Research Protocol

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Effectiveness of Injectable Platelet Rich Fibrin with Demineralised Freeze Dried Bone Allograft in Class II Furcation Defects: Protocol for a Randomised Clinical Trial

Dentistry Section

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ABSTRACT

Introduction: In cases of substantial furcation involvement, non surgical mechanical debridement results in disease progression. Surgery allows for root debridement, osseous recontouring, odontoblasts, and periodontal regeneration while maintaining periodontal attachment, to stop the disease process. The current study evaluates whether combining injectable-Platelet Rich Fibrin (i-PRF) mixed with Demineralised Freeze-Dried Bone Allograft (DFDBA) for treating class-II furcation diseases is successful.

Need for the study: In comparison to 'Open Flap Debridement' (OFD) alone, certain studies have shown that DFDBA results in a considerable and persistent increase in periodontal regeneration. The current study evaluates whether combining i-PRF mixed with DFDBA for treating class-II-furcation diseases is successful or not.

Aim: To compare evaluation of i-PRF with DFDBA compared to DFDBA alone in the treatment of the class-II furcation defects- a randomised controlled clinical trial.

Materials and Methods: The current investigation will be a randomised, double-blinded clinical trial, on total twenty-four patients of 'Class-II furcation' including the buccal/lingual sides of the tooth. By the completion of the first therapy and before the beginning of surgery, the chosen defects will be randomly allotted to group A and group B equally, using a computer-generated randomisation process employing a randomised table. Group A will operate with both i-PRF and DFDBA, whereas group B here will only be given DFDBA. To estimate outcomes, a re-evaluation will be done at six and nine months after starting therapy.

Keywords: Bone graft, Bone loss, Demineralised freeze-dried bone allograft, Injectable platelet-rich fibrin, Periodontitis, Periodontal regeneration

INTRODUCTION

Periodontitis is one of the chronic, multimicrobial, multifactorial inflammatory illnesses that affect the structures supporting the tooth in dentistry. It is linked to 'dysbiotic- (plaque) biofilms and progress for deterioration of periodontal tissues [1]. Non treated periodontal diseases turn into damaged periodontal- attachment and tooth-supporting structures, particularly in multirooted teeth. Only inter radicular 'clinical attachment loss' in furcation defect i.e., class-I is prevented by non surgical therapy, which includes the removal of supra with subgingival plaque and calculus [2].

Because of the ongoing connective tissue structure loss connection and alveolar bone resorption, 'Class-II furcation diseases' is a serious clinical worry [3]. For many years, periodontal treatment has focused on mechanical debridement to remove bacterial infection [4]. In cases of substantial furcation involvement, non surgical mechanical debridement frequently results in disease progression [5]. Surgery allows for root debridement, osseous recontouring, odontoblasts, and periodontal regeneration, while maintaining periodontal attachment, to stop the disease process [6].

Periodontal regeneration is one of the many areas that has benefited from tissue engineering, in which biomaterials (scaffolds), chemicals (growth factors), and stem cells are significant components in the regenerative procedure, which increases the quality and predictability of the technique [7]. Current or innovative biomaterials used in periodontal treatment often stimulate healing and reduce disease transmission risk. Growth factors are employed to stimulate periodontal tissues' inherent regeneration capacity [8].

The use of DFDBA for treating 'Class-II furcation' involvement has been studied extensively. DFDBA, which contains bone morphogenic proteins, is considered to promote host Mesenchymal Cells (MSCs) into osteoblasts, resulting in an osteoconductive and osteoinductive effect [9]. In comparison to OFD alone, certain studies or reviews have shown that DFDBA results in a considerable and persistent increase in bone fill. Collagen membranes by combining with bone grafts have been revealed to be effective for various periodontal abnormalities in studies [10]. The use of a membrane combining with a bone graft has the advantage of establishing clotting of blood stability [11].

The i-PRF is a newly researched or developed leucocyte-enriched platelet-rich concentrate that aids the regeneration of tissues and healing wounds more effectively. It has been observed that i-PRF has better antibacterial action against a variety of periodontal infections, as well as the ability to generate increased fibroblast migration and the production of high levels of growth factor [12]. Because of their 'supra-physiological' concentrations of their growth factors and the cells i-PRF is a very new form of concentrate rich in platelet that improves regeneration of the periodontal tissues. Although in the liquid phase, i-PRF generates a dynamic and fibrin gel that embeds platelets, leucocytes, 'Collagen type I-(COL1)', 'Osteocalcin-(OC)' and the growth factors, while allowing for gradual growth factor release. By boosting the reproduction of 'Human-MSCs and initiating osteogenic differentiation of MSCs, i-PRF may stimulate the regeneration capacity of intrinsic tissue [13].

Cone Beam Computed Tomography (CBCT) can be used to evaluate treatment outcomes, particularly to check healing following grafting or regeneration. This imaging technique can equally be used to measure the 'Gingival-tissue' as well as the dimensions of the 'Dentogingival unit' [14,15]. A 3D scans, such as CBCT, can provide details regarding faults that are not visible on 2D pictures [16]. Because CBCT is anticipated to identify marginal bone shapes, infrabony, and furcation defects, it can be used to assess and plan therapy for molars with furcation involvement [17].

To the best of our knowledge, this is the first clinical trial to use the biomaterials listed above. As a result, the current study evaluated whether combining i-PRF mixed with DFDBA for treating Class-II furcation diseases is successful.

Objectives

- For evaluation of the effectiveness of i-PRF mixed DFDBA in the therapy of Class-II furcation diseases in terms of radiographic bone fill, decrease in PPD and CAL gain.
- For evaluation of the effectiveness of DFDBA in the therapy of Class-II furcation diseases along the terms of radiographic bone fill, decrease in PPD and CAL gains through CBCT.
- To compare the efficiency of DFDBA along with 'i-PRF' in the therapy of Class-II furcation diseases regards to radiographic bone fill, decrease in PPD and CAL gain.

REVIEW OF LITERATURE

The use of DFDBA in the treatment of 'Class-II furcation' involvement has been widely researched. Certain research or reviews have demonstrated that DFDBA results in a significant and long-lasting increase in bone fill when compared to OFD alone, according to Mehta DB et al., (2018). DFDBA is still a feasible therapy option for periodontal attachment apparatus regeneration [10].

The PRF is an immune and platelet concentrate that offers various advantages over platelet-rich plasma, including easier processing, no biochemical alteration, and prolonged growth factor release. The ability of PRF to augment the regenerative effects of DFDBA in the treatment of mandibular degree Class II furcation defects was investigated by Basireddy A et al., [18]. They found that PRF appears to favour soft-tissue healing but has no additional benefit in bone regeneration when used in combination with DFDBA.

The i-PRF is a very new form of concentrate, rich in platelet that improves the regeneration of the periodontal tissues. Dsa E et al., (2020) evaluated the efficacy of i-PRF to PRF in the treatment of infrabony deformities. Total 54 sites were split into 3 groups; namely group 1: 'Open-flap debridement' only, group 2: OFD accompanied by i-PRF, and group 3: OFD accompanied by PRF [12]. When compared to group 1, group 2 and group 3 exhibited improved results in all parameters (PPD, RAL, PI, GI at baseline, six and nine months). OFD-41.59%, IPRF-72.75%, and PRF-62.11% defect depth reduction at nine months after the operation. i-PRF, PRF were showing good clinical outcomes and radiographic outcomes than OFD for treating infrabony periodontal disease or defects in periodontitis, according to the authors [12].

MATERIALS AND METHODS

The current investigation will be a randomised, double-blinded, clinical trial for a period of nine months on total 24 systemically healthy subjects of moderate to advanced range of chronic periodontal diseases and Class-II furcation diseases in lower (mandibular) buccal as well as lingual side of the tooth, aged between 30-50 years, undergoing periodontal therapy at the Department of Periodontics will be selected.

All procedures in the study involving human participants will be carried out in compliance with the Institutional and/or National Research Committee's Ethical standards, as well as 1964 Helsinki statement and its subsequent revisions or comparable Ethical standards. The Institutional Ethics Committee of Datta Meghe Institute of Medical Sciences, Sawangi, Meghe, Wardha, Maharashtra, India has accepted the study methodology under the Ref. no. DMIMS (DU)/IEC/2022/751; 14 February 2022 Trial Acknowledgement number: REF/2022/06/054903, This trial is registered with Clinical Trial Registration (CTRI) Number: CTRI/2023/02/049517.

Inclusion criteria:

- Class-II furcation diseases of lower molars having buccal/lingual involvement [3].
- A 3 mm horizontal furcation probing depth {Horizontal Defect Depth (HDD)}.
- Vertical furcation probing depth of less than 3 mm {Vertical Defect Depth (VDD)}.
- The proximal bone height of the selected tooth should be coronal to the inter-radicular bone level.
- An ample aggregate of keratinised tissue is present.
- The selected tooth should be having undamaged surfaces near the furcation area and respond to an electric pulp test.
- The chosen tooth's gingival edge should be to the coronal and directed toward furcation fornix.
- Selected patient who are systematically healthy.
- Radiographic evidence of molar furcation defects seen on buccal/lingual/mesiobuccal or distobuccal surfaces.

Exclusion criteria:

- Patients not adhering to the continuance of the periodontal maintenance program.
- Patients having a habit of smoking or consuming tobacco.
- Patients having mobility in a chosen tooth.
- Allergic reactions to the graft material which will be used for the procedure, local anaesthetics, chlorhexidine content, antibiotics drugs, or analgesics.
- Any previous periodontal regeneration procedures performed at chosen location or site.
- Females who are pregnant, childbearing or nursing.
- Infectious diseases in patients like hepatitis, Human Immunodeficiency Virus (HIV), or tuberculosis.
- Patients having a history of systemic illness, as well as pregnant women.

In a carefully designed chart, information regarding nutritional or dietary status, oral or dental hygiene practices, patients of systemic background, gingival as well as, the periodontal state will be recorded. For examination, mouth mirror and University of North Carolina-15 (UNC-15) probe will be used in patients. The purpose and plot of the study will be well-explained to subjects at the start and a signed informed consent from the patients will be taken.

Sample size calculation: Sample size formula for difference between two means is as below:

$$N=\frac{(z_{\alpha}+z_{\beta})^2(\delta 1+\delta^2_{2/k})}{\Delta^2}$$

Where;

N=12 patients needed in each group

A total of 24 systemically healthy subjects of moderate to advanced range of Chronic Periodontal Diseases and Class-II furcation diseases in lower (mandibular) buccal as well as lingual sides of tooth, aged between 30 to 50 years, undergoing periodontal therapy. This one is calculated by referring to the data of the earlier research by Bevilacqua L et al., 2020 and SPSS, version 27, open-source calculator- SSMean and the counting is 20, therefore a round figure of 24 samples will be taken for the present study [2].

Allocation concealment mechanism: On confirmation of eligibility, the clinical site furcation defects will be divided by computerised randomisation, according to case numbers. The defects will be split into group A and group B, each consisting of 12 defects. Generation of allocation sequence and enrollment of participants, the assignment of participants to interventions will be performed by authors in the Department of Periodontics and Implantology.

Intervention

Initial therapy: Before surgery, each patient will be given detailed instructions on how to maintain proper oral hygiene. By a thorough examination and diagnosis, Scaling and Root Planning (SRP) will be performed under local anaesthetic. Six weeks after starting treatment, a review will be performed to see how the patient is responding to the treatment and to determine whether periodontal surgery is necessary. To standardise probe location and angulations, a custom-made occlusal acrylic stent is to be constructed. The occlusal stent will be covering the occlusal side of the selected tooth, as well as, the occlusal side of one (tooth) distant and mesial to that until the coronal third of the tooth is involved. A reference point for the placement of the periodontal probe will be made at the deepest site of the affected tooth. Before initiating the surgery, furcation defects will be divided by computerised randomisation, according to case numbers. The defects will be split into group A and group B, each consisting of 12 defects. Group A will be managed by DFDBA combined with i-PRF, while group B will be managed by DFDBA alone.

All procedures will be done by one operator (US), while pretreatment and post-treatment clinical and radiographic assessments will be performed by another operator (PB), who will be blind to the type of treatment the patients will get. Patients will not be knowing in which group or treatment they will have. SRP will be conducted at baseline until the operator deemed the root surface to be smooth and clean (US). After treatment, no antibiotics or anti-inflammatory drugs were prescribed.

Clinical measurements: This evaluation criterion includes: i) Plaque Index (PI); ii) Papillary Bleeding Index (PBI); iii) Probing Pocket Depth (PPD); iv) Relative-Clinical Attachment Level (R-CAL); v) Relative Gingival Marginal Level (R-GML); and hard tissue evaluatory measurements include roof of furcation site to the crest of the bone and CEJ-D/CEJ-M: Cementoenamel Junction to mesial/distal (Interdental Bone Crest). All the clinical measurements will be taken on the day of surgery, and follow-up will be done after six months and nine months. The present study's primary end-point will be the radiographic bone fill of the defect, with outcomes including a decrease in PPD and a gain in CAL.

I) Indices: The full-mouth PI will be calculated before anaesthesia, at baseline, and after six months using the Plaque Index (PI) [19], whereas gingival inflammatory measurements will be done by using the Papillary Bleeding Index (PBI) [20].

II) Probing measurements: A surgical stent and a UNC-15 calibrated periodontal probe will be used to take the measurements (University of North Carolina, Hu-Friedy). The: i) Vertical-PPD (V-PPD); ii) R-CAL; iii) R-GML will be measured from three sites for each furcation surface: Distal/Mesial line angle, Midbuccal/Midlingual surface. For analysing the results, only the very deepest measurement (defect) will be measured. The UNC-15 probe will be inserted vertically into slots cut into that acrylic stent. A R-GML will be recorded from the stent's inferior border to the R-GML. The UNC-15 probe will be used to measure the bottom of the pocket and the stent's inferior border distance to determine the R-CAL. The V-PPD will be calculated from the pocket base to the margin of the gingiva. The Horizontal-PPD (H-PPD) will be measured by a curved colour-coded furcation probe. (0-3, 3-6, and 6-9 mm markings) [5].

Width of Keratinised Gingiva (WKG) will be determined by UNC-15 probe. The results of all measurements will be taken. Then the UNC-15 probe will be inserted vertically into slots cut into the acrylic-made stent, and an R-GML will be recorded from the stent's inferior border to the R-GML. The UNC-15 probe will be used to measure the distance between the pocket base and the stent's inferior border to determine the R-CAL. The V-PPD will be measured from the pocket base to the margin of the gingiva. The H-PPD will be measured using a curved colour-coded furcation probe (0-3, 3-6, and 6-9 mm markings) [5].

III) Radiographic Measurements: Vertical measurements component will be taken in the sagittal view by using CBCT:

- The Cemento-Enamel Junction (CEJ) was detected, and the horizontal line was drawn- from the mesial to the distal of the tooth, linking the CEJ.
- A Perpendicular Line was drawn- running from the centre of the tooth to the middle to the furcation until it reaches the alveolar crest area, and the alveolar crest area and the place where the above metioned line joins that distance measured as the first line.

In axial view, using CBCT, the horizontal component measurements were made:

- A line will be drawn on the most buccal end of one root till the other one.
- After that, a Perpendicular Line will be drawn in the center of the first line to the beginning of the bone trabeculae [21].

Surgical Procedure for group A: Patients will be asked to gargle for one minute with 0.2% of chlorhexidine-gluconate solution before the surgery. During the procedure, asepsis will be maintained. By nerve block and infiltration, xylocaine of 2% consisting of 1:80,000 epinephrine concentration anaesthetic solutions will be utilised to anaesthetise the area. Among all anaesthetics, lignocaine is regarded as the gold standard [22]. Intra-crevicular incisions will be made on the lingual or buccal locations of the affected tooth with surgical blade no. 12 or 15. To achieve primary wound closure, the incisions will be performed far interproximally for securing the interdental papillae. The flap will cover the area proximal and distal to the affected tooth.

A full thickness-mucoperiosteal flap will be raised at the afflicted site by a periosteal elevator instrument to reveal the underlying defect margin (24 G Hu-Friedy, USA). When the removal of the granulation tissue process is performed, considerable caution will be used to avoidance of perforation of the flap (papilla) loss. To remove the pocket epithelium, the flap will be reflected. Hand

instruments, ultrasonic instruments, and furcation curettes will be used to debride the denuded particles of root surface by using the dome of furcation. The root surfaces and furcation faults will be planned until a smooth, firm consistency is produced.

Intraoperative measures of horizontal, as well as vertical-depth at the furcation area, will be taken after irrigation with physiologic saline solution and attaining haemostasis: i) H-PPD: The furcation site will be measured horizontally at the deepest area by using a UNC-15 probe and another (probe) inserted at the prominence of the root surface as a reference to make a bridge with the probe; ii) V-PPD: The furcation site will be measured vertically at the furcation fornix's site at the deepest area (fixed reference point). The final and exact patient's eligibility for the study will be confirmed only, if the furcation defect depth is less than 3 mm vertically and horizontally.

Preparation of i-PRF: A standard protocol according to Mourão CF et al., is 10 mL of venous blood will be taken under aseptic circumstances by venipuncture of the (antecubital) vein and transferred to fresh sterile test tubes centrifuged at 700 rpm for three minutes to produce i-PRF. The i-PRF fluid, which is orange in colour, will be collected in a syringe and injected [13].

In group A, i-PRF will be injected into the furcation defect, followed by i-PRF mixed with DFDBA (manufactured by the Tissue Bank of Tata Memorial Hospital).

Surgical Procedure for group B: The site or location will be made completely isolated as well as haemostatic. DFDBA will be condensed, covered, and stabilised once the furcation defect has been completely debrided, and after this suture will be placed on the flap. A periodontal pack or coe-pack will be administered to the patient.

Postoperative care: A non steroidal anti-inflammatory and antibiotics will be provided for five days after the completion of surgery. Brushing will be avoided on the affected site and for almost 4-6 weeks and all the patients will be asked to gargle with (0.2%) Chlorhexidine gluconate twice in a daytime for atleast one minute. To avoid any damage to the pack, patients will be notified. After seven days, the periodontal (coe-pack) dressing and stitches will be removed, and the healing will be assessed. Irrigation (using saline), polishing will be performed using polishing paste, and rubber cup, with caution to avoid damage to the affected site. The patients will be asked to clean the area of the surgical site with the cotton pellets in an apical-coronal orientation, followed by a soft toothbrush. Follow-up will be done at six months and nine months.

Maintenance care: At six, and nine months after surgery, the subjects will be re-evaluated. At each follow-up appointment, patients will receive dental hygiene advice, as well as, a complete oral prophylaxis will be done using ultrasonic scalers. No clinical measurements will be taken during the first six months after the surgery.

Re-examination: At the sixth and nine month follow-up visit, a full review will be performed. All clinical variables will be assessed. Standardised radiographic evaluation will be conducted and a CBCT examination will be done for the same.

STATISTICAL ANALYSIS

The data will be analysed using SPSS (version 27.0). Power calculations will be performed using the Student's t-test before the study initiates [2].

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