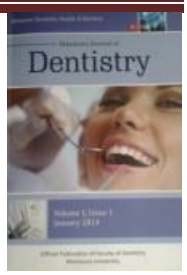
The Effect of Hyaluronic Acid in Combination With β-Tri-Calcium Phosphate in Regeneration of Periodontal Vertical Bone Defect in Periodontitis Patients



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Abstract:
Objectives: The aim of the present study is to evaluate the effect of hyaluronic acid (HA) in combination with Beta-tri-calcium phosphate (βTCP) in treatment of vertical bone loss in periodontitis patients clinically and radiographically.
Patients and methods: Forty patients diagnosed as stage III & grade B periodontitis with vertical bony defects and showed radiographic bone loss ≥5mm were selected from periodontology and oral medicine department faculty of dentistry Mansoura University. The patients were divided into four groups. Group I treated with combination of hyaluronic acid (HA) and Beta-tri-calcium phosphate (βTCP). Group II treated with HA alone, group III treated with βTCP alone and group IV treated by open flap debridement using minimally invasive surgical technique. Clinical periodontal parameters (plaque index PI, gingival index GI, clinical probing depth PD, clinical attachment level CAL) were taken from each patients at baseline , 3 months and 6 months after treatment. Periapical radiographs were taken for each patient to evaluate the radiographic bone level change before and after treatment. The radiographs were taken at baseline, 3 months and 6 months from surgery.
Results: Clinical periodontal parameters were significantly improved after treatment in all treatment groups compared to the baseline. There was a statistically significant difference among the treatment groups between the base line, after 3 months and after 6 months from surgery (p value ≤0.05). Regarding bone level there was gain in the bone level after treatment in all treatment groups with significant improvement in group I (HA+ βTCP). There was a statistically significant difference among the treatment groups between the base line, after 3 months and after 6 months from surgery (p value ≤0.05).
Conclusions: Combination of Hyaluronic acid and (βTCP) gave good results in the treatment outcomes as regard bone level and periodontal clinical parameters. Hyaluronic acid in combination with (βTCP) showed a synergistic effect on regeneration of vertical bony defects.
Keywords: *Periodontal regeneration, vertical bone loss, hyaluronic acid, beta tri-calcium-phosphate*.

Introduction

Periodontitis is a frequently occurring inflammatory disease caused by the pathogenic activity of bacteria of the dental biofilm. This disease affects the surrounding teeth tissues (periodontium) and leads to their destruction and even to tooth loss in drastic cases. Periodontitis associated with bone loss whether vertical or horizontal. Bone loss is characterized by supra-bony pocket due to equal loss of the alveolar bone, or is characterized by infra-bony pocket due to unequal loss of the alveolar bone.(1)

The clinical treatment strategies for periodontitis are focused on the reduction or elimination of the periodontal pathogenic condition (i.e., removal of bacterial infection and debridement of infected tissues) to stop the progress of the disease and heal the periodontal tissues. However, the healing pattern resulting from conventional treatment is variable and is often inconsistent with original periodontal tissues morphology and function. (2)

Regeneration of periodontal defects seeks to restore the periodontal structure into normal shape and function.(3) Current considerable efforts are being made to achieve regeneration. Key success of regeneration is the stability of the blood clot with selection of appropriate regenerative material. Several biomaterials are used as filling materials to treat defects and bone deficiencies after oral and maxillofacial injuries or pathological condition. Several bone regeneration enhancers such as platelet-rich plasma, platelet-rich fibrin, and hyaluronic acid have been used to accelerate bone healing and repair in the bone defects. (4)

Hyaluronic acid (HA) is a high-molecular weight polysaccharide ubiquitously distributed in the extracellular space HA has been reported to play critical roles in a wide variety of biological events, such as wound healing, chondrogenesis, osteogenesis, the immune response and migration cells.(5) HA demonstrated anti-inflammatory, antioxidant, and antibacterial properties in the treatment of periodontal diseases. In addition, due to its viscoelastic properties, it can be used as an adjunct to maintain space during the treatment of periodontitis. In an animal study, Sasaki and Watanabe (1995) studied the osteoinductive action of (HA) and found that it is capable of accelerating new bone formation through mesenchymal cell differentiation in bone wounds. (6)

Various bones and bone substitutes have been used in periodontal surgery with varying success. The objectives of these transplants are to fill defects in bone, facilitate healing of periodontium. As a material for bone regeneration, bio-ceramics usually have good osteoconductivity and bioactivity with a similar mineral phase of natural bone tissue. Among bio-ceramics, β-TCP was used.(7) Due to HA's unique viscoelastic and osteogenic properties and due

to the advantages of the use of , β-TCP in guided tissue regeneration (GTR), it was worse using both materials in order to obtain better results in treatment of periodontal defects.(8)

Patients and methods:

A total of forty patients with inclusion criteria: 1.Patient of both sexes above 20 years, 2. Individuals with probing depth ≥5mm. 3.Individuals with clinical attachment loss ≥ 5mm. 4.Individuals with vertical bony defects. Smoker, patients with systemic diseases, drug abusers and uncontrolled diabetic patients were excluded from the study. The patients were diagnosed as stage III & grade B periodontitis and showed radiographic bone loss ≥5mm. The patients were divided into four treatment groups.

Before any surgical procedures Phase I periodontal therapy consisted of debridement, scaling and root planning to remove all subgingival and supragingival deposits (plaque and calculus) and oral hygiene instruction were given to the patients in the form of teeth brushing, flossing and chlorohexidin mouth wash. Clinical assessment of situation after Phase I therapy were conducted to assess improvement in clinical signs and symptoms.

Aseptic field was required for all surgical procedures using povidine iodine. Local anesthesia using 4% articane with 1:100.000 epinephrines was applied for buccal infiltration injection of 1.5 ml and palatal infiltration injection of 0.3 ml. Inferior alveolar nerve block and Lingual nerve block were used for mandibular posterior segment.

All surgical procedures were performed using minimally invasive surgical technique. Blade number 15 was used to reach to infrabony defects through horizontal or oblique incisions at the base of the buccal papilla. Flap was extended mesiodistally and was modified according to the defect geometry then full thickness buccal flap was elevated to evaluate the periodontal defect. The interdental tissues were separated from underlying bone using sharp micro blade. The interdental tissue might be rolled at palatal side with full thickness flap if the periodontal defects reached to the palatal site.

All granulation tissues into the periodontal defect were removed using sharp micro scalers and curettes, then flushing of the defect with ultrasonic scaler. Then different regenerative materials were applied and packed into the periodontal defects according to the different treatment groups. Group I treated with combination of hyaluronic acid (HA) and Beta-tri-calcium phosphate (*β*TCP). Group II treated with HA alone and group III treated with *β*TCP alone. Group IV involved open flap debridement through minimally invasive surgical technique. Then intimate wound closure was conducted and post-operative medications and instructions were given to the patients.

Analytical statistics:

In the statistical comparison between the different groups, the significance of difference was tested using one of the following tests:

• Student's t-test (Paired): -Used to compare between mean of two related groups of numerical (parametric) data.

• Wilcoxon signed rank: Used to compare between two related groups of numerical (non-parametric) data. A P value <0.05 was considered statistically significant.

Results:

A total of forty patients with stage III & grade B periodontitis with periodontal infrabony defects (20 male and 20 female with age range from 25-55 years old) were chosen and examined from the Department of Periodontology and Oral Medicine, Faculty of Dentistry, Mansoura University. Patients were divided into four groups.

Table 1 summarizes the improvement in clinical periodontal parameters and bone level in Group I at baseline, 3 months and 6 months after treatment. In Group I (HA+ *β*TCP) the mean value of plaque index (PI) at the baseline before surgery was 1.34±0.32, after 3 months after surgery it was 0.43±0.22 while after 6 months it was 0.39±0.19. The mean value of gingival index (GI) at the baseline before surgery was 1.47±0.25, after 3 months after surgery it was 0.53±0.23 while after 6 months it was 0.516±0.22. The mean value of clinical probing depth (PD) at the baseline was 7.50±1.58, after 3 months after surgery it was 5.20±1.39 while after 6 months it was 2.60±1.51. The mean value of clinical attachment level (CAL) at the baseline before surgery was 8.0±1.76, after 3 months after surgery it was 5.0±1.7 while after 6 months it was 3.45±0.71. The mean value of bone level 7.5±1.8 at base line, after 3 months after surgery it was 4.10±1.52 while after 6 months it was 4.20±1.14.

Table 2 summarizes the improvement in clinical periodontal parameters and bone level in Group II at baseline, 3 months and 6 months after treatment. In Group II (HA) the mean value of plaque index (PI) at the baseline before surgery was 1.47±0.33, after 3 months after surgery it was 0.64±0.11while after 6 months it was 0.64±0.09. The mean value of gingival index (GI) at the baseline before surgery was 1.48±0.25, after 3 months after surgery it was 0.633±0.12while after 6 months it was 0.60±0.10. The mean value of clinical probing depth (PD) at the baseline was 5.70±0.95, after 3 months after surgery it was 3.0±0.94 while after 6 months it was 3.30±0.95. The mean value of clinical attachment level (CAL) at the baseline before surgery was 6.0±1.05, after 3 months after surgery it was 3.9±0.74while after 6 months it was 4.10±0.74. The mean value of bone level 5.80±0.79 at base line, after 3 months after surgery it was 2.20±0.92 while after 6 months it was 2.60±0.84.

Table 3 summarizes the improvement in clinical periodontal parameters and bone level in Group III at baseline, 3 months and 6 months after treatment. In Group III (*β*TCP) the mean value of plaque index (PI) at the baseline before surgery was 1.32±0.29, after 3 months after surgery it was 0.49±0.21while after 6 months it was 0.47±0.22. The mean value of gingival index (GI) at the baseline before surgery was 1.44±0.19, after 3 months after surgery it was 0.488±0.20 while after 6 months it was 0.46±0.18. The mean value of clinical probing depth (PD) at the baseline was 6.50±1.35, after 3 months after surgery it was 3.80±1.23 while after 6 months it was 3.80 ±1.32. The mean value of clinical attachment level (CAL) at the baseline before surgery was 7.0±1.94, after 3 months after surgery it was 4.80±1.81 while after 6 months it was 5.10±1.52. The mean

value of bone level 6.5±1.35 at base line, after 3 months after surgery it was 3.10±0.88 while after 6 months it was 3.50±1.08.

Table 4 summarizes the improvement in clinical periodontal parameters and bone level in Group IV at baseline, 3 months and 6 months after treatment. In Group IV (Minimally invasive surgical technique) the mean value of plaque index (PI) at the baseline before surgery was 1.49±0.35, after 3 months after surgery it was 0.65±0.11 while after 6 months it was 0.60±0.14. The mean value of gingival index (GI) at the baseline before surgery was 1.52±0.29, after 3 months after surgery it was 0.671±0.11 while after 6 months it was 0.46±0.18. The mean value of clinical probing depth (PD) at the baseline was 5.20±0.92, after 3 months after surgery it was 3.0±0.67 while after 6 months it was 2.90±0.74. The mean value of clinical attachment level (CAL) at the baseline before surgery was 5.20±1.23, after 3 months after surgery it was 3.60±0.97 while after 6 months it was 3.30±1.06. The mean value of bone level 5.20±0.92 at base line, after 3 months after surgery it was 2.20±0.79 while after 6 months it was 2.0±0.82.

Discussion

The goal of regenerative periodontal therapy is to restore the supporting structures of periodontium that are damaged due to periodontitis. Many treatment modalities have been used through years to treat periodontal defects ranging from non-surgical periodontal therapy to surgical periodontal therapy. Open flap debridement has been used alone or along with bone graft materials as a treatment of vertical bony defects. Later on guided tissue regeneration has been adopted and biologic mediators and barrier membranes have been incorporated into the process (9)

The purpose of the present study is to evaluate clinically and radiographically the effect of hyaluronic acid (HA) in combination with Beta-tri-calcium phosphate (βTCP) in treatment of vertical bone loss in periodontitis patients.

In the present study we found that there was a significant improvement in the plaque index (PI) and gingival index (GI) after 3 and 6 months following surgery in comparison to the base line in all treatment groups [table 1, 2, 3 and 4]. As regards the improvement in (Group I and II), this could be due to the application of hyaluronic acid (HA) with its healing potential and viscoelastic properties, so HA has bacteriostatic effect and anti-adhesive properties. This was consistent with Bertl et al (10)who found in their study that there was significant improvement in periodontal clinical parameter when it was used as adjunctive to non-surgical periodontal therapy. They stated that PI scores decreased from base line to 6 months, in case of continuous maintenance of good oral hygiene by the patients.

As regard (Group III and IV) the improvement in plaque index and gingival index could be attributed to using minimal periodontal surgical wound that was associated with limited post-surgical discomfort that allowed the patient to keep good oral hygiene (11). Also the application of small particle sizes of βTCP was associated with limited risk of infection and intimate wound closure (12)..

In the present study we also found that there was a significant improvement in the clinical probing depth (PD) and clinical attachment level (CAL) after 3 and 6 months following surgery in comparison to the base line in all treatment groups [table 1, 2, 3 and 4]. As regard improvement in (Group I), this could be due to the unique combination between HA and βTCP. This combination improved the osteogenic effect of grafting procedures and increased the regenerative potential of both materials so it produced synergistic effect regarding PD improvement. This was consistent with Jiang et al (8)They used combination of HA and βTCP in rabbit models, and their experimental study showed significant improvement in PD and CAL.

As regard (Group II) the improvement in clinical probing depth (PD) and clinical attachment level (CAL) could be attributed application of HA with its unique regenerative potential. Due to its viscoelastic behavior HA was easily invaded by host mesenchymal cell which gave osteoinductive potential with improved healing outcomes. This was consistent with Lobato et al. (13) who showed good periodontal clinical parameters when HA was used in periodontitis patients. As regard (Group III) the improvement could be due to the using of small particle sizes of βTCP which were able to enhance vascularization of the grafted site for graft integration so it improved the regenerative potential. This was consistent with Sun et al.(14) who studied the response of periodontal ligament to βTCP and showed good results as regard to pocket depth reduction and CAL gain. Regarding (Group IV) the improvement could be due to the using of minimally invasive surgical technique which created stable environment for blood clot stabilization which was essential for periodontal regeneration. This was consistent with Cortellini et al. (15) who used minimally invasive surgical technique alone without any graft as a treatment for infrabony defects and showed promising results.

The measurement of bone level in our study was done by using Periapical radiographs to assess the vertical bony defect depth. Each exposure was customized for every patient to standardize the periapical radiograph before and after treatment. Customized bite block was used for each patient and was used during pre-operative and post-operative exposure. Belgin and Ceren Aktuna(16) who used a periapical radiography to evaluate and assess changes in trabecular bone in periodontitis patients and they provided good diagnostic and quantitative information regarding the bone level.

In our study bone level showed significant increase after 6 months as compared to the baseline in Group I [table 1] .That could be related to the osteoinduction and bone formative capacity of HA and βTCP combination. The viscous and hydration properties of HA gave that combination perfect remodeling behavior and resorption time to be replaced with the host bone. These results came in agreement with Portron et al (17) who studied the effect of HA and βTCP combination in regenerative periodontal therapy. Also it was consistent with Motamedian et al (18) who showed good bone filling in extraction sockets after application of HA and βTCP in combination.

Bone level showed significant increase after 6 months as compared to the baseline in Group II [table 2] .This could be related to marvelous osteoinductive potential of HA. This

was consistent with Kim et al. (19) who showed improved bone filling after application of HA in bony defects. Bone level showed significant increase after 6 months as compared to the baseline in Group III [table 3]. This could be attributed to the osteoconduction behavior of βTCP as it acted as scaffold on which the new bone grew. This was in agreement with Franceschini et al. (20) who tested the repair of bony defects using βTCP and showed good results. Regarding group IV there was a significant increase after 6 months as compared to the baseline [table 4]. This could be attributed to the using of minimally invasive surgical technique as it provided space maintenance and blood clot stabilization that allowed the self-repair of the host bone. This was in agreement with Ortiz et al. (21) who used minimal flap to correct periodontal bony defects and showed good results regarding bone level gain.

Conclusion

Based on the results of the study it can be concluded that:

- Combination of Hyaluronic acid and (βTCP) gave good results in the treatment outcomes as regard bone level and periodontal clinical parameters.
- Hyaluronic acid in combination with (βTCP) showed a synergistic effect on regeneration of vertical bony defects.
- The application of Hyaluronic acid gel alone as a regenerative material in vertical bony defect gave a promising predictable outcome regarding bone level gain and periodontal clinical parameters improvements.

Although (βTCP) showed good results as regard improving bone level and periodontal clinical parameters, but it was not as effective as when it was combined with Hyaluronic acid.

Table 1: Mean &Standard deviation values of periodontal parameters indices and bone height level after 3 months and 6 months for group I

Group I	Base line (M ± SD)	After 3 months (M ± SD)	After6 months (M ± SD)	test of significance
PI	1.34±0.32^{ab}	0.43±0.22^{ac}	0.39±0.19^{bc}	p1<0.001* p2<0.001* p3=0.006*
GI	1.47±0.25^{ab}	0.53±0.23^{a}	0.516±0.22^{b}	p1<0.001* p2<0.001* p3=0.34
PD	7.50±1.58ab	5.20±1.39ac	2.60±1.51bc	p1<0.001* p2<0.001* p3<0.001*
CAL	8.0±1.76^{ab}	5.0±1.7^{a}	3.45±0.71^{b}	p1<0.001* p2<0.001* p3<0.001*
Bone Height	7.5±1.8^{ab}	3.10±1.52^{ac}	4.20±1.14^{bc}	p1<0.001* p2<0.001* p3=0.003*

P1: difference between Base line and after 3 months of treatment, **P**2: difference between Base line and after 6months of treatment, **P**3: difference between 3 months and 6 months after treatment

Test used for comparison between Tim: repeated measure ANOVA followed by post-hoe Bonferroni

*p value is highly significant at level ≤0.001

*p value is significant at level ≤0.05

M = Mean, SD = Standard deviation

Similar superscripted letters within same row denote significant difference between groups

Table 2: Mean &Standard deviation values of periodontal parameters indices and bone height level after 3 months and 6 months for group II

Group II	Base line (M ± SD)	After 3 months (M ± SD)	After 6 months (M ± SD)	test of significance
PI	1.47±0.33^{ab}	0.64±0.11^{a}	0.64±0.09^{b}	p1<0.001* p2<0.001* p3=0.91
GI	1.48±0.25^{ab}	0.633±0.12^{a}	0.60±0.10^{b}	p1<0.001* p2<0.001* p3=0.25
PD	5.70±0.95^{ab}	3.0±0.94^{a}	3.30±0.95^{b}	p1<0.001* p2<0.001* p3=0.08
CAL	6.0±1.05^{ab}	3.9±0.74^{a}	4.10±0.74^{b}	p1<0.001* p2<0.001* p3=0.16
Bone Height	5.80±0.79^{ab}	2.20±0.92^{a}	2.60±0.84^{b}	p1<0.001* p2<0.001* p3=0.10

P1: difference between Base line and after 3 months of treatment, **P**2: difference between Base line and after 6months of treatment, **P**3: difference between 3 months and 6 months after treatment
Test used for comparison between Tim: repeated measure ANOVA followed by post-hoe Bonferroni
*p value is highly significant at level ≤0.001
*p value is significant at level ≤0.05
M = Mean, SD = Standard deviation
Similar superscripted letters within same row denote significant difference between groups

Table 3: Mean &Standard deviation values of periodontal parameters indices and bone height level after 3 months and 6 months for group III

Group III	Base line (M ± SD)	After 3 months (M ± SD)	After 6 months (M ± SD)	test of significance
PI	1.32±0.29^{ab}	0.49±0.21^{a}	0.47±0.22^{b}	p1<0.001* p2<0.001* p3=0.09
GI	1.44±0.19^{ab}	0.488±0.20^{a}	0.46±0.18^{b}	p1<0.001* p2<0.001* p3=0.16
PD	6.50±1.35^{ab}	3.80±1.23^{a}	3.80 ±1.32^{b}	p1<0.001* p2<0.001* p3=1.0
CAL	7.0±1.94^{ab}	4.80±1.81^{a}	5.10±1.52^{b}	p1<0.001* p2<0.001* p3=0.08
Bone Height	6.5±1.35^{ab}	3.10±0.88^{a}	3.50±1.08^{b}	p1<0.001* p2<0.001* p3=0.34

P1: difference between Base line and after 3 months of treatment, **P**2: difference between Base line and after 6months of treatment, **P**3: difference between 3 months and 6 months after treatment
Test used for comparison between Tim: repeated measure ANOVA followed by post-hoe Bonferroni
*p value is highly significant at level ≤0.001
*p value is significant at level ≤0.05
M = Mean, SD = Standard deviation
Similar superscripted letters within same row denote significant difference between groups

Table 4: Mean &Standard deviation values of periodontal parameters indices and bone height level after 3 months and 6 months for group IV

Group IV	Base line (M ± SD)	After 3 months (M ± SD)	After 6 months (M ± SD)	test of significance
PI	1.49±0.35^{ab}	0.65±0.11^{ac}	0.60±0.14^{bc}	p1<0.001* p2<0.001* p3=0.012*
GI	1.52±0.29 ^{ab}	0.671±0.11 ^{ac}	0.46±0.18 ^{bc}	p1<0.001* p2<0.001* p3<0.001*
PD	5.20±0.92 ^{ab}	3.0±0.67 ^{a}	2.90±0.74 ^{b}	p1<0.001* p2<0.001* p3=0.34
CAL	5.20±1.23 ^{ab}	3.60±0.97 ^{a}	3.30±1.06 ^{b}	p1<0.001* p2<0.001* p3=0.08
Bone Height	5.20±0.92 ^{ab}	2.20±0.79 ^{a}	2.0±0.82 ^{b}	p1<0.001* p2<0.001* p3=0.51

P1: difference between Base line and after 3 months of treatment, **P**2: difference between Base line and after 6months of treatment, **P**3: difference between 3 months and 6 months after treatment
Test used for comparison between Tim: repeated measure ANOVA followed by post-hoe Bonferroni
*p value is highly significant at level ≤0.001
*p value is significant at level ≤0.05
M = Mean, SD = Standard deviation
Similar superscripted letters within same row denote significant difference between groups

Fig.1. showed infra bony defect related to the mesial surface of upper left central incisor.

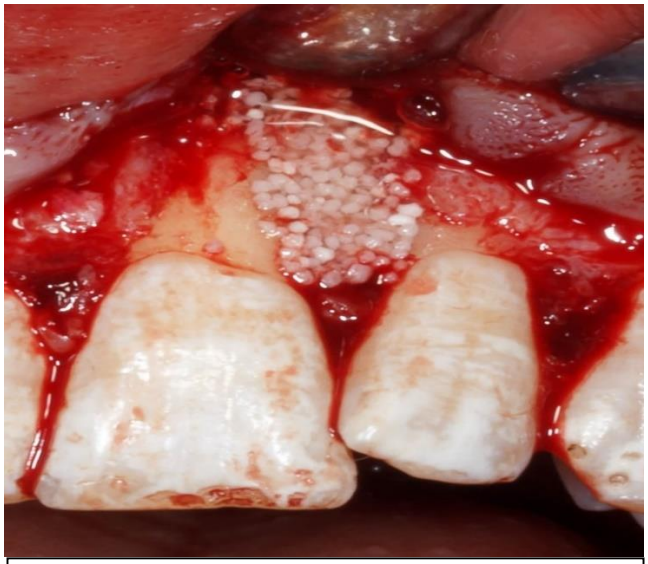

Fig.2. showed bone graft material (HA+ βTCP) into the periodontal bony defect

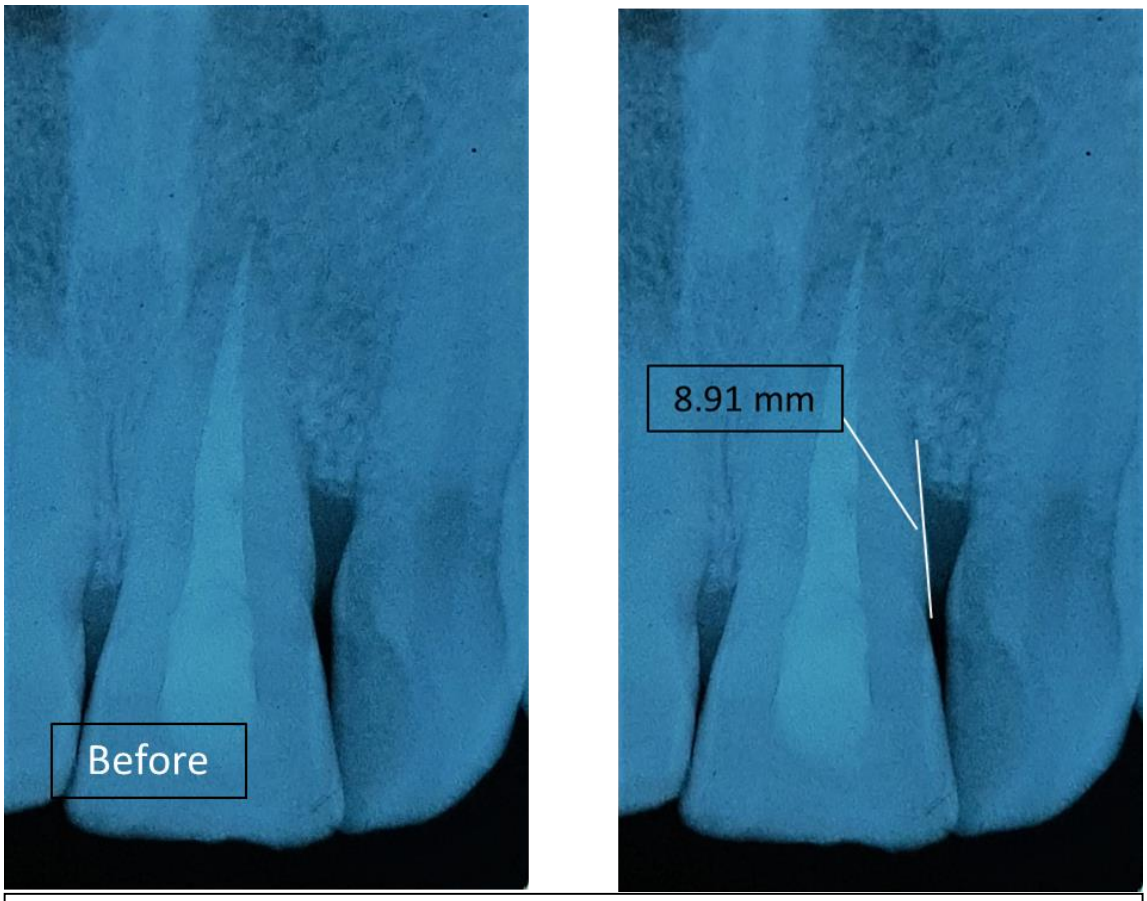

Fig.3. periapical radiograph showed the bone loss at the baseline before the treatment.

Fig.4. periapical radiograph showed the bone gain after 6 months after treatment.

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