International Journal of Applied Research

Dr. Nilima Rajhans
HOD, Department of Periodontics, Late Shri Yashwantrao Chavan Dental College and Hospital, Ahmednagar, Maharashtra, India

Dr. Joanna Shrisunder
Postgraduate Student, Department of Periodontics, Late Shri Yashwantrao Chavan Dental College and Hospital, Ahmednagar, Maharashtra, India

Dr. Aparna Pathare
Postgraduate Student, Department of Periodontics, Late Shri Yashwantrao Chavan Dental College and Hospital, Ahmednagar, Maharashtra, India

Dr. Tanuja Ugale
Postgraduate Student, Department of Periodontics, Late Shri Yashwantrao Chavan Dental College and Hospital, Ahmednagar, Maharashtra, India

Comparative evaluation of PRF in diabetics and smokers with chronic periodontitis

Dr. Nilima Rajhans, Dr. Joanna Shrisunder, Dr. Aparna Pathare and Dr. Tanuja Ugale

Abstract

Background: Platelet concentrates are autologous bioactive substances that have found multiple applications in the field of dentistry. The rationale behind this is to concentrate and extract certain elements from the patient's own blood sample, which can be used to improve healing and promote tissue regeneration. Diabetics and smokers are also known to be at a much higher risk of periodontal disease and also show delayed wound healing. This may lead to the failure of periodontal regenerative therapy. PRF, may serve to accelerate the healing process and give an improved outcome since it contains concentrated platelets and WBCs which play a pivotal role in wound healing.

Aim: The aim of this study was to analyze the platelet and the leukocyte counts in the PRF and the size of the PRF membrane formed in order to determine whether diabetes and smoking has an impact on the PRF formation.

Material and Method: This is a clinical study in which 30 chronic periodontitis patients from the OPD were enrolled. The study consists of three groups. Group 1 (Control) comprised of 10 systemically healthy patients with chronic periodontitis, Group 2 (Test 1) comprised of 10 chronic smokers with chronic periodontitis and Group 3 (Test 2) comprised of 10 chronic diabetics with chronic periodontitis. 15 ml of blood was drawn from all subjects to assess the size outcome, platelet, and leukocyte concentration and the size of the PRF clot formed.

Results: Diabetics showed comparatively higher platelet and WBC counts and also showed larger PRF clot dimensions on measurement.

Conclusion: The increased blood sugar levels led to increase in the leukocyte and platelet activity. This could be beneficial for usage in regenerative procedures and treatment of periodontal defects.

Keywords: Diabetics and smokers, chronic periodontitis, PRF

Corresponding Author:
Dr. Nilima Rajhans
HOD, Department of Periodontics, Late Shri Yashwantrao Chavan Dental College and Hospital, Ahmednagar, Maharashtra, India

Introduction

Platelet concentrates are autologous bioactive substances that have found multiple applications in the field of dentistry. The rationale behind this is to concentrate and extract certain elements from the patient's own blood sample, which can be used to improve healing and promote tissue regeneration [1].

It is well known that platelets and leukocytes play a very important role in wound healing. Platelets contain biologically active proteins which bind to the developing fibrin mesh. These proteins thus create a gradient for chemotaxis leading to the migration of stem cells to the area. These stems cells undergo the process of differentiation, and lead to healing by regeneration. Therefore, the use of autologous platelet concentrates is a promising treatment modality in the field of periodontal regeneration [2].

Smoking and diabetes are well known risk factors for periodontal disease. They increase the extent and severity of periodontitis by 3-10 times and hasten the destruction of periodontal tissues by a large extent [3, 4]. Periodontitis has been called the sixth complication of diabetes [6], having one-third of diabetics suffering from periodontitis [5]. Around 42% of periodontitis cases can be attributed to the habit of smoking [4]. Diabetics and smokers are also known to show delayed wound healing may lead to the failure of periodontal regenerative therapy. PRF, when used in regenerative therapy in the treatment of periodontal disease may serve to accelerate the healing process and give an improved outcome.

Therefore the goal of this study was to analyze the platelet and the leukocyte counts in the PRF and the size of the PRF membrane formed in order to determine whether diabetes and smoking has an impact on the PRF formation.

Materials and Method
This is a clinical study in which 30 chronic periodontitis patients from the OPD were enrolled. The study consists of three groups. Group 1 (Control) comprised of 10 systemically healthy patients with chronic periodontitis, Group 2 (Test 1) comprised of 10 chronic smokers with chronic periodontitis and Group 3 (Test 2) comprised of 10 chronic diabetics with chronic periodontitis.

The study included patients with chronic generalized periodontitis with a minimum of 20 teeth and having pocket probing depths of ≥6 mm, Clinical Attachment Loss (CAL) of ≥5 mm without a history of any periodontal therapy. The present study included patients with a history of smoking of more than 10 cigarettes per day for the past 5 yrs and patients with a history of type 2 diabetes for the past 10 years.

The present study excluded subjects with subjects with bleeding disorders or any systemic disorders, subjects with any diagnosed malignancy, subjects with decrease or increase in platelet count, pregnant and lactating mothers, subjects on antibiotics, anticoagulants, immunosuppressive, or cytotoxic medications from the past 6 months and subjects with adverse habits such as pan chewing and alcohol

Procedure
Periodontal examination was done using mouth mirror and UNC-15 Periodontal Probe to assess the Plaque Index by Silness and Loe in 1964 [7], Gingival Bleeding Index by Ainamo and Bay in 1975 [8], and PPD by Grant *et al*. in 1965 [9]. The probe was passed within the gingival sulcus along the circumference of the tooth. CAL was measured from the cemento–enamel junction to the base of the pocket using UNC-15 Periodontal Probe.[7] Three measurements were made on the buccal aspect and three on the lingual aspect of each tooth for PPD and CAL.

Under all aseptic conditions the blood sample was collected from each participant to prepare PRF for hematologic analysis. A 15 ml disposable syringe and tourniquet were used to draw 15 ml intravenous blood from antecubital vein. (Figure 1)

For the baseline WBC and platelet counts 3 ml of blood was separated. The blood was collected in an EDTA containing vacutainer tubes for whole blood analysis. WBCs were counted using the manual method using a Nebauers Chamber and platelet count was also measured manually using a commercial diluting system, hemocytometer and a microscope.

Preparation of PRF
The remaining 12 ml of blood was transferred into a sterile, non coated vacutainer tube for PRF preparation.

Preparation was done in a Remi C852 benchtop centrifuge. (Figure 2) The tube was placed into the centrifuge and a test tube filled with equal quantity of water was placed on the opposite side for balancing it. The machine cycle was set for 1300 rpm for 15 mins. This resulted in the separation of the contents of the tube into three layers due to the varying densities. The three layers consist of a plasma layer on top, a platelet rich layer in the middle and an RBC layer as the bottom most layer. (Figure 3) The platelet concentrate layer which is formed is separated by using a sterile tweezers and a scissors. (Figure 4)

It was not possible to measure the concentration of the platelets and the WBCs directly in the PRF clot obtained. Therefore, a formula was used in which the WBC and platelet counts in the serum were subtracted from the baseline WBC and platelet counts. This will give us the concentration of the WBCs and platelets in the PRF clot.

(Platelets/WBCs in PRF) = (Platelets/WBCs in Whole Blood/Baseline) - (PLTs/WBCs in Serum)

The dimensions of the PRF clot were measured using a vernier caliper and was recorded accordingly.

Fig 1: 15 ml of blood drawn under aseptic conditions

Fig 2: Remi C852 benchtop centrifuge
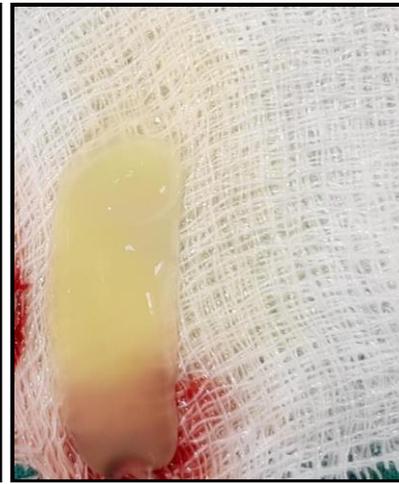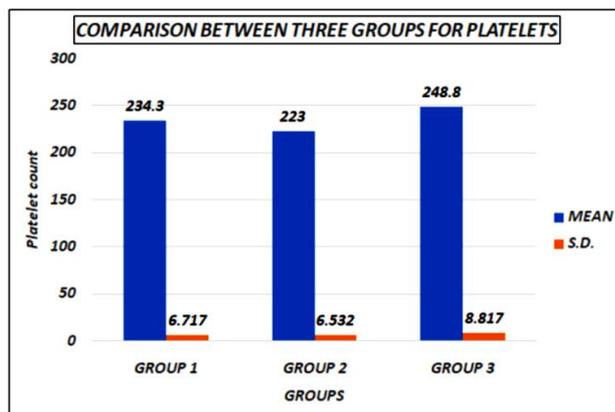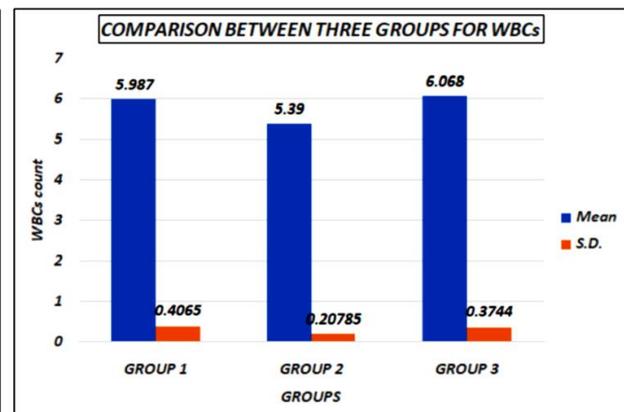
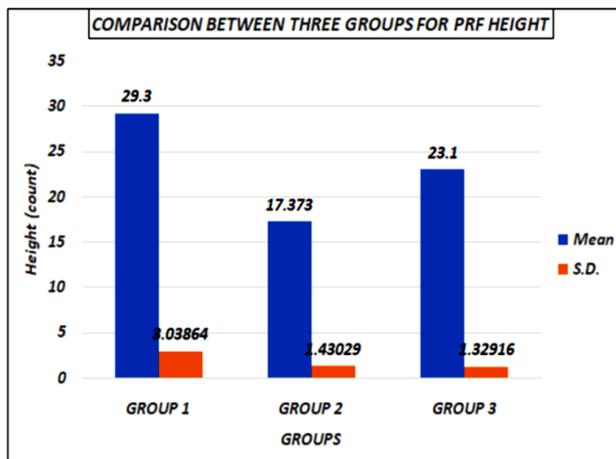**Fig 3:** Blood separated into three layers.   **Fig 4:** PRF membrane
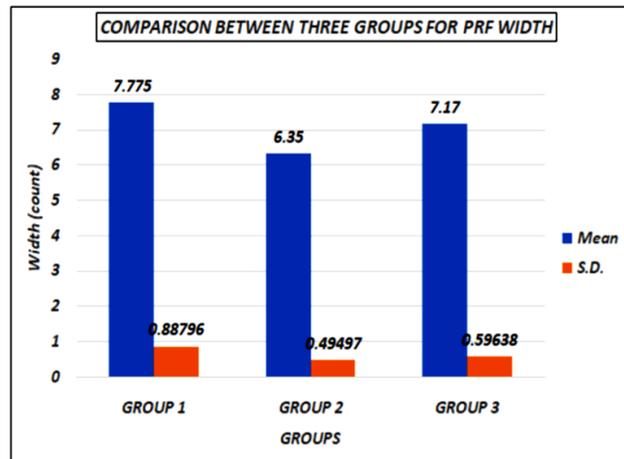

Graph 5: Compression between three groups for platelets

Graph 6: Compression between three groups for WBCs

Graph 7: Compression between three groups for PRF height

Graph 8: Compression between three groups for Width

Statistical Analysis

The IBM Statistical Package for the Social Sciences (SPSS) Statistics 21.0, United States, was used to analyze the data collected. Descriptive statisctics (mean and standard deviation) and inferential statistics (comparison using one way ANOVA followed by Tukeys Posthoc Correction) was done. Tukey's multiple post hoc procedures were performed to evaluate pair-wise difference if ANOVA was found to be significant.

Results

This cytological cross sectional study was undertaken to evaluate the formed PRF quantitatively by measuring the platelet and leukocyte concentration and the size of the PRF clot formed.

Platelet concentration in the PRF clot

The mean platelet concentrations of the three test groups are shown in the table given below. Statistically significant differences were seen between the platelet concentrations of each group in the PRF clot. The diabetics showed a significantly higher platelet concentration than that of smokers.

Table 1: Showing comparison of the platelet count in the three groups

Platelets						
Groups	N	Minimum	Maximum	Mean	Std. Deviation	F	P value
1	10	220	245	234.30	6.717		
2	10	210	234	223.00	6.532	20.771	.000
3	10	234	263	248.80	8.817		

Leukocyte concentration

The mean leukocyte concentrations of the three test groups are shown in the table given below. Statistically significant differences were seen between the leukocyte concentrations of each group in the PRF clot. The diabetics showed a significantly higher leukocyte concentration than that of smokers.

Table 2: Showing comparision of the WBC count in the three groups

WBC						
Groups	N	Minimum	Maximum	Mean	Std. Deviation	F	P value
1	10	5.30	6.50	5.9870	.40650		
2	10	5.00	5.70	5.3900	.20785	14.590	.001
3	10	5.60	6.66	6.0680	.37440		

Size of the PRF formed

On comparison of the height and width of the three groups it was found that there was significant difference in the dimensions of the clot formed.

Diabetics showed a comparatively larger clot size formation than that formed by chronic smokers. The comparison is shown in the table below.

Table 3: Showing comparison of the PRF clot height in the three groups

Height						
Groups	N	Minimum	Maximum	Mean	Std. Deviation	F	P value
1	10	25.00	36.00	29.3000	3.03864		
2	10	15.00	19.50	17.3730	1.43029	25.579	.000
3	10	21.00	25.50	23.1000	1.32916		

Table 4: Showing comparison of the PRF clot width in the three groups

Width						
Groups	N	Minimum	Maximum	Mean	Std. Deviation	F	P value
1	10	6.00	9.00	7.7750	.88796		
2	10	5.80	7.20	6.3500	.49497	13.164	.001
3	10	6.30	8.00	7.1700	.59638		

Discussion

It is a well-known fact that uncontrolled diabetes and chronic smoking are detrimental to proper wound healing. This may affect the success and outcome of the periodontal therapy.

Platelets and leukocytes are known to play a key role in wound healing and any differences in their concentration could have an impact on regenerative therapy.

In this study on comparison of the hematological parameters which included the platelet and the leukocyte concentration it was found that diabetics showed a higher platelet concentration than the other two groups. This was in accordance with the studies conducted by Kodiatte *et al*. in 2012 [10] Demirtunc *et al*., [29] and Zuberi *et al*. [12]. The platelet hyper-reactivity and increased baseline activation which is seen has a multi-factorial etiology in diabetic patients [13-17] It is associated with biochemical factors such as hyperglycemia and hyperlipidemia, insulin resistance, an inflammatory and oxidant state and also with increased expression of glycoprotein receptors and growth factors [13-17]. In contradiction to the present study no significant difference was seen in the platelet concentrations in diabetics when compared to controls in the study conducted by Jabir PK in 2019 [18]. Hekimsoy showed a decrease in the platelet concentration as compared to non diabetics which could possibly be attributed to the platelet survival period, the turnover rate and the rate of production [19].

The present study showed an increase in the leukocyte count in diabetics. This was in accordance with the studies conducted by Naredi *et al*. in 2017 [20] and Vozarova *et al*. seen due to the chronic low grade inflammation which leads to increase in insulin resistance [21]. However, a study by Oda *et al*. showed that there was no association seen with diabetes and an increase in the WBC count This study was conducted in Japanese subjects with a normal BMI and well controlled diabetes. This may account for the contradictory results [22].

The present study also showed that chronic smokers had lower platelet and leukocyte counts when compared to the other groups which was also seen in the study conducted by Das *et al*. [23], chow [24] Mohammad *et al*. [25] and Bellizi *et al*. [26] This however was contradicted by a study conducted by Roethig *et al*. [27] who found that smokers had a higher platelet count and that a lower exposure to cigarette smoke in long-term adult smokers led to statistically significant decreases of up to 9% in platelets within only 3 days.

On comparison of the size of the clot formation it was seen that patients with type 2 diabetes showed a significantly larger clot formation in height and width than smokers which was in accordance with the study conducted by Das *et al*. [23]. Another study by Miron *et al*. [28]. also stated that the clot formation was of a greater dimension in diabetics and particularly females. This could possibly be due to a lower hematocrit level in females which could account for the easier separation into the different layers by centrifugation as compared to males.

Conclusion

The results which were found in the study showed that patients with type 2 diabetes had a higher level of platelets and leukocytes than the control group and smokers. The increased blood sugar levels led to alterations in the leukocyte and platelet activity. This could be beneficial for usage in regenerative procedures and treatment of periodontal defects.

Further study on a large sample size is required into the application of PRF in the treatment of periodontal defects in diabetics and the outcomes of the treatment.

References

1. Mohan SP, Jaishangar N, Devy S, Narayanan A, Cherian D, Madhavan SS. Platelet-rich plasma and platelet-rich fibrin in periodontal regeneration: a review. Journal of Pharmacy & Bioallied Sciences. 2019 May;11(Suppl 2):S126.
2. Preeja C, Arun S. Platelet-rich fibrin: Its role in periodontal regeneration. The Saudi Journal for Dental Research. 2014 Jul 1;5(2):117-22.
3. Bergström J, Eliasson S, Dock J. A 10-year prospective study of tobacco smoking and periodontal health. Journal of periodontology. 2000 Aug;71(8):1338-47.
4. Tomar SL, Asma S. Smoking-attributable periodontitis in the United States: findings from NHANES III. Journal of periodontology. 2000 May;71(5):743-51.
5. Centers for Disease Control and Prevention. National diabetes statistics report: estimates of diabetes and its burden in the United States, 2014. Atlanta, GA: US Department of Health and Human Services. 2014 Jul;2014.
6. Löe H. Periodontal disease: the sixth complication of diabetes mellitus. Diabetes care. 1993 Jan 1;16(1):329-34.
7. Das N, Amaranath BJ. Quantitative evaluation of modified advanced platelet-rich fibrin buffy coat among diabetic patients and tobacco smokers with chronic periodontitis. Journal of Indian Society of Periodontology. 2022 Jan;26(1):24.
8. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. International dental journal. 1975 Dec 1;25(4):229-35.
9. Grant DA, Irving B, Stern IB, Listgarten MA. Periodontics. 6th ed. St. Louis: CV Mosby. Co: Mobsy; 1979. p. 525-7.
10. Kodiatte TA, Manikyam UK, Rao SB, Jagadish TM, Reddy M, Lingaiah HK, Lakshmaiah V. Mean platelet volume in type 2 diabetes mellitus. Journal of laboratory physicians. 2012 Jan;4(01):005-9.
11. Turaeva FA. Comprehensive treatment of generalized periodontitis using autotrombocytic mass. Pharma Innovation. 2020;9(6):599-600.
12. Zuberi BF, Akhtar N, Afsar S. Comparison of mean platelet volume in patients with diabetes mellitus, impaired fasting glucose and non-diabetic subjects. Singapore medical journal. 2008 Feb 1;49(2):114.
13. Colwell JA, Nesto RW. The platelet in diabetes: focus on prevention of ischemic events. Diabetes care. 2003 Jul 1;26(7):2181-8.
14. Vinik AI, Erbas T, Park TS, Nolan R, Pittenger GL. Platelet dysfunction in type 2 diabetes. Diabetes care. 2001 Aug 1;24(8):1476-85.
15. Schneider DJ. Factors contributing to increased platelet reactivity in people with diabetes. Diabetes care. 2009 Apr 1;32(4):525-7.
16. Kakouros N, Rade JJ, Kourliouros A, Resar JR. Platelet function in patients with diabetes mellitus: from a theoretical to a practical perspective. International journal of endocrinology. 2011 Oct;2011.
17. Yngen M, Norhammar A, Hjemdahl P, Wallén NH. Effects of improved metabolic control on platelet reactivity in patients with type 2 diabetes mellitus following coronary angioplasty. Diabetes and Vascular Disease Research. 2006 May;3(1):52-6.
18. Jabir PK. Platelet Count and its Correlation with Blood Sugar Level in Type 2 Diabetes Mellitus Patients. International Journal of Physiology. 2019;7(4):75-78.
19. Hekimsoy Z, Payzin B, Örnek T, Kandoğan G. Mean platelet volume in Type 2 diabetic patients. Journal of Diabetes and its Complications. 2004 May 1;18(3):173-6.
20. Naredi M, Jhavar D, Krishnan D. Study of relationship between WBC count and diabetic complications. Int J Adv Med. 2017 Jul;4(4):1128-32.
21. Vozarova B, Weyer C, Lindsay RS, Pratley RE, Bogardus C, Tataranni PA. High white blood cell count is associated with a worsening of insulin sensitivity and predicts the development of type 2 diabetes. Diabetes. 2002 Feb 1;51(2):455-61.
22. Oda E. High-sensitivity C-reactive protein, but not white blood cell count, independently predicted incident diabetes in a Japanese health screening population. Acta diabetologica. 2015 Oct;52(5):983-90.
23. Das N, Amaranath BJ. Quantitative evaluation of modified advanced platelet-rich fibrin buffy coat among diabetic patients and tobacco smokers with chronic periodontitis. Journal of Indian Society of Periodontology. 2022 Jan;26(1):24.
24. Chow CK. Circulating endothelial coagulative activation markers after smoking cessation: A 12-month observational study. Eur J Clin Invest. 1993;41:616-26.
25. Muhammad A. Cigarette smoking and hemostatic function. Am Heart J. 2013;115:267-71.
26. Bellizzi MC. The effect of smoking on mean platelet volume and lipid profile in young male subjects. Anadolu Kardiyol Derg. 1997; 8:422-25.
27. Roethig HJ, Koval T, Muhammad-Kah R, Jin Y, Mendes P, Unverdorben M. Short term effects of reduced exposure to cigarette smoke on white blood cells, platelets and red blood cells in adult cigarette smokers. Regulatory toxicology and pharmacology. 2010 Jul 1;57(2-3):333-7.
28. Miron RJ, Dham A, Dham U, Zhang Y, Pikos MA, Sculean A. The effect of age, gender, and time between blood draw and start of centrifugation on the size outcomes of platelet-rich fibrin (PRF) membranes. Clinical oral investigations. 2019 May;23(5):2179-85.
29. Demirtunc R, Duman D, Basar M, Bilgi M, Teomete M, Garip T. The relationship between glycemic control and platelet activity in type 2 diabetes mellitus. Journal of Diabetes and its Complications. 2009 Mar 1;23(2):89-94.