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IN VITRO EVALUATION OF PLATELET COUNT AND ANTIMICROBIAL EFFICACY OF INJECTABLE PLATELET-RICH FIBRIN VS. OTHER PLATELET CONCENTRATES

Dentistry

Dr. Anju K S Post Graduate Student, A. J. Institute of Dental Sciences Kuntikana, Mangalore, Karnataka

Dr. Nandini Manjunath Professor and Head of the Department of Periodontology, A. J. Institute of Dental Sciences, Kuntikana, Mangalore, Karnataka

Dr. Melba Liza D`souza Assistant professor Department of Periodontology A.J. Institute of Dental Sciences, Kuntikana, Mangalore, Karnataka.

ABSTRACT

Background And Objectives: Platelet concentrates are used in various medical procedures to promote soft- and hard-tissue regeneration. In recent times, their antimicrobial efficacy is also explored. However, various platelet concentrates have evolved which differ in the centrifugation protocols. One such recently introduced platelet concentrate is injectable platelet-rich fibrin (i-PRF) concentrate. Hence, the aim of the present study was to evaluate the antimicrobial property, and platelet count of i-PRF in comparison to other platelet concentrates, i.e., PRF, platelet-rich plasma (PRP), and control (whole blood).in chronic periodontitis patient. **Methodology:** Blood samples were collected from 16 patients who were diagnosed with chronic periodontitis (16 x 4 = 64 samples) of both genders between the ages of 30 and 60. Platelet concentrates were prepared using standardized centrifugation protocol and were divided in to four groups. Group I- i-PRF (16 samples), Group II -PRF (16 samples), Group III -PRP (16 samples), Group IV- Whole blood. Platelet count was evaluated by manual counting method using smear preparation of each sample. antimicrobial activity against periodontal pathogen was examined on blood agar (by streaking the plaque sample) using disc diffusion method by measuring zone of inhibition around each platelet concentrates. **Results:** The obtained results were tabulated and statistically analyzed with one-way ANOVA, $P < 0.05$ considered statistically significant. i-PRF concentrate had a considerably higher mean platelet count In comparison to prp, prf, and whole blood concentrates, differences were statistically significant. I-PRF concentrate also displayed a considerably greater mean Zone of Inhibition, in relation to prp, prf, and whole blood concentrates and the differences were statistically significant at ($p<0.001$), ($p=0.001$) and($p<0.001$). **Conclusion:** The results showed that i-PRF has maximum antimicrobial efficacy and higher platelet count in comparison to other platelet concentrates, there by indicating to have a better regenerative potential than other blood constituents.

KEYWORDS

platelet concentrates, i-PRF, PRF, PRP platelet count, zone of inhibition, antimicrobial efficacy,

INTRODUCTION

Periodontal disease is defined as a complex, multifactorial disease characterized by the loss of connective tissue attachment with destruction of periodontal tissues. The aim of periodontal therapy is to eliminate inflammatory process, prevent the progression of periodontal disease and also to regenerate the lost periodontal tissues. Periodontal regeneration is a complex multifactorial process involving biologic events like cell adhesion, migration, proliferation, and differentiation in an orchestrated sequence.1, Periodontal regenerative procedures include soft tissue grafts, bone grafts, root bio modifications, guided tissue regeneration, and combinations of these procedures[2]. Various biomaterials have been used for periodontal tissue regeneration in addition to autogenous and allogenic bone grafts but not a single graft material is considered as gold standard for the treatment of intrabony defects. There is evidence that the presence of growth factors and cytokines in platelets play key roles in inflammation and wound healing,[6].

Platelets are enucleated hematologic component derived from bone marrow precursor cells: megakaryocytes. The α granules are spherical or oval membrane-enclosed units with the cytoplasm. Diameter of the granules range from 200 to 500 nm each. TGF-β has chemotactic effect on osteoblastic cells and endothelial cells, inhibitory effects on osteoclasts, and initiates woven bone formation. The α granules after activation fuse with the cell membrane. Platelets also secrete fibrin, fibronectin, and vitronectin, which act as a matrix for the connective tissue and as adhesion molecules for more efficient cell migration[6,12]. Platelets play a key role in wound healing and hence wound healing after periodontal treatment can be accelerated by the use of platelet concentrates. Platelet concentrates have advanced from the first generation, platelet-rich plasma (PRP) to the second generation, platelet-rich fibrin (PRF). Previous research has demonstrated that PRF contains a greater number of GFs than PRP. It induces higher fibroblast migration and expression of the transforming growth factor-β1, the platelet-derived growth factor, and the vascular endothelial growth factor[14,15]. Thus, they ensure a better environment for regeneration and repair of the defects.[14]. PRP although being an autologous preparation requires the addition of thrombin and calcium for its activation. These additives can result in the development of antibody to the clotting factors V, XI, and thrombin, thereby adversely affecting the coagulation process and also can trigger an immune reaction. It is easy to prepare and has good handling characteristics. This fibrin network protects the growth factors from proteolysis. i-PRF was introduced based on the similar concept as that of PRF with added advantage of it being in injectable form. Its protocol is based on the concept that slower and shorter centrifugation spin results in a higher presence of regenerative cells with higher concentrations of growth factors[16,17]. Currently, PRF is widely utilized in the surgical treatment of periodontal intrabony defects, treatment of furcation defects, sinus lift procedures, and tissue engineering[14,18]. Since the standard PRF is not entirely appropriate for injection, I-PRF enables easier use of the platelet concentrate in a liquid state. After being generated during centrifugation, it maintains its liquid viscosity for about 15 minutes[14,19,20]. Initially, the PRF has been developed at high centrifugation speeds, enabling a formation of a fibrin clot, which could be utilized as a three-dimensional scaffold for the promotion of periodontal regeneration[14,21].

In addition to regenerative potential, the antibacterial activity of the platelet concentrates has been reported against staphylococcus aureus[22], Escherichia coli[23], Klebsiella pneumonia[24] and streptococcus oralis[25] among the other microorganisms. There is not much evidence for the antimicrobial activity of i-PRF against periodontal pathogens since i-PRF has been recently introduced[26]. Hence, the present study is designed to evaluate the platelet count, and antimicrobial efficacy of i-PRF in comparison with the other available platelet concentrates, i.e. Platelet rich fibrin (PRF), Platelet rich plasma (PRP), and control (whole blood).

MATERIAL AND METHODES

The patients were selected from the outpatient Department of Periodontics at A.J Institute of Dental Sciences, Mangalore. The sample size consists of 16 patients (16×4=64 samples) of both genders within the age group of 30 to 60 years. This study included patients in good general health who had been diagnosed with chronic generalized periodontitis with infra bony pockets and were scheduled for surgery. Ethical clearance was obtained from the ethical committee of A.J Institute of Medical Sciences and Research Centre. Prior to initiating the study, written informed consent was taken from all the participants after informing them about the purpose and procedure of the study. A

complete case history was recorded of all participants taking part in the study.

Patient having habit of tobacco chewing and smoking, Patient who had undergone for periodontal therapy in last 6 months, Patient with a history of infection or any antibiotic use for at least 3 months, Patient under anticoagulant or immunosuppressive therapy, Patient with bleeding or clotting disorders, Pregnant and lactating women were excluded from this study.

Sample Preparation

Plaque sample collection

Subgingival plaque samples were collected from the patients using an area-specific Gracey curette (Gracey Curette, Hu-Friedy Mfg. Co., LLC, Chicago, USA) from the deepest pocket site before scaling and root planning. The sample was immediately transferred to a 5-ml saline containing tube and vortexed for 5 minutes to obtain uniform solutions. Later, 0.1 ml of this suspension was used to plate blood agar using the streak method. (Department of Microbiology, A.J. Institute of Medical Sciences, Mangalore)

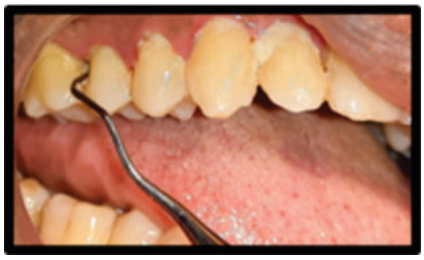


Fig 1 collection of plaque sample

Blood sample collection

A volume of 8 ml of venous blood was collected in a disposable syringe by venipuncture from the median cubital vein under asepsis conditions. 2 ml of blood was used for preparation of Injectable platelet rich fibrin (i-PRF), Platelet rich fibrin (PRF), and Platelet rich plasma (PRP); the remaining 2 ml with anticoagulant served as a control. During centrifugation, a tube containing 2 ml of saline served as a counterbalance.

Preparation of platelet concentrates

1. Injectable platelet rich fibrin preparation;

A volume of 2 ml blood collected in non-coated vacutainer without any addictives and centrifuged at 700 rpm for 3-4 min.

2. Platelet rich fibrin preparation;

A volume of 2ml blood collected in silicon-coated vacutainer without any addictives and centrifuged at 3000rpm for 10 min.

3. Platelet rich plasma preparation;

A volume of 2ml blood collected in 3.2% sodium citrate anticoagulant-coated vacutainer. Initially, soft spin at 1000rpm for 10 min, followed by centrifuging top layer of plasma at hard spin at 2000rpm for 20 min. Finally, half of the superficial plasma layer removed, and the platelet pellet suspended in the remaining half of the plasma volume was collected.

4. Control;

A volume of 2ml blood collected in ethylene diamine tetra acetic acid anticoagulant containing vacutainer which is not subjected to any centrifugation.

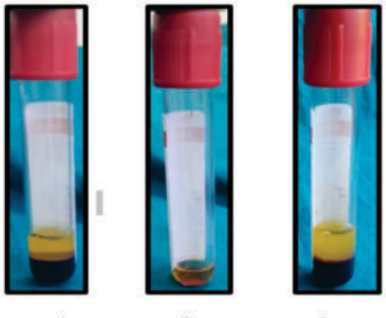
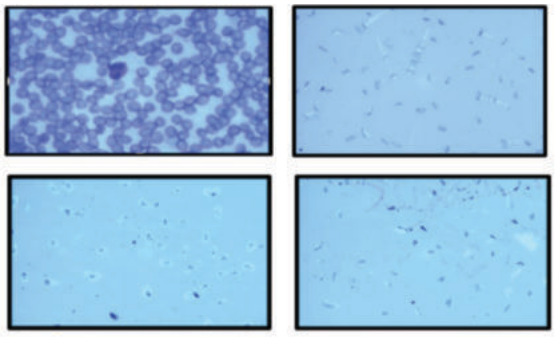


Fig 2 prepared platelet concentrates

Test Performed

1.Quantification of platelet

0.1 ml of all platelet concentrates was placed separately on a glass slide. Using the corner of a clean slide held at 45°, the drop of the sample was spread on that slide. Platelets were calculated by the traditional estimation method. All the slides were subjected to Leishman staining. In this method, 10 fields of the smear were evaluated under oil immersion. The average was then multiplied by 15,000. This gives the platelet count in cubic millimeters. The PRF platelet count was assessed indirectly by subtracting the platelet count in the residual serum (remaining after PRF preparation) from the platelet count of whole blood. Quantification of platelets was carried out in the Department of Oral Pathology, A.J. Institute of Dental Sciences, Mangalore)



Platelet count in a) whole blood b)i-PRF c)PRF d) PRP

Agar plate preparation

Wells are prepared in the inoculated blood agar plate with plaque solution.0.1 ml of I-PRF, PRF, PRP, and control is placed in the wells. The inoculated blood agar plates are then incubated aerobically for 48 hours at 37 °C. The same procedure is repeated for all the samples.

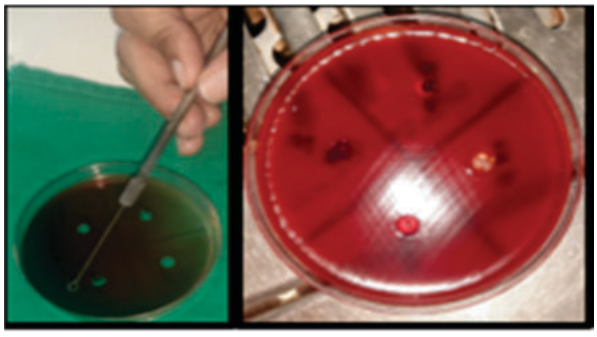


FIG 3 platelet count

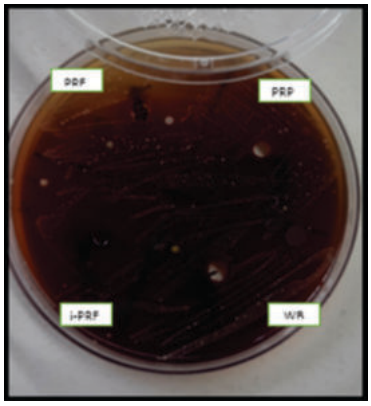


FIG 4 Agar plate preparation

Antimicrobial efficiency measurement

After 48 hours, antimicrobial efficacy was measured by measuring the mean zone of inhibition around each platelet concentrate's well using a divider and scale FIG 5 Zone of inhibition around each platelet concentrate

Statistical Analysis

Statistical Package for Social Sciences [SPSS] for Windows Version

22.0 Released 2013. Armonk, NY: IBM Corp., will be used to perform statistical analyses.

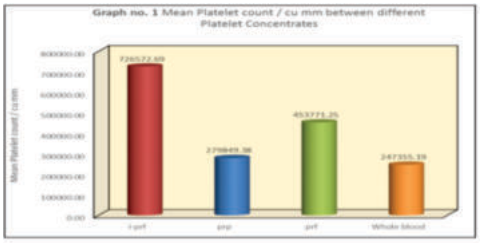
Descriptive analysis includes expression of Plate count & Zone of Inhibition in terms of Mean & SD for each platelet concentrate.
One-way ANOVA test followed by Tukey's post hoc test was used to compare the mean Plate count & Zone of Inhibition between different platelet concentrates.

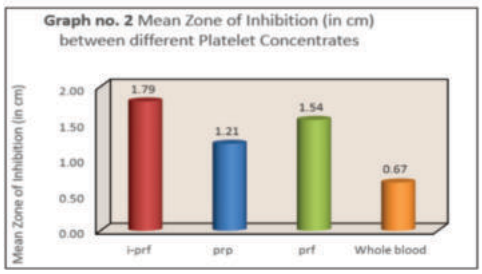
The level of significance was set at P<0.05.

RESULTS

The mean Plate count in i-PRF group was 726572.69 ± 125137.60, for PRP group was 279849.38 ± 118716.97, for PRF group was 453771.25 ± 118835.62 and for whole blood concentrate was 247355.19 ± 58788.01. This difference in the mean plate count b/w different platelet concentrates was statistically significant at p<0.001.(GRAPH NO 1)

The mean Zone of Inhibition in i-PRF group was 1.79 ± 0.19, for PRP group was 1.21 ± 0.23, for PRF group was 1.54 ± 0.15 and for whole blood was 0.67 ± 0.17. This difference in the mean Zone of Inhibition b/w different platelet concentrates was statistically significant at p<0.001(GRAPH NO 2)





DISCUSSION

Platelet concentrates are increasingly being used in periodontal surgeries, particularly because of their regenerative potential. Platelets play a vital role in wound healing. They release growth factors such as platelet derived growth factor, transforming growth factor TGF, vascular endothelial growth factor, epidermal growth factor, and insulin like growth factor on activation. Platelets provide matrix for connective tissue by secreting fibrin, fibronectin, and vitronectin, which also help in cell migration. In this way, platelets play an important role in proliferation of cells, synthesis of collagen, and formation of osteoid.

Hence, over the years, various techniques were developed for the platelet concentrates. Miron et al.[26] in 2017 presented iPRF which is in the injectable form, as one of the recent modifications of PRF. The protocol followed was that of centrifugation of venous blood at a very low speed of 700 rpm at 60 g for a short centrifugation time of 3 min15.

Wend et al. 2017 used three different techniques to achieve the greatest number of cells in the i-PRF. The procedure that yielded the most cells was 700 rpm at 60 g for 3 minute. In this investigation, we used the same methodology to obtain the i-prf. The platelet concentrates being autologous minimize the chances of infection and development of antibodies, but in PRP preparation, usually, thrombin and calcium are required which can lead to the development of antibodies against the clotting factors V and XI and thrombin. This can adversely affect the coagulation process and also lead to an immune reaction. PRF, on the other hand, is the second generation platelet concentrate introduced by Choukron 2001, which does not involve any use of bovine thrombin or anticoagulant, considerably reducing the biochemical handling of blood as well as risks associated with the use of any additives. In PRF, fibrinogen slowly and naturally gets polymerized to fibrin using the thrombin that is physiologically available and thereby forms architecture that helps in wound healing and prevents the proteolysis of growth factors. PRF also helps in microvascular iation, thus helping in cell migration3. The fibrin matrix of PRF contains a large quantity of platelet and leukocyte cytokines and they are progressively released over time [7]11 days as the fibrin network disintegrates[26].

i-PRF is based on the similar concept as PRF, but is available in an injectable form.This injectable PRF has the advantage that it can be used alone or in combination with other biomaterials easily. It is proposed to contain a higher number of regenerative cells and a higher concentration of growth factors because of the slower and shorter centrifugation spin[17]. Furthermore, it has been observed that i-PRF forms a small clot because of its fibrin components which behaves as a dynamic gel containing cells and releases additional growth factors even beyond 10 days. PRP, however, dissolves by that time[15]. Evaluation of platelet counts can be done by various devices. We used the peripheral smear method.

Webb et al. 2004 found no statistically significant difference between the peripheral smear approach and the automated cell counter method for estimating platelet count. Similarly, in a 2015 study, Bajpai et al evaluated platelet count by peripheral smear method and automated cell counter, there was no significant difference between the two methods. The authors concluded that platelet quantification by peripheral smear is effective as a quick and low-cost means of assessing platelet count. Wend et al. 2017 utilised an automated device to count platelets, however Karde et al. 2018 used the smear method since they considered it to be a reliable and cost-effective technique[16]. In the present study, all the samples except PRF were available in liquid form which could be easily smeared on glass slide and stained. As PRF was available in a clot form, its platelet count was indirectly calculated by evaluating the platelets available in residual serum. Similar evaluation was performed by Suchetha et al for estimation of platelet count in PRF clot[35]. In the current study, i-prf had the highest platelet count, followed by prf, prp which was statistically significant. The procurement protocol provided an additional benefit in terms of higher platelet count[17]. Karde, et al. 2018 compared the antimicrobial property, and platelet count of i-PRF in comparison to other platelet concentrates, i. e. , PRF, platelet-rich plasma PRP, and control whole blood in chronic gingivitis patient they concluded that i-PRF has maximum antimicrobial efficacy and higher platelet count in comparison to other platelet concentrates, thereby indicating to have a better regenerative potential than others[16]. In Recent study 2022 Sari et al evaluated the effects of demographic and clinical parameters on the number of platelets in PRP. The results of the correlation analysis revealed no statistical significance relationship between age and BMI with regards to platelet count in PRP. Nayak A,et al 2021 done a Comparative Evaluation of Platelet Count in Whole Blood and Injectable Plateletrich Fibrin they concluded that IPRF has double the number of platelets than those seen in whole blood. There have been a few researches that have investigated platelet concentrates for their antibacterial activity, although most of these studies have not examined platelet concentrates in-depth. The largest zone of inhibition in our investigation was demonstrated by i-PRF[1]. 79cm, followed by PRF[1]. 54cm, PRP 1. 21, and whole blood 0. 67. In one study by Karde et al. in 2017[16], the antibacterial activity of PRP, PRF, and i-PRF was tested on the supragingival plaque, and it was observed that i-PRF showed a maximum zone of inhibition followed by PRP and then PRF. i-PRF, being recently introduced, is not much explored for its antibacterial activity.

Limitations Of The Study

- Smaller sample size
- This is an in-vitro study, its findings need be examined and validated in more in-vivo trials.
- Identification of specific bacteria inhibited by all the test samples was not performed.
- Comparative evaluation of release of growth factors could not be performed
- It is necessary to assess antibacterial activity in a dynamic environment.
- Errors can be expected in case of platelet count in PRF as its available in clot form, we used indirect method in our study.

CONCLUSION

To our knowledge, this is the first study in which three platelet concentrates PRP, PRF, and i-PRF have been compared for their platelet count and antibacterial activity in chronic periodontitis

patients. This might be a valuable adjunctive property to the enhancement of tissue regeneration. As a future perspective both the PRF and i-PRF need to be analyzed over a longer period for their antimicrobial activity and platelet count. This being an in vitro study, its results should further be evaluated and validated through more in vivo studies.

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