examination showed a pattern of bone formation with a large number of graft particles surrounded by newly formed bone that was then close together without forming a gap in the scaffold area. The newly formed osteoblasts in the area adhere directly to the bone on the surface of the bone graft particles. Trabecular stromal cells and blood vessels were found in the marrow spaces and new blood vessel formation was found in the new bone formation and mineralization process.[29]

Osteocalcin is known to have an important role in cementogenesis, osteoblast differentiation and bone mineralization, which is also expressed in hard tissue metabolism. The expression of osteocalcin levels in the gingival crevicular fluid is related to the bone graft resorption process by osteoclasts followed by the bone formation process by osteoblasts as indicated by the presence of osteocalcin expression secreted by osteoblasts. Therefore, the presence of countless number of

osteoblasts will lead to an increase in osteocalcin levels.[21] The difficulty in sample collection of osteocalcin levels of the gingival crevicular fluid using paper points on day-7 after surgery can be a consideration for the time of sample collection for further studies.

CONCLUSION

The minimally invasive surgical technique in the treatment of infrabony pocket with the addition of hydroxyapatite and β-tricalcium phosphate combination was effective in increasing alveolar bone density from day-0 to 90, increasing osteocalcin levels in gingival crevicular fluid from day-0 to 14, and reaching the peak of osteocalcin levels on day-7. The minimally invasive surgical technique in the treatment of infrabony pockets with the addition of hydroxyapatite and β-tricalcium phosphate combination between times of observation could lead to a greater increase in alveolar bone density and osteocalcin levels of gingival crevicular fluid than that of open flap debridement.

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