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A Study to Estimate and Compare Platelet Derived Growth Factor and Vascular Endothelial Growth Factor in Platelet Rich Fibrin Across the Spectrum of Ages in Healthy Volunteers

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Abstract

Context: Platelet-rich fibrin (PRF) is a 2nd generation platelet concentrate. It contains high levels of growth factors (GF) and is used to treat various diseases.

Aims: To estimate the concentrations of Platelet-derived growth factor (PDGF) and Vascular endothelial growth factor (VEGF) in PRF and to compare them in different age groups.

Settings and Design: Samples were collected randomly from 28 healthy subjects. Subjects were divided into 2 groups: Group A- 18 to 27 years and group B- 28 to 65 years.

Methods and Material: PRF preparation- 10ml of whole blood was collected without anticoagulant and was centrifuged at 700 g for 12 minutes. The PRF clot was formed in the middle of the tube. The PRF clot was then centrifuged at 10,000 g for 15 minutes for obtaining the PRF release and was frozen at -80^{0}C. Estimation of PDGF and VEGF was done using ELISA method and were evaluated.

Statistical analysis used: Descriptive statistics like mean, median, standard deviation and ranges were calculated. An unpaired t-test was used to compare Group A and Group B.

Results: The mean concentrations of both growth factors were higher in group A. The difference in the concentration of VEGF was statistically significant (P Value- 0.0478).

Conclusions: A major advantage of PRF is its autologous nature. However, the concentrations of these two growth factors were higher in the younger age group which supports the use of allogeneous PRF in treatment of older patients.

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Keywords: Platelet Rich Fibrin; Enzyme Linked Immunosorbent Assay; Platelet Derived Growth Factor; Regenerative Medicine; Vascular Endothelial Growth Factor.

Key Messages: Considering the higher growth factor concentration in the younger age group, this study may influence our opinions towards using allogenic PRF for therapy in the older population. However, a larger case control investigation is necessary to substantiate the findings.

INTRODUCTION

- Growth factors act by binding to specific receptors and influencing the expression of genes that have various roles incell cycle, promoting replication, preventing apoptosis and biosynthesis of cellular components required for a mother cell to replicate .One of the major roles of the growth factors is to stimulate the activity of proteins required for cell growth, survival, and division.[1]
- PRF is an autologous fibrin matrix. The PRF contains abundant platelets and cytokines. Anticoagulants such as bovine thrombin or any other jellifying agent are not required for PRF preparation.[2]
- PRF is used by various specialties for different treatment modalities. There has been relatively limited research into the chemical properties of PRF in comparison to clinical studies.
- This study highlights two of the major growth factors found in the PRF involved in wound healing and quantifying them helps us understand about PRF better.

SUBJECTS AND METHODS

Sample collection and grouping:

Single anticoagulated whole blood sample of 10ml was collected from 28 healthy asymptomatic consenting subjects, by trained technicians, under aseptic conditions. The samples were separated into two groups viz. group A including ages from 18 to 27 years and group B including ages from 28 to 45 years. Each group comprised 14 samples.

PRF PREPARATION

The collected sample was centrifuged at 700 g for 12 minutes. The PRF clot was formed in the middle of the tube, just between a jelly like red blood cell layer at the bottom and serum at the top. The PRF clot formed was then transferred into another tube and then was centrifuged at 10,000 g for 15 minutes for obtaining the PRF releasate. The PRF releasate was transferred to labeled eppendorf tubes and was frozen at -80^{0}C (Fig. 1 & 2).

ESTIMATION OF PDGF AND VEGF

Estimation of PDGF and VEGF were done using two site sandwich ELISA method. (Kits used: KTE63444 Human Platelet Derived Growth Factor (PDGF) ELISA Kit (Abbkine Scientific Co., Ltd., Wuhan, China) and KET6033 EliKine™ Human VEGF ELISA Kit (Abbkine Scientific Co., Ltd., Wuhan, China)). The stored releasate was thawed and then was subjected to ELISA. Results were reproduced into a 4-Parameter Logistic Regression curve and concentrations were determined using Gain Data (Arigo laboratories Corp., Taiwan. https://www.arigobio.com/elisa-calculator).

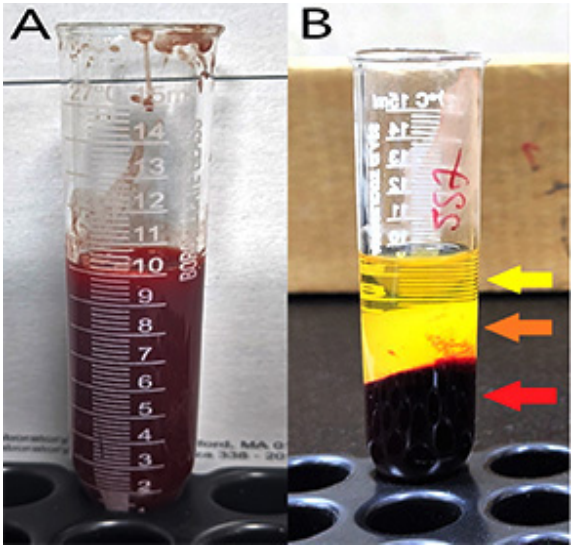


Fig. 1: A - 10 ml fresh blood collected in glass tube without anticoagulant; B - After the first centrifugation at 700 g for 12 minutes (Yellow arrow - Supernatant serum, Orange arrow - Platelet rich fibrin (PRF), Red arrow - Coagulated RBCs)

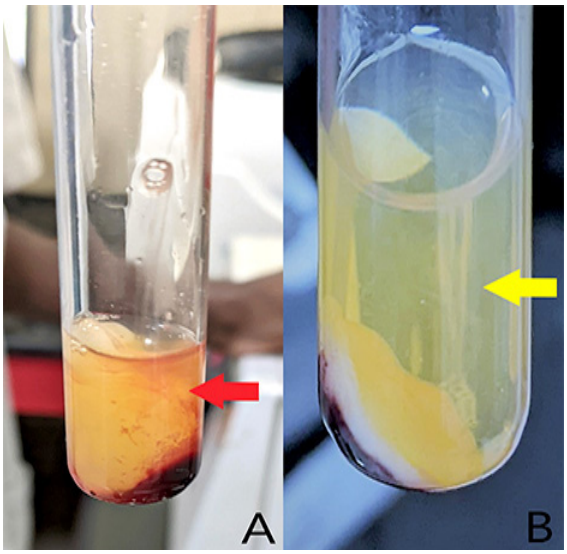
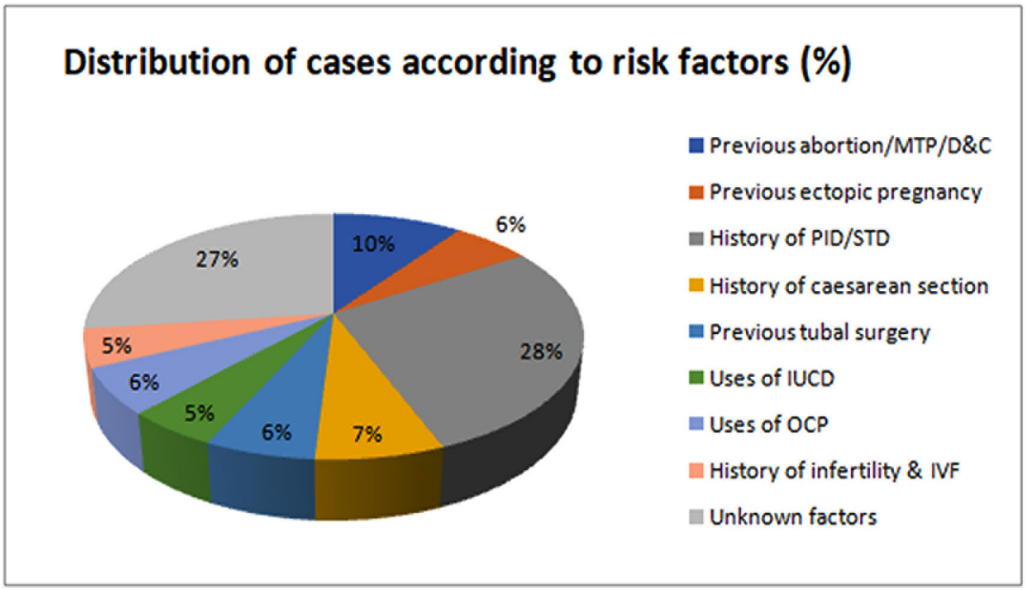
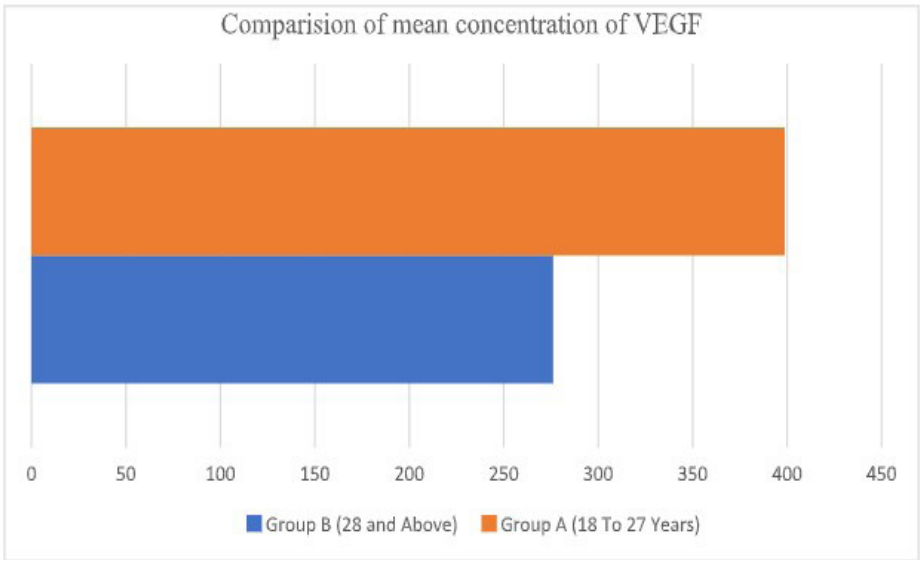


Fig. 2: A - PRF separated after first centrifugation. B - PRF releasate after second centrifugation at 10,000 g for 15 minutes



Graph 1: Comparison of mean concentration of VEGF between the two age groups Group A and Group B



Graph 2: Comparison of mean concentration of PDGF between the two age groups Group A and Group B

ETHICS

The study was initiated after getting approval from the Institutional Human Ethics Committee. The Institutional Human Ethics Committee Registered with DCGI (Reg. No: ECR/212/Inst/TN/2013) IORG & OHRP (IORG0010384) approved the study. Informed consent was obtained from every subject. It is a minimally invasive procedure; No harm was caused to the subjects. Subjects had freedom to withdraw from the study, and this was informed to them.

STATISTICS

Descriptive statistics like mean, median, standard deviation and ranges were calculated. An unpaired t-test was used to compare Group A and Group B to determine if there is a significant difference between the two.

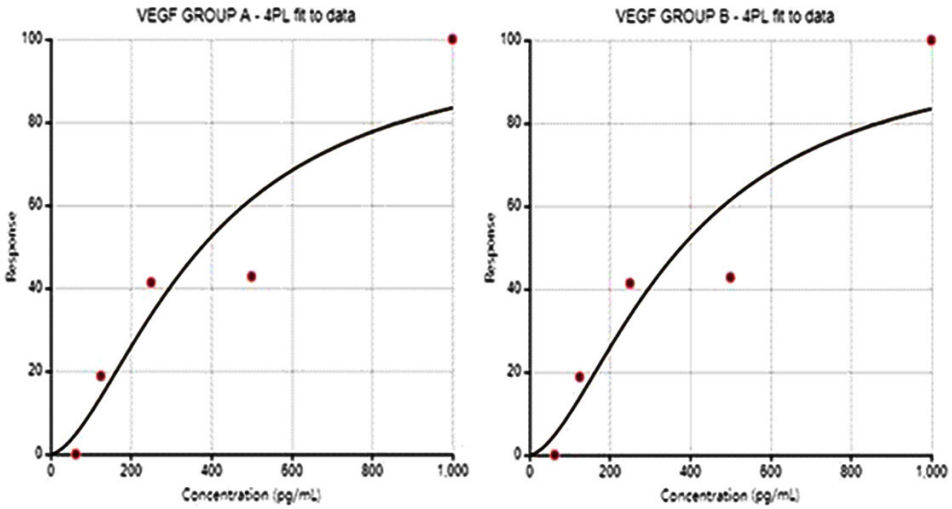


Fig. 3: VEGF 4PL graph

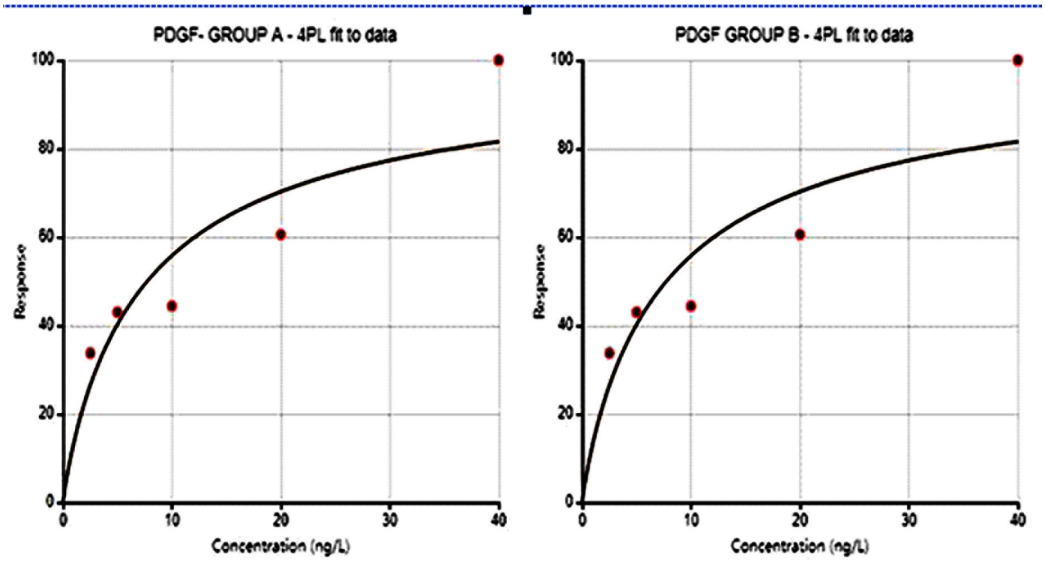


Fig. 4: PDGF 4PL graph

Hypotheses for the tests are as follows:

The null hypothesis (H_0): The average of VEGF for group A equals the average of VEGF for group B.

The alternative hypothesis (H_1): The average of VEGF for group A does not equal the average of VEGF for group B.

RESULTS

Estimation of VEGF

The calculated P value comparing group A and group B using unpaired t test was 0.0478. As the P Value was 0.0478 and was less than the significant value 0.05 the null hypothesis (H_0) was rejected. (Table 1)

Table 1: Statistical Data- VEGF

Statistical Data VEGF		
Mean	398.7335	276.147
Median	356.129	233.883
Std. D	180.8041018	126.8981941
P Value	0.047873499	

Estimation of PDGF

The calculated P value comparing group A and group B using unpaired t test was 0.1496. As the P

Table 2: Statistical Data PDGF

Statistical Data PDGF		
Mean	1.945428571	1.323785714
Median	1.3795	1.165
Std. D	1.393636677	0.715821548
P Vaule	0.149670953	

Value was 0.1496 and was more than the significant value 0.05 the null hypothesis (H_0) was not rejected. (Table 2)

COMPARISON OF GROWTH FACTORS CONCENTRATIONS OF GROUPS A & B

The mean concentrations of both growth factors were higher in group A. The difference in the concentration of VEGF was statistically significant (Graphs 1 & 2).

DISCUSSION

PRF clot is formed by natural polymerization during centrifugation. It is characterized by slow release of growth factors and matrix glycoproteins for a duration of ≥ 7 days. The natural fibrin architecture of PRF is responsible for the slow release. In PRP, there is a brutal platelet activation, contiguous release of growth factors, and very light fibrin network formation which prevents the slow release seen in PRF. Fibrin matrix is the determining element responsible for the therapeutic potential of PRF. The soluble fibrinogen modifies into insoluble fibrin and polymerizes into a cicatricial matrix. Centrifugation causes natural and slow polymerization of fibrin forming a homogeneous 3-dimentional organization. The platelets and glycans chains incorporate in these fibrin meshes. The fibrin matrix is elastic, flexible and strong. The fibrin matrix in PRP is rigid due to the increased thrombin used. Disadvantages of PRF: The PRF contains circulating immune cells and antigenic molecules and also increased risk of transmission of infectious agents that prevents it to be used as an allogenic therapeutic agent.[2]

Multiple growth factors combined together have shown to quicken bone repair, promote fibroblast proliferation, increase tissue vascularity, collagen formation, mitosis of mesenchymal stem cells and endothelial cells all of which play key roles in bone formation.[4]

PRF assures a second intention healing of the soft tissues and better healing of soft tissues. There is no risk of membrane infection or bone loss on exposure to PRF.[5] The mean wound healing rate is higher in tissues treated with PRF.[6]

Nishimoto et al[7] undertook a study similar to the present study in which he estimated PDGF and TGF-β in both PRP and PRF and concluded that the growth factors were concentrated in PRF; this study has estimated VEGF instead of TGF-β and the sample size was larger. This study done by Nishimoto et al estimated the growth factor in different levels of the PRF (top, middle, bottom, supernatant and red clot) whereas in this study we have centrifuged the PRF further to yield PRF releasate to measure the growth factors.

Yao Su et al.[3] did a similar study to ours estimating many growth factors and protein profiles in PRF supernatant but the samples were replicates and were not from different individuals. This study focused on the growth factor concentrations at different points of time. Considering the results of the study by Yao Su et al, it would have been better if we had applied the same. However, due to the limitations in the availability and the cost of the ELISA kits it was not done in our study.

Judith et al[8] studied with an objective to create an a cellular matrix that mimicked the extracellular matrix of an injured area and assess its potential for in vivo dermal regeneration. The relevance of this work lies in the observation that the biomimetic and chemotactic actions of monomeric MOLs in conjunction with PDGF improved wound healing. Our study has shown that the PDGF levels are considerably higher in younger individuals. Judith et al has shown that utilization of Collagen–Chitosan PDGF has better clinical outcomes. The use of products similar to this in older individuals must be extensively studied.

Nister et al.[9] estimated and compared the concentration of PDGF in vivo in neoplastic and non-neoplastic brain lesions. All PDGF isoforms were tested in fluids from cystic lesions and cerebrospinal fluid. In cyst fluids from several astrocytomas, one metastatic melanoma, one metastatic lung adenocarcinoma, and one intracerebral abscess, high concentrations of PDGF were found. PDGF was also found in some non -neoplastic lesions, most notably an intracerebral abscess. In addition to its healing properties, PDGF has been observed in neoplastic lesions; still it has to be determined whether PDGF is increased in blood in the presence of a neoplasm. A study comparing the levels of PDGF in PRF or serum of patients with neoplasms to healthy individuals may reveal some meaningful results.

Considering the study results, it is reasonable to claim that the concentration and efficiency of PRF obtained from younger individuals is greater than that of the older individuals. This result piques our desire to investigate further the concentration variations and their therapeutic applications. The most significant aspect of this study is the assessment of the risk versus benefit ratio of autologous PRF preparation. In addition, further research with a bigger study population and other types of studies are required to establish the risk to benefit ratio. A cohort study is also required to discover more about other factors that might impact growth factor concentrations in the PRF besides age.

The evaluation of both growth factors revealed that the younger age group (group A) had a mean concentration that was greater than that of the older age group (group B). Only VEGF had a statistically significant difference between groups A and B. Although group A had a higher mean PDGF concentration than group B, the difference was not statistically significant.

PRF's autologous nature is one of its advantageous characteristics. The mean concentration of the major growth factors in wound healing (PDGF and VEGF) was higher in younger individuals, according to this study. This raises the question of whether allogeneic PRF is significantly more effective for older patients and whether the risk associated with its allogeneic nature outweighs the risk associated with autologous PRF. A larger case control study is required for explanation.

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