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Observational Survey to Assess Platelet Concentration and Anti-microbial Potency of Different Blood Extracts

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Abstract

Introduction: Platelet concentrates have gained popularity in periodontal regenerative therapy because of its autologous nature. it promotes wound healing after surgical periodontal therapy**.**
Aim- to study the platelet count and antimicrobial efficacy of various platelet concentrates, such as PRP, PRF and IPRF.
Methodology: An Observational Study was carried out at Department of Periodontology & Implantology at Babu Banarasi Das College of Dental Sciences. Sample size was 45. Ten ml of blood was drawn from the patient out of which 3ml each of blood will be used for PRP, PRF and I-PRF preparation and remaining 1ml of blood was used for determining the platelet count. For I-PRF preparation, 3ml of blood wias used, d centrifuged at 700 rpm for 3 minutes. For PRF preparation, Agar plates will be inoculated with plaque sample of same patient and will be labelled and divided into 3 compartments. Wells was prepared in the inoculated agar plate and 0.1 ml of PRP, PRF and I-PRF will be placed in those wells. Inoculated blood agar plates was then incubated aerobically at 37℃ for 24 hours to 48 hours.
Results- The platelet count of i-PRF was statistically significant when compared to control ($P < 0.001$). It was also significant when compared to PRP ($P < 0.01$) and PRF ($P < 0.001$). Mean zone of inhibition around i-PRF ($P < 0.01$) and PRF ($P < 0.05$) reached statistical significance. Although a distinct zone of inhibition was seen with PRP, it was not statistically significant ($P > 0.05$).

Conclusion- In the present study, although a distinct zone of inhibition was obtained with all test samples, it was significant with only i-PRF and PRF.

Keywords: Platelet Concentrates, Antimicrobial Efficacy, PRP, PRF And IPRF

INTRODUCTION

Periodontitis is an inflammatory disease characterized by the progressive destruction of supporting structures of teeth, it is caused by a specific microorganism or a group of microorganisms[1]. This causes progressive attachment loss and bone loss. Therefore, periodontal therapy is aimed at regenerating the lost periodontal structures.

Recently, platelet concentrates have gained popularity in periodontal regenerative therapy because of its autologous nature. The rationale behind its regenerative potential is the presence of various growth factors in the α–granules of platelets which are released at the local site on their activation. Thus, it promotes wound healing after surgical periodontal therapy. Besides this, they also show anti-inflammatory properties, thereby reducing postoperative pain and swelling. [2]

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. Thus, they play a crucial role in cell proliferation, collagen synthesis, and osteoid formation.[2]

Recent studies have shown that platelet concentrates are being used for management of periodontal diseases.[1]

Basically, human blood consists of four main components that are- platelets, white blood cells, red blood cells and plasma. These platelets present in the blood release growth factors at the site of injury and play a major role in wound healing.

In addition to the regenerative potential, the antibacterial activity of the platelet concentrates has been reported against several microorganisms such as Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, and Streptococcus oralis.

The leukocytes in PRF are known to exhibit antimicrobial activity. However, there is not much evidence for the antibacterial activity of I-PRF against these periodontal pathogens since I-PRF has been recently introduced. I-PRF is being studied for its regenerative potential and release of growth factors and owing to its ease of preparation and capability to be used with other biomaterials, its other properties including the antibacterial property also need to be explored.[1]

Aim of the study was to study the platelet count and antimicrobial efficacy of various platelet concentrates, such as PRP, PRF and IPRF. And objectives were to analyse the number of platelets in PRP, PRF and IPRF plus to analyse the antimicrobial activity of various platelet concentrates on bacterial plaque.

METHODOLOGY

An Observational Study was carried out at Department of Periodontology & Implantology, Department of Oral Pathology and Microbiology, at Babu Banarasi Das College of Dental Sciences, Lucknow. Sample size was 45. Subjects with normal periodontium, patients with gingivitis, patients with periodontitis and between 18- 50 years of age were included in the study. At the same time individuals with smoking habit or tobacco chewing habit, with any systemic disease, pregnant or lactating women and individuals using antibiotics or anti-inflammatory drugs since last 6 months were excluded. The patient's was explained about the procedures and the possible outcomes in their vernacular language as well as written informed consent was obtained. The demographic data of all the subjects was recorded on the customized case history proforma. Ten ml of blood was drawn from the patient out of which 3ml each of blood will be used for PRP, PRF and I-PRF preparation and remaining 1ml of blood was used for determining the platelet count as per the criteria of Kour P, et al. [1] For I-PRF preparation, 3ml of blood wias used, this

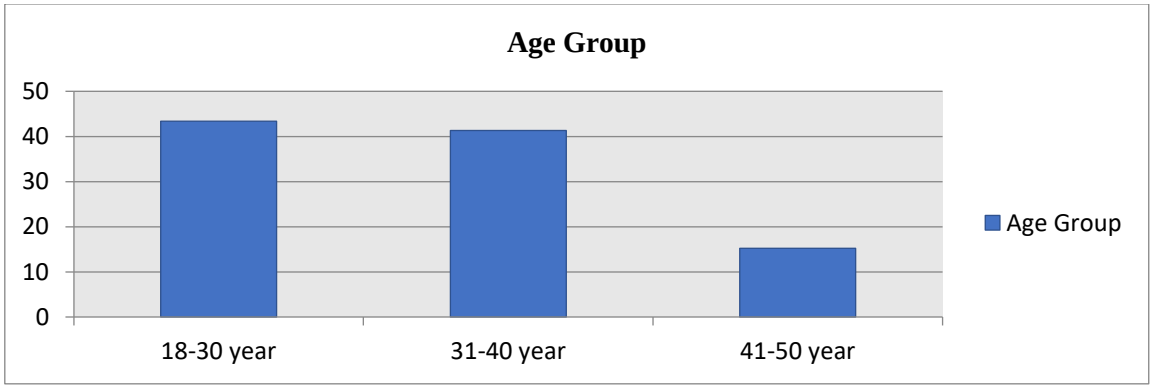
blood will be collected in a vacutainer without any additives and centrifuged at 700 rpm for 3 minutes. For PRF preparation, 3ml of blood was collected in a glass collecting tube without any additives and centrifuged at 3000 rpm for 10 minutes. For PRP preparation, blood was collected in collecting tube containing 3.2% sodium citrate and centrifuged at 1000 rpm for 13 minutes. The top layer of plasma will be removed. After this 2nd round of centrifugation will be done at 2000 rpm for 10 minutes. The upper half of the plasma will be discarded and the lower half will be used to check the antimicrobial activity.[1] Agar plates will be inoculated with plaque sample of same patient and will be labelled and divided into 3 compartments. Wells was prepared in the inoculated agar plate and 0.1 ml of PRP, PRF and I-PRF will be placed in those wells. Inoculated blood agar plates was then incubated aerobically at 37°C for 24 hours to 48 hours and then the zone of inhibition was accessed.[2] All the patients visiting the OPD of Periodontology and Implantology Department will be screened clinically for Normal Periodontium, Gingivitis and Periodontitis as per criteria given by Armitage (1999).[3]

Data was entered in the Microsoft excel spread sheet. Descriptive statistics like percentages and frequency distribution was calculated. Inferential statistics like one-way ANOVA (Analysis of Variance) will be used to find out whether there is any statistical difference between the means of three or more independent groups and post-hoc Tukey's test to check the difference between the variables using SPSS.

RESULTS

The present study has been done to assess the platelet count and antimicrobial efficacy of various platelet concentrates, such as PRP, PRF and IPRF. To analyze the number of platelets in PRP, PRF and IPRF and also the antimicrobial activity of various platelet concentrates on bacterial plaque.

Graph 1 Age Group distribution among studied population



Graph-2 Gender distribution among studied population

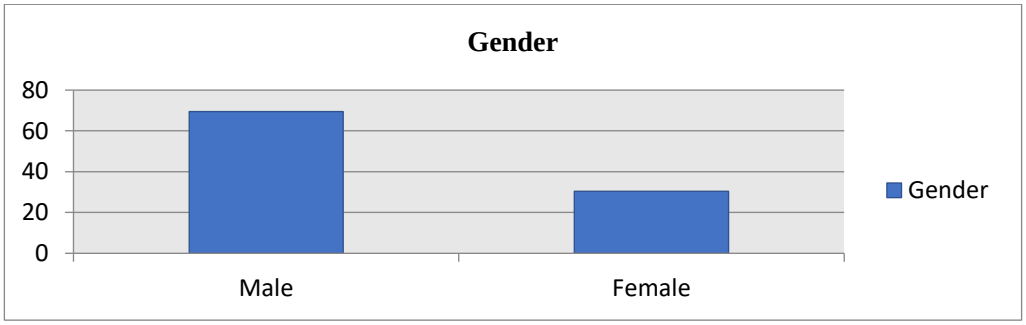


Table-1 Quantification of platelets

	Platelet count/mm^3	p-value
i-PRF	1,434,000 ± 75,233	$P < 0.001$
PRP	1,343,000 ± 81,486	
PRF	249,000 ± 61,319	
Control	291,000 ± 51,575	

The platelet count of i-PRF was statistically significant when compared to control ($P < 0.001$). It was also significant when compared to PRP ($P < 0.01$) and PRF ($P < 0.001$). This indicates that i-PRF has maximum number of platelets in comparison to other concentrates.

Table-2 Evaluation of antimicrobial potential

Antimicrobial Efficacy	Mean zone of inhibition (in cm)	p-value
i-PRF	1.42 ± 0.25	$P < 0.01$
PRP	1.3 ± 0.16	$P > 0.05$
PRF	1.02 ± 0.12	$P < 0.05$

The antimicrobial efficacy was demonstrated by appearance of a clear zone of inhibition around the samples. Mean zone of inhibition (in cm) around i-PRF, PRF, and PRP were 1.42 ± 0.25, 1.3 ± 0.16, and 1.02 ± 0.12, respectively. Mean zone of inhibition around i-PRF ($P < 0.01$) and PRF ($P < 0.05$) reached statistical significance. Although a distinct zone of inhibition was seen with PRP, it was not statistically significant ($P > 0.05$). These results indicate that i-PRF has a significant inhibitory effect on growth of oral bacteria in comparison to other platelet concentrates.

Table-3 Evaluation of Gingivitis Patients

Gingivitis Patients			
Parameters	**N**	**Mean**	**Std. Deviation**
BOP	15	2.52	.510
PPD	15	2.96	.841
CAL	15	1.56	.507
Whole blood	15	202515.12	45676.134
PRP	15	1865649.52	266897.538
PRF	15	2397508.00	381263.907
I-PRF	15	3068625.60	258936.375

The periodontitis patients were 15 out of 45 that is total sample size. We found that the mean of bleeding of probing was 3.15 with standard deviation of 1.040 while mean of pocket probing depth was 3.95 with

standard deviation of 1.638. The mean of CAL was 2.35 with standard deviation of 1.268 and mean of whole blood was 209558.45 with standard deviation of 47542.014as shown Table 3.

Table-4 Evaluation of Periodontitis patients

Periodontitis patients			
Parameters	**N**	**Mean**	**Std. Deviation**
BOP	15	3.15	1.040
PPD	15	3.95	1.638
CAL	15	2.35	1.268
Whole blood	15	209558.45	47542.014
PRP	15	1931008.10	276244.256
PRF	15	2506691.50	355532.141
I-PRF	15	3130957.50	265201.795

The healthy patients were 15 out of 45 that is total sample size. We found that the mean of bleeding of probing was .00 with standard deviation of .000[a] while mean of pocket probing depth was 1.38 with standard deviation of 619. The mean of CAL was 1.13 with standard deviation of .719 and mean of whole blood was 1572781.25 with standard deviation of 466820.523 as shown Table 4.
The mean of PRP was 2187260.63 with standard deviation of 696143.908, mean of PRF was 2665323.75 with standard deviation 762564.486 and also mean of I-PRF was .89 with standard deviation .248 as shown Table 4.

Table-5 Evaluation of Healthy patients

Healthy Patients			
Parameters	**N**	**Mean**	**Std. Deviation**
BOP	15	.00	.000[a]
PPD	15	1.38	.619
CAL	15	1.13	.719
Whole blood	15	1572781.25	466820.523
PRP	15	2187260.63	696143.908
PRF	15	2665323.75	762564.486
I-PRF	15	.89	.248

Discussion

Periodontal regeneration involves various biologic events such as cell adhesion, migration, proliferation, and differentiation in an orchestrated sequence. Regenerative procedures with the use of soft- and hard-tissue grafts are performed to attain periodontal restoration. Platelets play a vital role in wound healing. They release growth factors such as platelet-derived growth factor (PDGF), transforming growth factor (TGF-β), and insulin-like growth factor once they are activated. 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. Thus, they play a crucial role in cell proliferation, collagen synthesis, and osteoid formation. This led to the development of numerous techniques of autologous platelet concentrates over the years such as PRP and PRF.[4,5,6]

PRP although being an autologous preparation requires the addition of thrombin and calcium for its activation. These additives can result in the development of antibodies to the clotting factors V, XI, and thrombin, thereby adversely affecting the coagulation process and also can trigger an immune reaction. PRF is the second generation platelet concentrate introduced by Choukron (2001). It is easy to prepare and has good handling characteristics. It does not involve any use of bovine thrombin or anticoagulant which considerably reduces the biochemical handling of blood as well as risks associated with the use of any additives. PRF itself contains physiologically available thrombin that is responsible for slow polymerization of fibrinogen into fibrin resulting in a physiologic architecture favorable to wound healing. This fibrin network protects the growth factors from proteolysis. Besides, PRF also favors the development of microvascularization leading to a more efficient cell migration.[7]

I-PRF was introduced based on the similar concept as that of PRF with added advantage of it being in injectable form. This injectable form of PRF can be utilized alone or combined easily with various biomaterials. 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.[8]

In a recently conducted study, it was observed that PRP slowly dissolved over a period while i-PRF formed a small clot as a result of fibrin components that acted as a dynamic gel with cells likely contained within its hydrogel. It was therefore hypothesized that an additional release of growth factors from i-PRF can be excepted beyond 10 days although PRP was found to be dissolved by that time.[9]

Evaluation of platelet counts can be done by various automated devices. However, in a study done by Bajpai *et al*. to estimate the platelet count by peripheral smear method and by automated cell counter, it was observed that there was no significant difference in the two methods. The authors concluded that the method of platelet estimation by peripheral smear is useful as a rapid, cheap method to assess platelet count.[10]

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.[11]

In the present study, it was observed that i-PRF had highest number of platelet count and it was statistically significant. This could be attributed to the low centrifugation speed and time resulting in their higher number. Ghanaati *et al*. introduced the "low-speed concept" for blood centrifugation whereby lower

centrifugation speeds were shown to contain higher numbers of cells including leukocytes before the formation of a fibrin clot.[8]

In the present study, it was observed that i-PRF and PRP showed 503% and 464% rise in number of platelets. PRF clot showed the concentration of about 87% of platelets when compared to whole blood. Preparations with higher platelet counts are known to release more growth factors. Miron *et al*. demonstrated that in general PRP had higher early release of growth factors whereas i-PRF showed significantly higher levels of total long-term release of these factors. It was concluded that i-PRF can release higher concentrations of various growth factors and induce higher fibroblast migration and expression of PDGF, TGF-β, and collagen1.[12]

Studies evaluating the antimicrobial efficacy of platelet concentrates such as PRP and PRF have been previously carried out. i-PRF being recently introduced is not much explored. In the present study, it was observed that i-PRF showed maximum zone of inhibition around oral microflora, i.e., 1.42 ± 0.25 (in cm). The order of zone of inhibition from highest to lowest was i-PRF > PRF > PRP. In one study, antimicrobial efficacy of PRP and PRF was performed against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*. It was seen that *P. gingivalis* and *A. actinomycetemcomitans* were inhibited by PRP but not by PRF.[13] In another study, PRF demonstrated clear zone of inhibition against subgingival plaque sample and minimum amount of turbidity using PRF was confirmed by colorimetric analysis.[14]

In the present study, although a distinct zone of inhibition was obtained with all test samples, it was significant with only i-PRF and PRF. Various mechanisms have been hypothesized regarding the mechanism of antibacterial effect of platelet-derived preparations such as generation of oxygen metabolites, including superoxide, hydrogen peroxide, and hydroxyl free radicals; binding, aggregation, and internalization of microorganisms, thereby enhancing the clearance of pathogens from the bloodstream; release an array of potent antimicrobial peptides.[53] Yeaman proposed that direct interaction of platelets with microorganisms, participation in antibody-dependent cell cytotoxicity and engulfment by entrapped white blood cells within PRF could result in direct bacterial killing. Besides this release of myeloperoxidase, activation of the antioxidant responsive elements and antigen-specific immune response have also been suggested.[15]

CONCLUSION

In the present study, although a distinct zone of inhibition was obtained with all test samples, it was significant with only i-PRF and PRF.

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