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Original Research

Quantitative assessment of Platelets and antimicrobial efficacy of different platelet concentrates in Gingivitis patients: A comparative clinico-microbiological study

[1]Dr Bindiya Udhani, [2]Dr.Hiral Parikh, [3]Dr. Shilpa Duseja, [4]Dr. Divya Singh, [5]Dr. Hiren Rana

[1]Postgraduate student, [2]HOD & Professor, [3]Professor, Department of Periodontology, Narsinhbhai Patel Dental College and Hospital, Sankalchand Patel University, Visnagar, Gujarat, India;
[4]Private Practitioner, Department of Periodontology, SCM Dental Clinic, Jaipur, Rajasthan, India;
[5]Dental Practitioner, Department of Periodontology, Bharuch, Gujarat, India

ABSTRACT:

Background: 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. **Aim:** The aim of the present study was to evaluate the platelet count of different concentrates and compare their antimicrobial properties. **Materials and Method:** Blood samples were obtained from 20 chronic generalized marginal gingivitis patients. Platelet concentrates were prepared using standardized centrifugation protocol. Platelet count was evaluated by manual counting method using smear preparation of each sample. Subsequently, antimicrobial activity against oral bacteria was examined on blood agar using disc diffusion method to quantify the inhibitory effects. **Results:** Statistical significance was analyzed by one-way analysis of variance (ANOVA). P <0.05 was considered statistically significant. Mean zone of inhibition around i-PRF (P< 0.01) and PRF (P < 0.05) showed statistical significance. Although a distinct zone of inhibition was seen with PRP, it was not statistically significant (P > 0.05). i-PRF showed statistically significant difference (P < 0.001) in platelet count when compared to control. It was also significant when compared to PRP (P < 0.01), PRF (P < 0.001). **Conclusion:** i-PRF has maximum antimicrobial efficacy and higher platelet count in comparison to other platelet concentrates, thereby indicating to have a better regenerative potential then others.

Key words: Antimicrobial, periodontitis, platelet counts, platelet-richplasma

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Corresponding author: Dr Bindiya Udhani, Postgraduate student, Department of Periodontology, Narsinhbhai Patel Dental College and Hospital, Sankalchand Patel University, Visnagar, Gujarat, India

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INTRODUCTION

Periodontitis is an inflammatory and a polymicrobial disease affecting, supporting structures around the teeth resulting in progressive attachment loss, bone loss and pocket formation. The principle behind this oral systemic connection is dissemination of locally produced proinflammatory mediators such as C-reactive proteins, IL-1β, IL-6 and tumor necrosis factor alpha etc.[1]

Various approaches have been applied for the treatment of periodontitis which aim at controlling the infection and are important for proper wound healing and subsequent regeneration of periodontal tissues, but there is always a risk of bacterial contamination with surgical procedures because even after that bacteria may still be able to survive and infiltrate into deeper tissues.

Periodontal wound healing requires a sequence of interactions between epithelial cells, gingival fibroblasts, periodontal ligament cells, and osteoblasts. The disruption of vasculature during wound healing leads to fibrin formation, platelet aggregation, and release of several growth factors into tissues from plateletsthrough molecular signals which are primarily mediated by cytokines and growth factors. There is evidence that the presence of growth

factors and cytokines in platelets play key roles in inflammation and wound healing.[2]

Platelets are sub cellular fragments derived from megakaryocytes in the bone marrow, circulating in the blood as small discs having a precise and reproducible structure. A single megakaryocyte can give rise to 1000- 3000 platelets. Megakaryocytes are rare myeloid cells (constituting <1% of these cells) that reside primarily in the bone marrow. The platelets are very small, non-nucleated, about 3 m in diameter, and consist of cytoplasm enclosed within a cell membrane. They are anucleate cytoplasmic fragments basically derived from bone marrow megakaryocytes. They enclose many granules, few mitochondria and two prominent membrane structures, the surface-connected canalicular system and the dense tubular system [2]. The life span of a normal platelet is about 7-12 days, and they are destroyed by the macrophages in the spleen. The platelet in peripheral blood is heterogeneous with respect to size, density, and staining characteristics. Their morphology also varies greatly depending on the methods by which they are examined, and the anticoagulant used. In wet preparations, they are colorless, moderately refractile bodies that are discoid or elliptical. These granules may be so tight in the central portion of the platelet that may give the appearance of the nucleus.

Platelets are multifunctional and play a key role in many physiological processes (e.g. wound repair, immune response) apart from their well-known roles in hemostasis and thrombosis. Platelets perform a pivotal role in hemostasis and wound healing. The growth factors released by them are well recognized reservoir of healing cytokines.

In recent times, these platelet concentrates have gained popularity in periodontal regenerative therapy because of its autologous nature. The rationale behind this is presence of various growth factors in the α–granules of platelets which are released at the local site on their activation. Beside this they have anti-inflammatory properties which reduce the postoperative pain and swelling [1,3]

The journey starts from the development of platelet concentrates originating as key concept for fibrin adhesives development which were quite popular in 1970s in Europe. Platelet concentrates; concept was first started in the field of hematology. In 1970s the term PRP was coined to describe plasma with a platelet count many folds above that of peripheral blood count, which was earlier used to treat patients with thrombocytopeniaby transfusion products . Later in same era Fibrin glue was made by polymerizing fibrinogen with thrombin and calcium. Fibrin Sealants are human plasma derivatives that mimic the final stages of blood coagulation, forming a fibrin clot. It is used as topical haemostatic and tissue sealing agent. However, due to low concentration of fibrinogen in donor plasma, the quality and stability of fibrin glue was suboptimal and these products were associated with a risk of cross infection[4,5].

Knighton*et al*1 was the first to demonstrate that platelet concentrate successfully promote healing and termed it as “Platelet Derived Wound Healing Factors (PDWHF)”, which were successfully used initially for the treatment of ulcers of skin. Whitman first introduced the Platelet Rich plasma (PRP) in field of oral and maxillofacial surgery. Platelet Rich Plasma (PRP) is a form of platelet concentrate whose regenerative potential is due to the release of various growth factors.[6,7] They improve wound healing by increasing the levels of growth factors at the wound site after degranulation of the platelets.[8] Recently, it is demonstrated that Platelet Rich Plasma (PRP) exerts positive effects on gingival fibroblasts, oral osteoblasts, and periodontal ligament (PDL) fibroblasts [9-11]to facilitate complete periodontal regeneration. However, the precise role of platelet concentrates of periodontal tissue regeneration needs to be clarified.[12] An antimicrobial effect of PRP has also been reported against Staphylococcus aureus, Escherichia coli, and Klebsiella pneumonia indicating that PRP is potentially useful to fight against postoperative infections. However, the disadvantage of using PRP is that its properties can vary depending on the concentration of platelets, amount of leukocytes, the type of activator used, and time of placement of fibrin scaffold after clotting.[12]

Platelet-rich fibrin (PRF), the second-generation PC, has been introduced by Choukroun et al. in 2000.[14] It contains platelets and GFs in the form of fibrin membranes prepared from the patient’s own blood free of any anticoagulant.[15] PCs accelerate the wound healing after periodontal treatment. In addition, there is release of certain substances from platelets that promote tissue repair, angiogenesis, inflammation, and immune response. Platelets also contain biologically active proteins. The binding of these secreted proteins with a developing fibrin mesh or to the extracellular matrix can create chemotactic gradients aiding the recruitment of the stem cells, stimulating cell migration, differentiation, and promoting repair. PRF has a dense fibrin network with leukocytes, cytokines, structural glycoproteins, and also GFs.[13] Leukocytes that are concentrated in PRF scaffold play an important role in GF release,[14] immune regulation, anti-infectious activities,[15] and matrix remodeling during wound healing. The slow polymerization mode of PRF and cicatricial capacity create a physiologic architecture favorable for wound healing.[16]

Injectable PRF (I-PRF) is one of the recently introduced platelet concentrates. As the name suggests, it is available in injectable form and coagulates few minutes after the injection. It is also called “blood concentrate” because in addition to platelets and leukocytes, it also contains stem cells and endothelial cells.[17,18]

So, this study was carried out to evaluate the platelet count of different platelet concentrates and compare

the antimicrobial efficacy of different platelet concentrates with amongst each other.

AIM

The aim of the present study was to evaluate the platelet count of different concentrates and compare their antimicrobial properties.

OBJECTIVES

- To evaluate the platelet count of different platelet concentrates.
- To evaluate the antimicrobial efficacy of Platelet Rich Fibrin (PRF) against periodontal pathogens.
- To evaluate the antimicrobial efficacy of Platelet Rich Plasma (PRP) against periodontal pathogens.
- To evaluate the antimicrobial efficacy of Injectable-Platelet Rich Fibrin (I-PRF) against periodontal pathogens
- To evaluate the antimicrobial efficacy of whole blood against periodontal pathogens
- To compare the antimicrobial efficacy of all platelet concentrates amongst each other.

MATERIALS AND METHODS

TYPES OF STUDY

A Clinico-microbiological cross sectional study collecting blood samples of 20 patients with marginal gingivitis. Patients with marginal gingivitis were selected and study was approved by ethical committee of Narsinhbhai Patel Dental College and Hospital (NPDCH), Visnagar, before the commencement of the study.

SELECTION OF SUBJECTS

The subjects were examined under the inclusion and exclusion criteria and they were selected for study. Subjects who agree to participate in the study were asked to sign (thumb impression in case of illiterate subjects) the written consent. The selection of patient was done according to inclusion and exclusion criteria and subjects who agree to participate in the study were asked to sign the written consent.

SELECTION CRITERIA

INCLUSION CRITERIA

1. Age above 25 years irrespective of gender.
2. Systemically healthy patients.
3. Subjects with generalized chronic marginal gingivitis with Gingival Index ≥ 1 and probing depth ≤ 3mm.

EXCLUSION CRITERIA

1. Pregnant or lactating women.
2. Subjects with habit of smoking or tobacco chewing.
3. Subjects with history of injection or any antibiotic use in last months.
4. Subjects with history of any anticoagulant or immunosuppressive therapy.
5. Patient not ready to fill the consent form.

STUDY DESIGN

- Blood samples of 20 patients with chronic generalized marginal gingivitis were collected above age group of 25 years irrespective of gender.
- Samples were analyzed for quantitative assessment of platelet count and antimicrobial activity of platelet concentrates.

CLINICAL EXAMINATION

- Detailed Medical and Dental history.
- Necessary blood investigations.
- Here patients were examined and data collection was done at baseline.

CLINICAL PARAMETERS

GINGIVAL INDEX (LOE AND SILLNESS, 1964)

The bleeding is assessed by probing gently along the wall of soft tissue of the gingival sulcus. The scores of the four areas of the tooth can be summed and divided by four to give the Gl for the tooth. The Gl of the individual can be obtained by adding the values of each tooth and dividing by the number of teeth examined. The Gingival Index may be scored for all surfaces of all or selected teeth or for selected areas of all or selected teeth. The Gl may be used for the assessment of prevalence and severity of gingivitis in populations, groups and individuals.

The Gl has been used frequently in clinical trials of therapeutic agents. The sensitivity and reproducibility is good provided the examiner's knowledge of periodontal biology and pathology is optimal.

INDEX SCORE= Total Score/ No of teeth examined

Interpretation: 0.1-1.0 – Mild Gingivitis 1.1-2.0 – Moderate Gingivitis 2.1-3.0 – Severe Gingivitis

Scores	Gingival status	Criteria
0	Normal gingiva	Natural coral pink gingival with no e/o inflammation
1	Mild inflammation	Slight changes in color, slight edema. No bleeding on probing
2	Moderate inflammation	Redness, edema and glazing. Bleeding upon probing
3	Severe inflammation	Marked redness and edema/ ulceration/tendency to bleed spontaneously

RESULTS

This was a comparative clinico - microbiological study designed to evaluate the quantitative assessment of platelets and antimicrobial efficacy of different platelet concentrates in gingivitis patients:

Twenty systemically healthy patients of age group above 25 years irrespective of gender suffering from

generalized chronic gingivitis were selected amongst the patients. The data for clinical parameters of 20 patients was collected and quantification of platelets of different platelet concentrates was checked. Descriptive statistics, Mean, Standard deviation and one way ANOVA test and Unpaired't' test was analysed using SPSS version 20.0.

IV: Demographic data of study population

Gender	Number	Percentages	Age in years	
			Mean	SD
Male	10	50	28.60	3.59
Female	10	50	29.00	3.52
Total	20	100	28.80	3.47

SD: Standard Deviation

The above table shows that out of 20 study subjects, 10 (50%) were male and 10 (50%) were female. Mean age was 28.80 ± 3.59 years for all study subjects, 28.60 ± 3.59 years for male study subjects and 29.00 ± 3.52 for female study subjects. The mean for both gender is shown in above table.

V: Quantification of platelets in different groups.

Groups	Number	Platelet count		P Value
		Mean	SD	
I-PRF	20	1131500	35433.40	≤ 0.05*
PRF	20	539000	23597.50	
PRP	20	629300	13833.97	
CONTROL	20	329800	14222.29	

Level of Significance P ≤ 0.05, * Significant, ** Non Significant

The above table shows that platelet count was highest in I-PRF group (P ≤ 0.05*) followed by PRP group, following the PRF group and then Control group respectively. Statistically, significant difference was observed in Platelet count amongst all groups shown in table 2.

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 for autologous platelet concentrates over the years leading to multiple types of preparations.

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 et al (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.[19]

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.[20]

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 expected beyond 10 days although PRP was found to be dissolved by that time.[23]

Evaluation of platelet counts can be done by various automated devices. However, In a study done by **Bajpai*et al.*(2015)**, 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 author concluded

that the method of platelet estimation by peripheral smear is useful as a rapid, cheap method to assess platelet count.[21]Here in the present study, the evaluation of platelet count was done by automated cell counter.

Here in this study, the platelet count of different platelet concentrates were checked and mean zone of inhibition was checked to know the antimicrobial efficacy of different concentrates. To the best of our knowledge, this was the first study to quantify the platelet counts of different platelet concentrates and to evaluate the antimicrobial efficacy of different platelet concentrates in gingivitis patient.

Patient with above age of 25 years irrespective of gender having chronic generalized gingivitis with Gingival Index ≥ 1 and probing depth ≤ 3mm were selected.

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* (2020)** 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.[24] In the present study, it was observed that i-PRF group($P \leq 0.05$*) showed highest number of platelets followed by PRP and than in PRF and control group **(Table V and Graph II).**PRF clot showed the highest concentration of platelets when compared to whole blood. Preparations with higher platelet counts are known to release more growth factors. **Miron*et al*.(2007)[22]** 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 collagen. **Nayak A et al (2021)[22]** in his study suggested that The platelet count in I-PRF was significantly more when compared with that of WB. The results of there study suggest that the I-PRF has a richer concentration of platelets when compared to the WB.

Yeaman et al (2015) 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 antigenspecific immune response have also been suggested.[25]

Therefore, the results from the previous and present study shows that platelet count and antimicrobial efficacy was high in i-PRF when compared with PRF and PRP.

CONCLUSION

Thus it can be concluded that the use of platelet concentrates in periodontal regenerative therapy is increasing nowadays. With i-PRF, its application can be more feasible and minimally invasive without using any additives for its preparation.

Within the limitations of the study, i-PRF had the highest number of platelets. Its antimicrobial potency was also highest when compared to other platelet concentrates. Since there is limited literature related to this novel platelet concentrate further research is required to explore its properties. More *in vitro* and animal studies are required to establish its healing and regenerative efficiency.

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