Original Article

Fibrin Network Pattern Changes of Platelet-rich Fibrin in Chronic Periodontitis Patients with and without Diabetes Mellitus

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Abstract

Introduction: Platelet-rich fibrin (PRF) has been widely applied for periodontal regeneration due to its simplicity, autologous nature and economics. This study aims to evaluate the variations in the fibrin network patterns of the PRF clot, which was isolated from chronic periodontitis patients with and without diabetes mellitus (DM). **Materials and Methods:** This study included 90 participants undergoing periodontal treatment therapy. The patients were divided into three groups: Group A ($n = 30$), chronic periodontitis patients without Type 2 DM; Group B ($n = 30$), chronic periodontitis patients with Type 2 DM (haemoglobin A1c [HbA1c], 6.5%–6.9%) and Group C ($n = 30$), chronic periodontitis patients with Type 2 DM (HbA1c ≥ 7%). The clinical parameters and blood samples were collected, and PRF preparation was done. Histological slide analysis was done in the stained section of the PRF clot and examined for the presence of dense and loose fibrin network patterns with the entrapments of platelets. **Results:** There was a significant difference in the dense and loose fibrin framework between chronic periodontitis patients without type 2 DM, chronic periodontitis patients with type 2 DM of 6.5–6.9 HbA1c and chronic periodontitis patients with type 2 DM of >7 HbA1c. **Conclusion:** Within the limitations of this study, future studies are needed to find the clinical outcomes of various PRF preparations in diabetic patients and other systemic factors that influence the fibrin network pattern, so that better regenerative outcomes can be achieved in patients with systemic diseases.

Keywords: Chronic periodontitis, diabetes mellitus, fibrin network pattern, platelet-rich fibrin

Introduction

Periodontitis is a chronic inflammatory disease characterised by the destruction of the teeth-supporting structures,[1] affecting 10%–15% of adults and negatively impacting the quality of life.[2] In the early 1990s, periodontitis was considered as the sixth complication of diabetes Mellitus.[3,4] Type 2 Diabetes Mellitus patients are 2.8 times at increased risk of developing Periodontitis and 4.2 times more affected by alveolar bone loss than non-diabetic patients.[5]

According to the US National Health and Nutrition Examination Survey III, adults with an haemoglobin A1c (HbA1c) level of >9% had more severe periodontitis than those without diabetes.[2] In 2007, Lalla *et al*. established that the periodontal destruction rate was related to inappropriate glycaemic control in diabetic patients.[6] Healing after periodontal treatment is impaired in diabetic patients. Wound healing is defective, including impaired neutrophil activation and responses, fibroblast migration and proliferation and angiogenesis in the diabetic condition.[7,8]

In periodontal regeneration, various biomaterials based on endogenous regenerative technology were used in addition to autogenous and allogenic grafts.[9] Platelets secrete fibrin, fibronectin and vitronectin, which act as a matrix for the connective tissue and cell adhesion molecules, which is more efficient for cell migration. Growth factors and cytokines in

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platelets play key roles in inflammation and wound healing. This has led to using platelets as therapeutic tools to improve tissue repair, particularly in periodontal wound healing.[10-12]

Platelet-rich fibrin (PRF), described by Choukroun *et al*.,[13] is a second-generation platelet concentrate, a physiological bio-scaffold rich in integrated platelets and leucocyte cytokines. It leads to the formation of a fibrin network that favours cytokine entrapment and cell migration.[13,14] PRF has been widely applied due to its simple, autologous and economical properties.

The PRF clot forms a strong natural fibrin matrix, which concentrates almost all the platelets and growth factors of the blood harvest and shows a complex architecture as a healing matrix with unique mechanical properties. PRF enhances wound healing and regeneration better than without use.

Many factors affect fibrin formation and structure, such as genetic factors, acquired factors such as abnormal concentration of thrombin and factor XIII in plasma, blood flow, platelet activation, oxidative stress, hyperglycaemia, hyperhomocysteinaemia, medications and cigarette smoking.[15] Regarding PRF, various studies have been performed to better understand the characteristic features of platelets and white blood cells (WBCs) and their role in regeneration.[16] However, the patterns and arrangement of fibrin networks within the PRF clot of diabetics and their capacity to entrap the platelets and WBCs have not been highlighted. Thus, this study aims to evaluate the variations in the fibrin network patterns of the PRF clot, which was isolated from chronic periodontitis patients with and without DM.

Materials and Methods

The histological study was used to compare the features of fibrin network patterns in chronic periodontitis patients without DM, chronic periodontitis patients with controlled (HbA1c 6.5%–6.9%) and uncontrolled diabetes (HbA1c 7%). The study was conducted after getting informed consent from the participants. This study included 90 participants undergoing periodontal treatment therapy at the Department of Periodontics in Vivekanandha Dental College for Women, Tamil Nadu. The institutional review board reviewed and approved this study on January 2023 (VKM/ETH/2023/289). It was carried out according to the Helsinki Declaration. The inclusion criteria included chronic periodontitis patients and clinical attachment loss of 3–4 mm, involving >30% of the sites, minimum of 20 natural teeth, age 30–65 years, type 2 diabetes HbA1c controlled 6.5%–6.9%, HbA1c uncontrolled >7% and normal platelet count. The exclusion criteria included patients with alcohol/smoking or tobacco use in any form, systemic disease other than type 2 DM, patients with major complications of DM (i.e., cardiovascular and peripheral vascular diseases), platelet and coagulation disorders and pregnancy/lactating women.

The patients were divided into three groups: Group A ($n = 30$), CHRONIC periodontitis patients without type 2 DM; Group B ($n = 30$), chronic periodontitis patients with type 2 DM (HbA1c 6.5%–6.9%) and Group C ($n = 30$), chronic periodontitis patients with type 2 DM (HbA1c ≥ 7%). Clinical parameters such as plaque index, gingival index, gingival bleeding index and number of sites with probing depth of 3–4 mm were recorded for the patients.

A blood sample of each volunteer was obtained from the antecubital vein of the subject's right or left arm. A volume of 10 mL of blood was collected from patients with 10 mL injectors. 10 mL of blood was transferred into the silica test tubes and centrifuged at 2800 rpm for 12 min. After centrifugation, PRF clots were removed using a sterile tweezer, the red blood cell base layer was separated using scissors and the clot obtained from PRF was used in light microscopy.

Histological Procedure for Light Microscopy

Fixing was done on PRF clots transferred into perforated stainless-steel cassettes placed in a 10% formalin-containing container. The fix was done for 24 h to prevent cell death and preserve the biological tissues. Tissue processing was done after 24 h of fixation, and the cassette-containing clot was subjected to dehydration, clearing and infiltration of wax processing through solutions such as 10% formalin, 60%, 70%, 80%/, 90% and 100% isopropanol alcohol, xylene and paraffin wax in an orderly manner. For embedding fibrin clot in paraffin wax, sectioning was done using a rotary microtome and dewaxing of the slide was done by heating about 55°C to eliminate wax in the slide warming table. Tissue staining was done using haematoxylin and eosin and followed by numbering the slides for identification. Histological slide analysis was done in the stained section of the PRF clot of all the patients, who were examined for dense and loose fibrin network patterns with the entrapments of platelets. WBC cells were analysed, which were categorised into mild, moderate and severe forms by focusing the following features using a light microscope at ×20 and ×40 magnification.

Results

The results obtained in this study were statistically analysed. To compare the groups, Kruskal–Wallis test was applied for multiple pairwise comparisons. To compare proportions between study and control groups, Chi-square test was applied. To analyse the data, SPSS (IBM SPSS Statistics for Windows, Version 23.0, Armonk, NY, USA: IBM Corp. Released 2015) was used and 5% ($\alpha = 0.05$) was fixed as the significance level.

In the histological section of PRF, all participants showed the outermost layer with a dense fibrin network and inner layers with a loose fibrin network with entrapment of platelets and WBCs.

The presence of dense and loose fibrin networks was analysed, and the percentage difference was investigated. The difference between Group A, Group B and Group C fibrin matrix was compared. The differences and percentage variation were compared in chronic periodontitis patients.

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Table 1: Comparison between Group A, B and C

	Entrapment of WBCs and platelets in dense fibrin	Entrapment of WBCs and platelets in loose fibrin	*P*	Dense fibrin network	Loose fibrin network	*P*
Group A	73.17	19.10	0.00*	74.50	61.83	0.00*
Group B	45.67	57.50		42.30	46.42	
Group C	17.67	59.90		19.70	28.25	

*Significant <0.05. WBCs: White blood cells

Significant differences in dense and loose fibrin framework between chronic periodontitis patients without type 2 DM, chronic periodontitis patients with type 2 DM of 6.5–6.9 hba1c and chronic periodontitis patients with type 2 DM of >7 HbA1c were found. Similar significant entrapment of cells was found between all three groups, as shown in Table 1.

Discussion

The present clinical research demonstrated the variation in the fibrin meshwork pattern of PRF in diabetic individuals through histological analysis. As per our knowledge of the literature, this was the first study of its kind that deviated from the thinking of understanding the pattern of a fibrin clot in diabetic individuals with chronic periodontitis. It histologically evaluated the change in dense and loose fibrin network patterns with the entrapment of cells in both controlled and uncontrolled DM. The knowledge of estimating the fibrin clot variation of platelets concentrates in chronic periodontitis with and without DM was because it contains a high concentration of growth factors and plays an immense role in the regeneration of the lost tissues.[17,18]

PRF is characterised by the prolonged release of growth factors for over 300 min after its preparation; it also releases leucocytes and cytokines from the fibrin mesh, which lasts for >7 days.[19] It is also biocompatible and able to release growth factors and cytokines, increasing the healing of hard and soft periodontal tissues.[20]

Yajamanya *et al*., 2016,[21] in a histological analysis, showed that the fibrin network's density was reduced in quantity and quality in older patients compared to younger patients. Denser fibrin network pattern and a larger amount of WBC and platelet entrapment were seen in the PRF obtained from younger individuals as compared to older individuals.[19] From the study, it can also be stated that there are significant intergroup differences in the patterns of fibrin network arrangement and their ability to entrap the platelets and WBCs observed as the age progresses, i.e. from 20 to 55 years and above. According to this study, diminished cellular components and the presence of thin, loose fibrin could be attributed to systemic diseases such as uncontrolled DM when compared to controlled DM, which showed a moderate form of dense fibrin pattern.

Chatterjee *et al*., in 2018,[17] showed histologically the fibrin network pattern in the hypertensive and smokers showed less prominent fibrin borders between the cellular structure in both the platelet concentrates, but sufficient meshwork was formed in Titanium Platelet Rich Fibrin (TPRF) when compared to PRF. Following this study, a thick border of dense fibrin network pattern, along with more entrapment of platelets and WBCs, was seen in chronic periodontitis patients without DM. Still, the diabetic group showed a thin border of the fibrin network with less entrapment of cells.

Anirban Chatterjee *et al*., 2019,[21] evaluated the fibrin network pattern changes in healthy, gingivitis and generalised chronic periodontitis. They showed a thick, dense fibrin matrix with well-organised cellular components in TPRF clots compared with PRF groups. The PRF clots obtained from chronic periodontitis patients showed 83.3% of severe dense fibrin network pattern and 16.7% of mild loose fibrin network pattern. The entrapment of WBCs and platelets was found to be 76.7% in dense fibrin network whereas 23.3% in loose fibrin network. Systemic diseases such as DM have altered the fibrillar cell matrix, thus leading to impaired wound healing. In controlled DM, 56.7% of loose fibrin was dominant compared to mild dense fibrin at 40%. The loose fibrin network of mild form at 76.7% was more prominent, with 53.3% of no dense fibrin network formation in uncontrolled diabetic individuals. The platelets and WBC entrapments of 66.7% were significantly more in the dense fibrin network when compared to loose fibrin in controlled diabetics, whereas uncontrolled showed 70% of entrapment of cells in thin borders of loose fibrin.

To our understanding, the study's limitations included wide variations in age groups, sex distribution and the usage of antidiabetic drugs that could alter the fibrin clot. A histologically correct appreciation of the changes in individual fibrin strand morphology, thickness and difficulty in identifying and separating WBC and platelets within the fibrin meshwork may be noted.

Conclusion

Given this study's limitations, future studies are needed to determine the clinical outcomes of various PRF preparations in diabetic patients and other systemic factors that influence the fibrin network pattern to achieve better regenerative outcomes in patients with systemic diseases.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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