BMP2 Concentration in Gingival Crevicular Fluid as an Osseointegration Biomarker in Dental Implant

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Abstract

To date, there is no definitive guidance in determining the appropriate time to insert a restoration for dental implant; therefore, the idea that different methods may possibly give different results with regard to the success of osseointegration has emerged. The time of restoration insert should be based on the cellular activities that play a role in bone-healing, which influence both the mineralization process of the bone matrix and osteoblast activity. It is believed that some proteins that are the products of genes that play active roles in the bone regeneration process are biomarkers with regard to the readiness of bone in receiving the occlusal loading that occurs with restoration. Therefore, the analysis of biomarkers and their roles in the osseointegration process is necessary. Bone morphogenetic protein (BMP2) is an enzyme secreted by osteoblastic cell, and can be found in high concentrations. The expression of this enzyme by osteoblastic cell is very important in the mineralization process of bone. Alkali phosphatase expression has been reported as an initial marker of differentiation and mineralization in the bone-healing process, and may also be a determining factor in osteoblast activity. Although BMP is a member of the transforming growth factor-β superfamily; it plays an important role in the bone-healing process. This protein is expressed in repairing bone, has osseoinductive potentiality, and is secreted in any cell differentiation and cell development processes. Several factors play roles in the success of osseo integration, including immunologic reaction, gene expression, and cellular mechanisms that involve BMP, vascular endothelial growth factor, fibroblast growth factor, and other cellular markers.

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Introduction

Implant is now a solution in addressing missing tooth problems. In addition to its benefit in restoring mastication and phonetic functions, as well as confidence, the dental implant is also the best restoration with regard to esthetics and comfort. However, with the conventional method (delayed louding), two surgical steps are required in the treatment process. One surgical step in engrafting a dental implant is development by attaching the supra-structure immediately after the implant body is inserted. This technique is known as immediate loading, and is quite promising due to its benefits, such as requiring less treatment time and less surgery, well-preserving the esthetic condition of the gingival, greater comfort and safety, as a result of the presence of fixed prostheses, and indirectly providing greater patient satisfaction.[1] However, the degree of success of dental implant treatment using the immediate loading method remains controversial.

To date, no evidence-based study can answer the questions regarding for how long osseointegration takes place and when is the appropriate time to give the load to the dental implant.[1,2] Several experts have different opinions concerning the immediate loading method. Some have stated that insertion of the restoration via immediate loading should be conducted on the same day as the implant placement,[3] others believe that the insertion of

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the restoration should be carried out in the first week,[4] while yet others have asserted that the insertion of the restoration via immediate loading should be carried out within the 2 weeks following implant placement.[5]

There is no definitive guidance in determining the appropriate time of inserting a restoration for dental implant treatment; therefore, the idea that different methods may possibly give different results with regard to the success of osseointegration has emerged. The time of insertion of restoration should be based on the cellular activities that play a role in bone-healing, which influence both the mineralization process of the bone matrix and osteoblast activity. It is believed that some proteins that are the products of genes that play active roles in bone regeneration process are biomarkers with regard to the readiness of bone in receiving occlusal loading that occur with restoration. Therefore, the analysis of biomarkers and their roles in the osseointegration process is necessary.[1]

The purpose of the present study was to analyze whether or not the differentiation in the time of restoration insertion, and the differentiation in occlusal contact in the dental implant placement, using the immediate loading method, could lead to the alteration of bone morphogenetic protein (BMP2) expression as a biomarker of osseointegration between the dental implant and alveolar bone, compared with delayed loading. It was expected that the method of dental implant treatment with the most appropriate restoration insertion time could be identified, thus providing evidence in support of the success of dental implant treatment. This would be shown by the occurrence of osseointegration between the dental implant and alveolar bone. In addition, it was expected that the level of BMP2 in gingival crevicular fluid would be identified as a prognostic factor that determines the appropriate time of restoration insertion and as a biomarker of osseointegration.

If the present study shows that the BMP2 expression could be used as a biomarker of osseointegration, then it will constitute a new non-invasive method that can sooner measure the occurrence of osseointegration. Furthermore, it is expected that this study will provide a new method that can be implemented in the development of dental science, particularly in prostodontics and implantology, as there is currently no non-invasive indicator for use in determining the success of dental implant treatment.

Methods

Animal testing was used to identify whether BMP2 could be a biomarker of osseointegration of dental implant treatment using immediate loading compared with the conventional method (delayed loading). The implant placement was carried out in the Laboratory of Primate Study Center/Laboratorium Pusat Studi Satwa Primata (PSSP) IPB, Bogor. This laboratory is customarily used to conduct studies in primates, and has received an accreditation from the Association for Assessment and Accreditation of Laboratory Animal Care International.

Study Sample

Three *Macaca fascicularis* primates aged 6 years, weighing approximately 3.5–4 kg, and in a similar general condition were used. These animals were quarantined for a period of 35 days in the PSSP IPB, Bogor.

The study samples were titanium dental implant specimens that were engrafted into the jaws of the animals and the specimens were then divided into treatment groups and a control group. In every animal, five treatments were tested. The implant placement used in the treatment groups was the immediate loading method, meaning that as soon as the implant was engrafted, the restoration was also immediately inserted. In the control group, the restoration was not provided over the dental implant.

The study groups were as follows:

Group 1. A control group. The restoration was not provided over the dental implant.

Group 2. The insertion of restoration with normal occlusal contact was carried out on the same day as dental implant placement (ILNC 1).

Group 3. The insertion of restoration with normal occlusal contact was carried out on Day 14 after implant placement (ILNC 14).

Group 4. The insertion of restoration with light occlusal contact was carried out on the same day as dental implant placement (ILLC 1).

Group 5. The insertion of restoration with light occlusal contact was carried out on Day 14 after implant placement (ILLC 14).

The Dental Implant Placement

Titanium implant placement was carried out in the jaws of the animals, 2 months after the tooth extraction. Prior to carrying out the procedure, the animals were anesthetized using intravenously administered ketamine (15 mg/kg). Approximately 5 minutes later, when the sedative was taking effect, general anesthesia was intravenously administered (4 mg/kg) via bolus propophol 1.8 ml. The implant placement was then performed without flap opening (flapless).

The first step was to determine the location of implant placement by marking the gingival with dental explorer. In order to ensure that the location of the engrafted dental implant was in the correct, as planned, place a surgical template was necessary as guidance to where the implant should be inserted at the edentulous area on the right and left sides of mandible, including the second premolar, first molar, and third molar regions.

On the five spots where drilling was to be carried out, a sufficient amount of soft tissue was then cut in order to not only facilitate drilling, but also to ensure that pieces of soft tissue did not fall into the hole for implant placement.

The control and treatment groups were rotated to avoid bias. For example, in the first animal, the implant in the right second premolar region acted as a control, and implants in other regions acted as treatment. After the implant was engrafted, the insertion of restoration in the treatment group was performed by applying light occlusal contact at the right side of the mandible and normal occlusal contact at the left side.

In the first molar region, the restoration was inserted immediately after implant placement on the same day (Day 1 immediate loading). In the third molar region, the restoration was inserted on Day 14 (Day 14 immediate loading).

The method used to determine whether the contact was normal or light was carried out using articulating paper. In order to obtain normal occlusal contact, articulating paper of a 12-micron thickness was used, while for light contact, 60 micron articulating paper was used.

In the second animal, the dental implant in the left first molar region was used as a control, while Day 1 immediate loading was applied in the second premolar region and Day 14 immediate loading was applied in the third molar region.

In a similar manner to the first animal, light contact was used on the right side of the mandible, while normal contact was used on the left side.

With the third animal, group rotation was carried out. The animals were intramuscularly injected with *Intramox* within 3 days to prevent infection.

The Measurement of BMP2 Concentration

The measurements of BMP2 concentration taken from the gingival crevicular fluid were carried out at 1 week, two weeks, 1 month, 2 months, and 3 months after the implant placement, using the enzyme-linked immunosorbent assay (ELISA) method. The reagent used was a Quantikine ELISA kit (R and D System, Minneapolis, USA).

Samples taken from the animals were collected in 1.5 mL micro tubes and stored at a temperature of -20° C until the ELISA was performed. The results of the measurements were BMP2 concentrations in pg/mL.

Results

1.Data Analysis of BMP2 Concentration

The measurements of BMP2 concentration were taken at the beginning (before treatment), at the first week, second week, first month, second month, and third month after treatment.

A total of 15 samples of BMP2 concentration were collected, which consist of five treatment groups of that were measured six times, as the periods described above.

BMP2 Concentration Differences between the Observation Times, Based on Treatment Groups. The BMP2 concentrations obtained from the treated animals are shown in Table 1.

Table 1 shows that the mean and median BMP2 concentration values at the initial measurement were similar in the five treatment groups, that were 221 and 245. The lowest mean concentrations at the initial measurement were observed in the ILNC14 and control groups, being 226.50 in both.

The highest mean was found in the ILLC14 group, and was 233.83. With regard to standard deviations, the largest variations were observed in the ILNC14.

Table 1. Distribution of BMP2 Concentrations between the Measurement Times, Based on Treatment Groups.

BMP2	Initial	Day 7	Day 14	Day 30	Day 60	Day 90
ILNC1						
Median	223.5	288	379.55	390	388	400
Mean	229.83	291.15	366.85	392.50	338.58	412.00
[SD]	[13.19]	[6.43]	[38.12]	[11.95]	[113.27]	[30.81]
ILNC14						
Median	223.5	331.25	346	337.5	440.56	880.83
Mean	226.50	305.42	341.17	351.16	482.27	902.91
[SD]	[17.20]	[52.50]	[48.43]	[44.59]	[91.78]	[93.91]
ILLC1						
Median	223.5	301	389	421	587.56	766
Mean	229.83	290.00	383.33	418.67	606.35	736.50
[SD]	[13.19]	[19.05]	[22.05]	[27.57]	[78.95]	[62.24]
ILLC14						
Median	223.5	240	389	462.5	581.25	755.83
Mean	233.83	277.13	375.67	524.67	614.92	756.00
[SD]	[12.09]	[66.58]	[33.55]	[165.74]	[207.81]	[83.08]
Control						
Median	223.5	265	301	322	337.5	830.5
Mean	226.50	280.08	307.08	380.08	412.00	822.39
[SD]	[17.20]	[56.17]	[88.58]	[184.85]	[143.12]	[21.99]

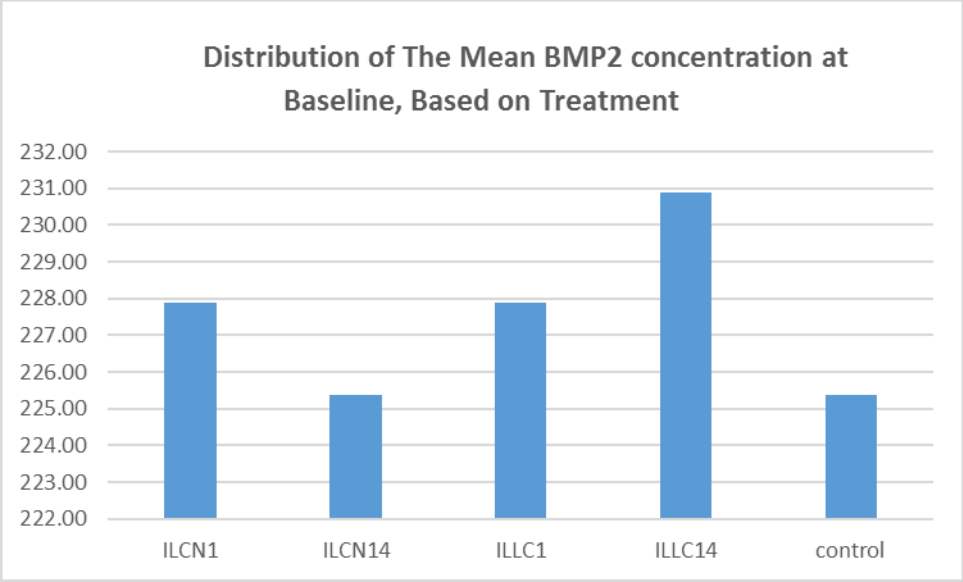

Figure1. Distribution of the Mean BMP2 Concentration at Baseline

On Day 7 after treatment, the mean and median minimum and maximum BMP2 concentration values varied among the treatment groups. The standard deviations showed that the ILNC1 group had the lowest variation in BMP2 concentration (6.43), while the highest variation, which was three-fold higher, was found in the ILLC1 group (19.05). However, other treatment groups were associated with fairly big variation values, the highest of which (66.58) was observed in the ILLC group. The lowest mean BMP2 concentration was found in the ILLC14 group (277.13), and the highest was observed in the ILNC14 group (305.42). When compared with

the initial (pre-treatment) measurements, there were increases in BMP2 concentrations in all treatment groups. In the Day 14 measurement, the lowest variation value of BMP2 concentration was observed in the ILLC1 group (22.05) while the highest was found in the control group (88.58). The means of the concentrations were higher in all treatment groups compared with previous measurements (initial and Day 7). The highest mean of BMP2 concentration was observed in the ILLC1 group (383.333), and the lowest was shown in the control group (307.08).

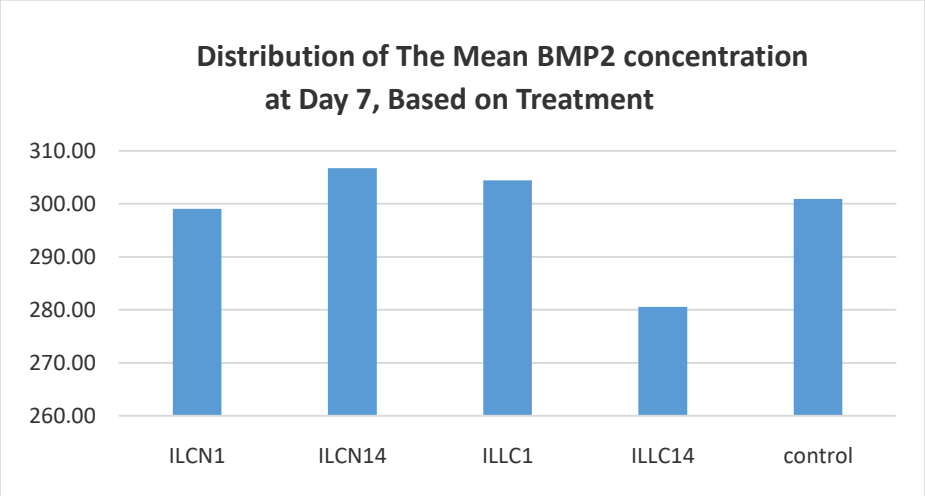

Figure 2. Distribution of the Mean BMP2 Concentration at Day 7

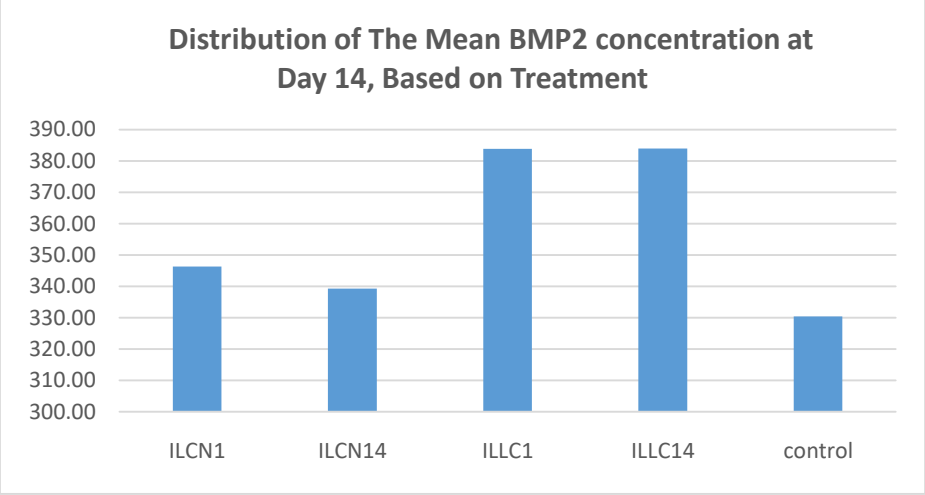

Figure 3. Distribution of the Mean BMP2 Concentration at Day 14

BMP2 concentrations at the Day 30 measurement had varied values based on their standard deviations. The lowest variation was shown in the ILNC1 group (11.95), while the highest was observed in the control group (184.85). The means of the five groups were higher compared with the means of previous measurements. The lowest mean was found in the ILNC14 group (351.16), and the highest mean was shown in the ILLC14 group (524.67).

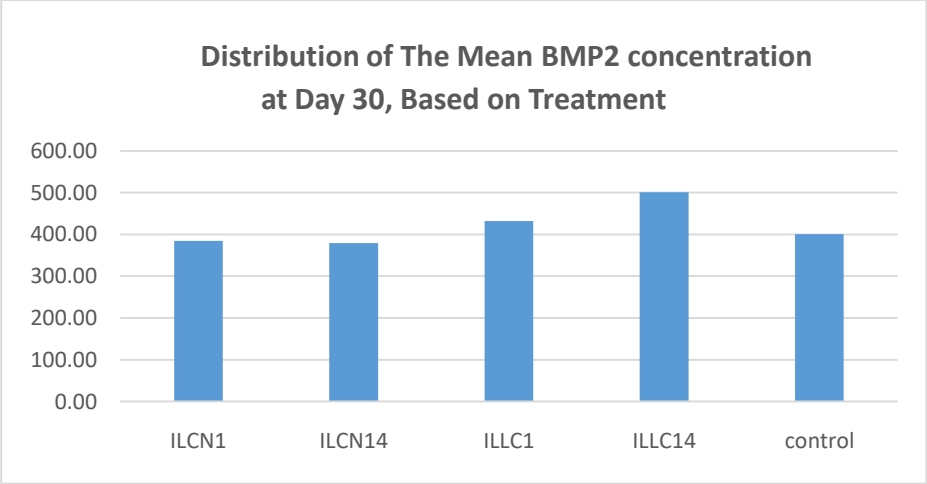

Figure 4. Distribution of the Mean BMP2 Concentration at Day 30

In the Day 60 measurement, there were fairly large variations in BMP2 concentration values. The lowest variation was observed in the ILLC1 group (78.95), and the highest was shown in the ILLC14 group (207.81). The lowest mean of BMP2 concentration was found in the ILNC1 group (338.58). The control and ILNC14 groups had means below 500, whereas the ILLC1 and ILLC14 groups had means above 600. If they were compared with the means obtained from the previous measurement (Day 30), the BMP concentration means were increased, with the exception of the ILNC1 group, in which it was decreased.

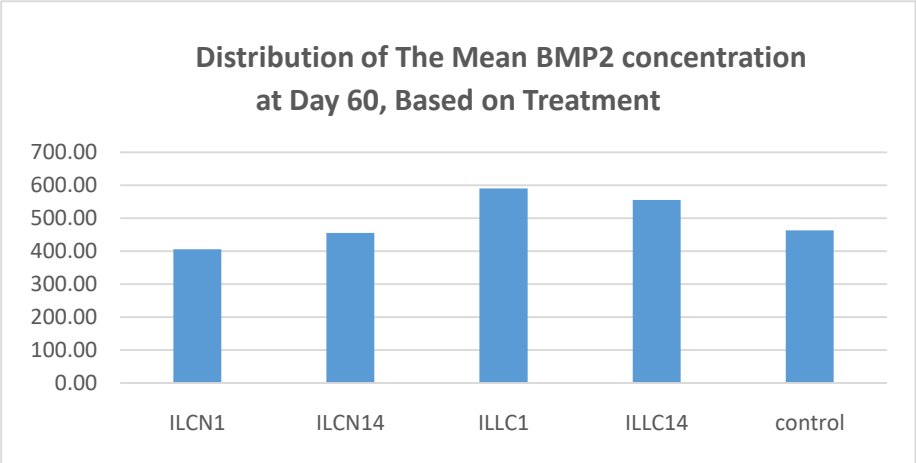

Figure 5. Distribution of the Mean BMP2 Concentration at Day 60

At Day 90, BMP2 concentrations showed fairly big variations in values among the five treatment groups. The lowest variation was observed in the control group (21.99), followed by the ILNC1 group (30.81). The ILNC14 group showed the highest variation (93.91) among the five groups. Based on mean value, the ILNC1 group had the lowest mean (412.00), while the ILNC14 group had the highest mean (902.91). Compared with the previous measurement (Day 60), all of the groups showed an increase in their mean values. The means of the ILNC1, ILLC1,

and ILLC14 groups were increased by approximately 100, and the mean control and ILNC14 group values were increased almost two-fold, compared with the previous measurement.

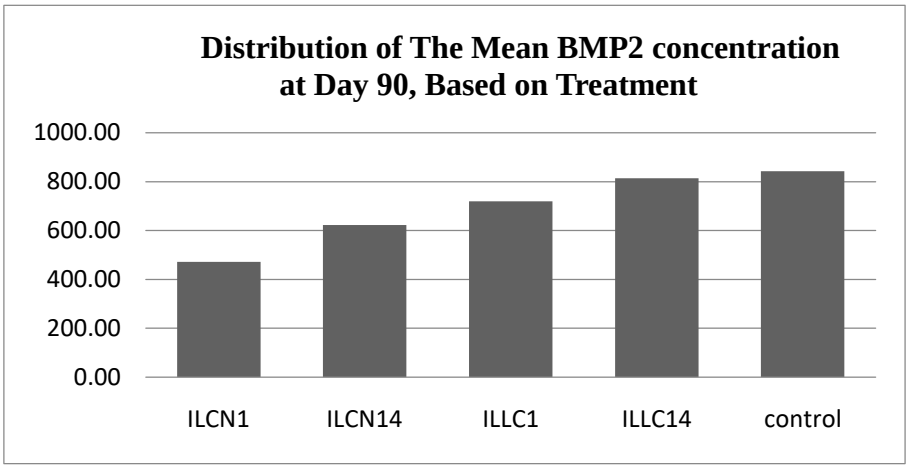

Figure 6. Distribution of the Mean BMP2 Concentration at Day 90

2. Equivalence Test of BMP2 Concentration at the Initial (Pre-treatment) Measurement, Based on Treatment Groups.

The equivalence test was carried out with the aim of observing the equivalence in value at the initial measurement before treatment was carried out. This was conducted to ensure that the value changes that were observed after treatment genuinely occurred as a result of the treatment and not because they were already different from the beginning (before treatment). The test was carried out using analysis of variance (ANOVA) of BMP2 concentration from the initial measurement because there were more than two independent groups.

The assumptions in the ANOVA were normal data distribution and similarity in the BMP2 concentration variability in all of the treatment groups. The result of the normality test for BMP2 concentration at the times of measurements, based on treatment groups, showed that data were normally distributed, and that variants of BMP2 concentrations from the initial measurement were similar among the treatment groups. The ANOVA results showed that the means of BMP2 concentration did not differ significantly among the five treatment groups ($p>0.05$). This showed that the five groups began with similar BMP2 concentrations (Table 2).

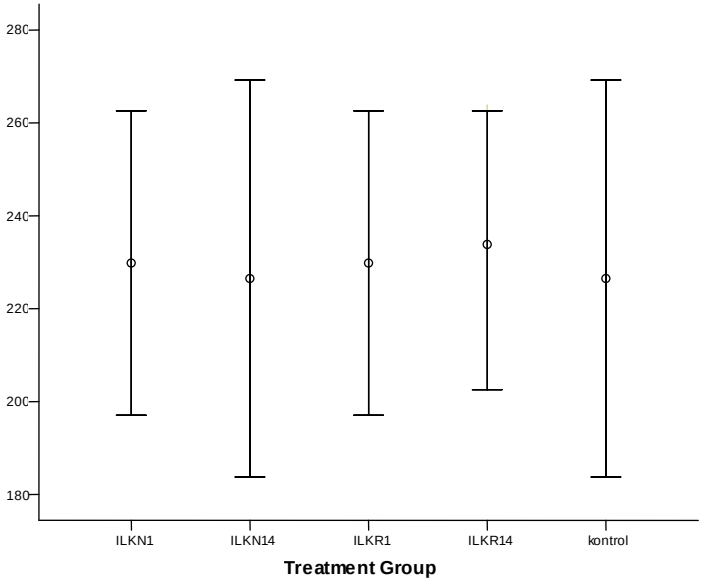

Figure 7. Error Bar

Table 2. The results of the ANOVA.

ANOVA	SS	df	MS	F	P
Inter-group	110.40	4	27.60	0.13	0.969
Within-group	2171.50	10	217.15		
Total	2281.90	14			

Discussion

The present study found that there was a significant increase in BMP2 concentration at 3 months of healing following treatment. In accordance with the results of preliminary studies, it was shown that BMP2 has good potential as a predictor and osseointegration biomarker, such that the two parameters are sufficient, particularly since the two proteins are indicators early in the mineralization process, which is indispensable as a prediction factor.[1,2,5,6]

BMP2 plays a role in the process of differentiation when there is injury due to drilling. The statistical analysis showed that BMP2 has osteogenic potential.[2,5] The result of preliminary study showed that BMP2 can accelerate mineralization processes occurring in bone around dental implants.[7,8]

The fact that the BMP2 concentrations tended to consistently increase indicates the presence of this protein as a potential biomarker of osseointegration. Zhang et al. stated that an analysis of the correlation between the materials to be used as biomarkers and other examination results should be conducted to support the diagnosis of a disorder or condition.[9,10] They saw a correlation of salivary biomarkers with clinical features occurring in periodontal disease.[10] Therefore, BMP2 concentration should be correlated with other markers to provide further evidence of its capacity as an osseointegration biomarker.[11]

In the present study, treatment effectiveness could be observed on the basis of the difference value (delta) between the initial (pre-treatment) measurement and the measurements after treatment, and by comparing these among the five treatment groups. For every measurement, the BMP2 concentration was compared with the pre-treatment measurement, based on treatment groups. This was carried out with the aim of identifying the optimal effective treatment time.

The BMP2 measurements showed an increased concentration on Day 14 compared to Day 7, following implant placement. The same was observed with Day 30 compared to Days 14 and 7.

The concentrations measured on Days 60 and 90 were also higher. However, the inter-group BMP2 concentration analysis showed that a significant difference was only obtained in the ILNC1 group compared to control (Day 1 immediate loading with normal occlusal contact).

Thus, it can be suggested that the insertion of restoration with normal occlusal contact should not be carried out on the same day as implant placement. It means that, in this group only, the osseointegration that occurreddid not have the same quality as in the control group.

Conclusion

The present study showed that BMP2 increases significantly as the osseointegration process takes place. The concentration was highest in the third month, meaning that osseointegration had already occurred by this time. It can therefore be concluded that BMP2 concentration, as extracted from gingival crevicular fluid, could be used as a biomarker of osseointegration in dental implant treatment.

In addition, statistical analysis showed that there was no significant difference in the implant placement between the immediate loading method and delayed loading. Further, there was no significant difference between light occlusal contact and normal occlusal contact, with the exception of the ILNC1 group.

Thus, it can be concluded that dental implant is the best solution in coping with lost tooth problems. Immediate loading for implant placement is the newest method, and is quite promising due to its benefits, such as requiring less time for treatment, less surgery, the well-preserved esthetic condition of the gingival, greater comfort and safety, due to the presence of fixed prostheses, and providing greater patient satisfaction.

Acknowledgement

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