Clinical and Radiographic Evaluation of Influence of Autologous Platelet Concentrates on Healing of Intra-Bony Defects

Amjad Shaikh[1], Abdul Salik[2], Amol Manoj Karande[3], Aashima Gupta[4], Anoop Gore[5], Nitin Lokhande[6]

ABSTRACT

Introduction: An important aim of periodontal therapy is to obtain a reduced pocket depth with gain in attachment apparatus. Various bone graft materials have been searched since decades, β-Tricalcium phosphate (TCP) has been shown to stimulate bone formation. Platelet-rich plasma (PRP) / Autologous Platelet Concentrate (APC) is a concentrated suspension of growth factors that has been demonstrated to induce healing and regeneration of tissues, including those in the periodontal region. The aim of this study was to investigate the influence of autologous platelet concentrate on the periodontal regeneration when used in combination with bone graft material.

Material and Methods: Fourteen patients with thirty intrabony defects were recruited for the study. Patients were randomly divided into test group (this site received β-TCP mixed with APC), control group (this site received β-TCP without APC). Clinical examination of probing pocket depth (PPD), clinical attachment level (CAL) and gingival recession (GR) were recorded at baseline, 3 months, 6 months and 9 months.

Results: Both test and control group showed statistically significant; reductions in Probing pocket depths (PPDs), recession coverage and gain in clinical attachment level (CAL) after 3, 6, and 9 months compared with the baseline situation. Inter-group comparison revealed statistically significant increase in CAL and recession coverage in test site as compared to the control site. The difference in probing depth reduction between two groups was non-significant.

Conclusions: Autologous Platelet Concentrate enhances periodontal regeneration when used in combination with bone graft material.

Keywords: Autologous Platelet Concentrate, β-Tricalcium phosphate, periodontitis, angular defect, clinical attachment level.

INTRODUCTION

Periodontitis is infectious disease damage to the periodontium in the form of periodontal attachment and bone loss.[1] The treatment of periodontal diseases falls into two distinct categories. The first is the treatment of the infections that causes the disease. The second is the regeneration or replacement of tissues that were destroyed by the infection. In the last two decades, there has been an exponential increase in the development and utilization of new materials and procedures to regenerate the periodontal attachment and alveolar bone.

An important goal of periodontal therapy is to obtain a reduced pocket depth with gain in attachment in order to arrest further disease progression. Usually, this goal can be accomplished by non-surgical therapy in patients with moderate periodontitis, whereas in advanced cases, particularly in the presence of intrabony defects, the treatment must be supplemented with periodontal surgery.

The techniques currently and routinely used in the field of regenerative periodontics include; Bone grafts, Guided tissue regeneration, Growth factors, Tissue engineering. Platelet rich plasma (PRP) / Autologous Platelet Concentrate (APC) has various growth factors which enhance the healing of wound and rapid regeneration of lost supporting periodontal structure.[2] This finding led to the use of APC to get more predictable results.

Various bone graft materials have been searched since decades, the focus began to shift to calcium phosphate ceramics in the 1970s. Tricalcium phosphate (TCP) has been shown to stimulate bone formation, and is comparable or in most cases superior in this regard to hydroxyapatite.[3] It has been shown to stimulate bone formation to a greater extent than hydroxyapatite, but to a much lesser extent than Bioglass.[3,4] The calcium phosphate in the TCP is mostly the β–whitlockite form and hence it is also referred to as the β-TCP. This graft is thought to stimulate bone formation. It is also the only known graft material to resorb at a rate that parallels new bone formation.[5]

Platelet-rich plasma (PRP) / Autologous Platelet Concentrate (APC) has been defined as the volume of autologous plasma that has a platelet concentration above the baseline. PRP is a concentrated suspension of growth factors that has been demonstrated to induce healing and regeneration of tissues, including those in the periodontal region.[6] Promising results in new bone formation by PRP were first demonstrated in 1998when treating mandibular defects by using a combination of autogenous bone graft with PRP.[7] The radiographic maturation rate was 1.62 to 2.16 times faster than the group without PRP. Platelet rich plasma is procured from the whole blood which is taken from the patient before the surgery and is then centrifuged. The concentration of platelets is then mixed with calcium chloride.[8] After activation, the alpha granules of platelets release a myriad of proteins and growth factors, which are important in the healing process.[9] Beta tri calcium bone graft in combination with platelet rich plasma is a very good alternative for new bone regeneration.[10]

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Tissue engineering is a newly emerging biomedical technique that involves the artificial manipulation of cells to promote tissue and organ regeneration. Tissue engineering *in vivo* utilizes the natural healing process of the body, which subsequently achieves natural regeneration of tissues and organs.[11] Regenerative periodontal therapy using Autologous Platelet Concentrate (APC) and different types of bone substitutes with or without guided tissue regeneration (GTR) has been proposed as one of the modalities to enhance the outcome of regenerative surgery.
Therefore the aim of this study was to investigate the influence of autologous platelet concentrate on the periodontal regeneration when used in combination with bone graft material.

MATERIAL AND METHODS

The study was designed as a randomized clinical case-controlled study, which was approved by the Institutional Ethical and review Committee of the institution. The sample selection was done by simple random sampling technique. Fourteen patients with thirty defects were recruited from the out-patient Department of Periodontology and Implantology of the Hospital. Out of the fourteen patients seven were females and seven were males with age ranging from 20 years to 55 years (mean age 34 ± 10.57 years).
Each patient showed at least one pair of deep intra-bony, inter-proximal periodontal defects, except in one patient who is having two pairs of defects each on different quadrants, with a probing pocket depth (PPD) of at least 6 mm, and radiographic evidence of angular bone loss of at least 4 mm at baseline. None of the intra-bony defects showed a furcation involvement.
Patients were explained about the study and written consents were obtained from the patients. Inclusion criteria for patient selection were; chronic periodontitis, presence of at least two intrabony defects, one on each quadrant or contra lateral sides of same arch with probing pocket depths (PPD) of at least 6 mm and radiographic evidence of angular bone loss of at least 4 mm at baseline, patients who were free from systemic diseases, patients who had a good level of oral hygiene maintenance.
Patients were excluded if they showed; unacceptable oral hygiene maintenance after phase I therapy, mobility of the affected tooth which was more than 1 mm in total, prosthetic restoration or endodontic treatment on the affected tooth, allergy to medicine, periodontal therapy in the last 6 months, pregnant and/or lactating mothers, habit of smoking.
Thirty selected sites from 14 patients were randomly divided into Experimental Site A and Experimental Site B according to the type of treatment to be received, each site in different quadrants. All patients received initial periodontal therapy consisting of oral hygiene instructions, supra and subgingival scaling and root planing prior to the beginning of the study.
Experimental site A (test group): This site received β-tricalcium phosphate bone graft (β-TCP) mixed with Autologous Platelet Concentrate (APC).
Experimental site B (control group): This site received β-tricalcium phosphate bone graft (β-TCP) without Autologous Platelet Concentrate (APC).
In order to standardize the probing technique, customized acrylic stents with guiding grooves was provided. University of North Carolina Probe (UNC-15) along with occlusal stent was used to measure the different clinical parameters and recorded to the nearest millimeter. Clinical and radiographic examination was performed both at baseline and after 3,6 and9 months after surgery.
Clinical examination of probing pocket depth (PPD), clinical attachment level (CAL) and gingival recession (GR) were recorded for the assessment of the healing results after the regenerative therapy. Hard tissue changes following therapy in the defect region were assessed by using radiovisiography (RVG).

STATISTICAL ANALYSIS

All the clinical parameters recorded were subjected to the following statistical analysis. To analyse the effect of the treatment in both the groups over a study period of 9 months at the regular intervals from baseline paired "t" test was applied for all the parameters. Intergroup comparisons were made using independent "t" test and at 48 degrees of freedom and at 95% confidence interval.

RESULT

A total of 14 subjects with 30 defects were enrolled in the present study. They were grouped as: Experimental site A (test group): This site received β-tricalcium phosphate bone graft (β-TCP) mixed with Autologous Platelet Concentrate (APC). Experimental site B (control group): This site received β-tricalcium phosphate bone graft (β-TCP) without Autologous Platelet Concentrate (APC).
The results are described in the given tables as; Table.1 shows intra-group comparison from baseline to 9 months in test and control group for reduction in probing pocket depth, and intergroup comparison between test and control group from base line to 9 month for mean reduction in probing pocket depth. Table. 2 shows intra-group comparison from baseline to 9 months in test and control group for gain in clinical attachment level, and intergroup comparison between test and control group from base line to 9 month for mean gain in clinical attachment level. Table. 3 shows intra-group comparison from baseline to 9 months in test and control group for change in gingival marginal level, and intergroup comparison between test and control group from base line to 9 month for mean change in gingival marginal level.

DISCUSSION

Findings from animal studies have provided histologic evidence of periodontal regeneration following short-term application of polypeptide growth factors like platelet-derived growth factor (PDGF) and insulin-like growth factor (IGF).[12-15] Platelet-rich plasma (PRP), an autologous volume of plasma with a high platelet concentration, has been shown to contain a high concentration of several growth factors.[16]
Material widely employed in dental and periodontal surgery is ß-tricalcium phosphate (ß-TCP) a purified, microcrystalline porous form of calcium phosphate with a Ca/PO4 ratio similar to natural bone. Histological studies in animals and humans have demonstrated that ß-TCP is biocompatible, may be incorporated into host bone, remodeled and eventually replaced by the host bone.[17,18]
In the present study, the APC was produced by withdrawing 10 ml of blood from the anticubital region of the patient's forearm using a 21 gauge needle and syringe and collected into the

10 ml blood collection tube coated with 3.2% sodium citrate solution used as an anticoagulant. 21 gauge needle was used to avoid rupture of platelets. Although EDTA gave higher yield of platelets than citrate, 3.2% sodium citrate was preferred. The importance of this relates to the fact that growth factors are actively extruded from the platelets during exocytosis. After proper mixing, the tubes were placed in centrifugation machine (Manual Centrifugation Machine- e-tek) and two cycles of rotation were carried out, first 1000 rpm for 13 minutes and then 2000 rpm for 10 minutes respectively. It was the modification of technique followed in another study after various trials.[19]

Before application to the test defect, the platelet concentrate was re-activated by adding a 10% calcium chloride solution in a 6:1 ratio. This ratio was determined as the minimal amount of $CaCl_2$ neutralizing the citrate effect and causing the clotting of the platelet concentrate. This corresponds to the data of Marx et al.[16] Other authors used ratios of 5:1–3:1 for reactivation.[20,21]

A multicentre study reported positive clinical and radiographic healing outcomes using β-TCP, combined with recombinant PDGF in intra-bony periodontal defects.[22] Additionally, an animal study found strong indications by histomorphometric and densitometric analysis, that platelet concentrates may accelerate the remodelling process of β-TCP and facilitate the formation of hard tissue similar to autologous bone.[23]

In the present study, both test and control procedures provided statistically significant; reductions in Probing pocket depths (PPDs), recession coverage and gain in clinical attachment level (CAL) after 3, 6, and 9 months compared with the baseline situation.

Inter group comparison revealed statistically significant increase in clinical attachment gain and recession coverage in test site as compared to the control site at the end of 9 month. The PPD reduction at 3, 6 and 9 months postoperatively was greater in test sites than control site. However, the mean difference between the two groups was statistically not significant.

In both groups, at the end of 9 months, all the sites gained ≥ 3 mm of CAL except in two control sites. Around 53.3% of the test and all the control sites revealed a clinical attachment gain of ≥ 4 mm at the end of 9 months. In former studies, a CAL gain of at least 4 mm was found in 51.6%[24], 38.7%[25], 69%[26] cases. In the present study, there was clinically accelerated gain of CAL and reduction in recession in sites where APC was used in contrast to sites where APC was not used at the end of 9 months postoperatively. The observations of the present study were in agreement with another study by Nevins et al.[22] who observed clinically an accelerated CAL gain and reduced increase in REC 3 months after combined therapy with recombinant PDGF and β-TCP compared with periodontal defect fill with β-TCP alone. Defect fill is the expected desirable outcome of periodontal regenerative therapy. Radiographic parameter is one of the important parameter to check the amount of defect fill and is correlated with other clinical parameters to know the actual regeneration of periodontal supporting structures.

Furthermore, the radiographically recorded area of intrabony defect fill in both test and control sites was around 80%, while it was only between 30% and 40% in a previous study using GTR membranes alone.[24] However, neither the clinical measurements nor the radiographic analysis can distinguish between the true periodontal regeneration and the defect fill by the radiopaque TCP granules. The true healing mode can only be verified by histological means. A further reason for the relatively good attachment gains may be the relatively high baseline depth of defect in the present study. Previous studies have shown a positive correlation between an increased baseline depth of the intra-osseous component and the final regeneration outcome.[25,26]

CONCLUSION

Highly significant reduction in probing pocket depth took place in both the groups over a nine month time period however the comparison between groups was not significant. There was significant gain in clinical attachment level in test site as compared to control site at the end of nine months, although gain in clinical attachment level was highly significant in both the groups. Comparison between the two groups revealed greater recession coverage at the end of nine months in test group. Radiographically, a highly significant percentage of intrabony defect fill was noticed without any significant difference between the two groups.

REFERENCES

1. Socransky SS, Haffajee A D. The nature of periodontal diseases. Ann Periodontol. 1997;2:3-10.
2. The American Academy of Periodontology. Position paper – The potential role of growth and differentiation factors in periodontal regeneration. J Periodontol. 1996;67:545-53.
3. Fetner AE, Hartigan MS, Low SB. Periodontal repair using PerioGlas in nonhuman primates: clinical and histologic observations. Compendium. 1994:932;935-8; quiz 939.
4. Wilson J, Low SB. Bioactive ceramics for periodontal treatment: comparative studies in the Patus Monkey. J Appl Biomater. 1992;3:123-9.
5. Mayubala, M Shivkumar, R. V. Sureshkumar, T P Sastry. Bioactivity and Osseointegration Study of Calcium Phosphate Ceramic of different chemical composition. J Biomedical Materials Research. 2002;63:2:200-8.
6. Arlson N E, Roach R B Jr. Platelet rich plasma; Clinical Applications in Dentistry. J Am Dent Assoc. 2002;133:1383-6.
7. Marx R E, Carlson E R, Eichstaedt R M et. al. Platelet rich plasma- Growth Factor enhancement for one Grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998; 85:638-46.
8. Eduardoalitua. Plasma rich ingrowth factors; Preliminary Results of Use in the preparation of future sites for implants. Int. Journal of Oral Max. Implants. 1999;14:529-35.
9. Grageda E. Platelet Rich Plasma and Bone Graft Materials. Implant Dent. 2004;13:301-9.
10. Christgau M et al. Influence of autologous platelet concentrate on healing on intrabony defects following guided tissue regeneration therapy: A randomized Prospective Clinical Split-Mouth Study. J Clin Periodontol. 2006;33:12:908-21.
11. Tabata Y. Recent Progress in Tissue Engineering. DDT. 2001;6:483-7.
12. Lynch SE, Williams RC, Polson AM, Howell TH, Reddy MS, Zappa UE, Antoniades HN. A combination of platelet derived and insulin derived growth factor enhances periodontal regeneration. J Clin Periodontol. 1989;16:545-8.
13. Wang HL, Pappert TD, Castelli WA, Chiego Jr. DJ, Shyr Y and Smith BA. The effect of platelet derived growth factor on the cellular response of the periodontium: An auto-

radiographic study on dogs. J Periodontol. 1994;65:429-36.
14. Rutherford R B, Niekrash CE, Kennedy JE,Charette MF. Platelet derived and insulin like growth factors stimulate regeneration of periodontal attachment in monkeys. J periodontal res. 2002;27:285-90.
15. Giannobile WV, Finkelman RD and Lynch SE. Comparison of canine and non-human primates animal models for periodontal regenerative therapy: Results following the single administration of PDGF/IGF-I. J Periodontol. 1994;65:1158-68.
16. Marx, R. E., Carlson, E. R., Eichstaedt, R. M., Schimmele, S. R., Strauss, J. E. and Georgeff, K. R. Platelet-rich plasma: growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998;85:638-46.
17. Saffar JL, Colombier ML and Detienville R. Bone formation in tricalcium phosphate filled periodontal intrabony lesion. Histological observation in humans. J Periodontol. 1990;61:209-16.
18. Buser D, Hoffmann B, Bernard JP, Lucci A, Mettler D, Schenk RK. Evaluation of filling materials in membrane protected bone defects. A comparative histo-morphometric study in the mandible of miniature pigs. Clin Oral Impl Res. 1998;9:137-50.
19. Gonshor A. Technique for producing platelet rich plasma and platelet concentrate.: Background and process. Int J Periodont Rest Dent. 2002;22:547-57.
20. Appel, T. R., Pötzsch, B., Müller, J., von Lindern, J.-J., Bergé, S. J. and Reich, R. H. Comparison of three different preparations of platelet concentrates for growth factor enrichment. Clin Oral Impl Res. 2002;13:522–8.
21. Kassolis, J. D., Rosen, P. S. and Reynolds, M. A. Alveolar ridge and sinus augmentation utilizing platelet-rich plasma in combination with freeze-dried bone allograft: case series. J Periodontol. 2000;71:1654–61.
22. Nevins, M., Giannobile, W. V., McGuire, M. M., Kao, R. T., Mellonig, J. T., Hinrichs, J. E., McAllister, K. S., McClain, P. K., Nevins, M. L., Paquette, D. W., Han, T. J., Reddy, M. S., Lavin, P. T., Genco, R. J. and Lynch, S. E. Platelet-derived growth factor stimulates bone fill and rate of attachment level gain: results of a large multicenter randomized controlled trial. J Periodontol. 2005:76;2205-15.
23. Kovacs, K., Velich, N., Huszar, T., Fenyves, B., Suba, Z. and Szabo, G. Histomorphometric and densitometric evaluation of the effects of platelet-rich plasma on the remodeling of beta-tricalcium phosphate in beagle dogs. Journal of Craniofacial Surgery. 2005:16;150-4.
24. Christgau, M., Bader, N., Felden, A., Gradl, J., Wenzel, A. and Schmalz, G. Guided tissue regeneration in intrabony defects using an experimental bioresorbable polydioxanon (PDS) membrane. A 24-month split-mouth study. J Clin Periodontol. 2002:29;710-23.
25. Tonetti, Cortellini, Lang. Clinical outcomes following treatment of human intrabony defects with GTR/Bone replacement material or access flap alone. A multicentre randomized controlled clinical trial. J Clin Periodontol. 2004;31:770-6.
26. Sculean, A., Chiantella, G. C., Windisch, P., Arweiler, N. B., Brecx, M. and Gera, I. Healing of intra-bony defects following treatment with a composite bovine-derived xenograft (Bio-Oss Collagen) in combination with a collagen membrane (Bio-Gide PERIO). J Clin Periodontol. 2005:32;720-4.
27. Tonetti, M. S., Pini-Prato, G. and Cortellini, P. Factors affecting the healing response of intrabony defects following guided tissue regeneration and access flap surgery. J Clin Periodontol. 1996:23;548-56.
28. Kornman, K. S. and Robertson, P. B. Fundamental principles affecting the outcomes of therapy for osseous lesions. Perio. 2000 2000:22;22-43.

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