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Growth Factor Identification Based on Speed And Duration of Centrifugation in Platelet Rich Plasma

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Abstract

Background: This study aims to determine the appropriate preparation of simple PRP protocol to yield the maximum growth factor concentration, specifically for PDGF and TGF-β1.
Methods: Blood samples were collected from 5 healthy volunteers who signed informed consent for participation in the study. The samples then processed by single centrifugation at 4 four different speed (600, 800, 1000, and 1200 rpm) for 4 different centrifugation times (8, 10, 12, 14 minutes). The Platelet Derived Growth Factor (PDGF) and Transforming Growth Factor β1 (TGF- β1) concentrations were determined by enzyme-linked immunosorbent assay (ELISA). Blood routine test analysis were measured by hematology analyzer.
Results: The 8 min with 600 rpm centrifugation protocol resulted in a slightly greater release of TGF-β1 (7,127 ng/mL) and PDGF (473,909 pg/mL). While the other samples resulted from 50,296 pg/mL (14 min with 1200 rpm) until 406.883 pg/mL (8 min with 800 rpm) of PDGF. The other results of TGF-β1 vary from 0,558 ng/mL (14 min with 1200 rpm) to 6,322 ng/mL (10 min with 600 rpm).
Conclusion: The highest concentration of PDGF and TGF-β1 were obtained from centrifugation process at 600 rpm for 8 minutes. Meanwhile, the lowest concentration of PDGF and TGF-β1 were obtained from centrifugation process at 14 min with 1200 rpm.
Keywords: PRP, PDGF, TGF- β1

Introduction

Blood with more platelets than usual is known as platelet-rich plasma. PRP injections are utilized for a variety of ailments, including injuries and musculoskeletal pain, as well as for cosmetic operations. Platelets that have been exposed to the bloodstream due to endothelial injury are activated during wound healing by coming into contact with collagen. After aggregation, platelets release their granule content and release cytokines and intercellular mediators that have been retained in the cytoplasm[1,2]. By centrifuging a greater volume of a patient's own blood, platelet-rich plasma (PRP) is a preparation of autologous human plasma with an elevated platelet concenration. It is directly collected from the patient's blood and given back to them (autologous). They also have a lot of growth factors and anti-inflammatory, healing, and pro-regenerative qualities, which help the body recover tissue damage[2,3].

Platelet-rich plasma (PRP) is one of the products formed from platelets; it can be utilized with or without prior platelet activation. Since the 1970s, these preparations have been in use, and since the 1990s, their popularity has grown[4]. PRP has since been prepared in a variety of ways, including commercial systems and traditional blood centrifugation; it can also be activated by adding collagen, calcium, and/or thrombin; it can be applied as a gel or as platelet suspension; and the methodology is still being expanded[5,6].

According to several studies, the higher concentration of growth factors in platelet-rich plasma may accelerate or hasten the healing process, reducing the amount of time that injuries take to heal, the intensity of the pain, and even promoting hair development. All wound healing processes are initiated by seven essential protein growth factors that are actively released by platelets and are concentrated via centrifugation in order to distribute supraphysiologic concentrations of these cytokines and growth factors to an injured area and

promote the body's natural healing process [7,8]. They are transforming growth factor-β (TGF-β), platelet-derived growth fator (PDGF), insulin-like growth factor (IGF-I, IGF-II), fibroblast growth factor (FGF), endothelial growth factor (EGF), vascular endothelial growth factor (VEGF), and platelet-derived endothelial growth factor (PDGF)[7].

Several hospitals are nowadays using PRP as one of medication to heal patients from various illness and trauma. Promising outcomes have been shown when platelet-rich plasma (PRP) is applied to various tissues, including bone and cartilage lesions that are both acute and chronic [9]. In chronic tendinopathies, numerous cytokines present in platelet-rich plasma are implicated in signaling pathways that transpire throughout the inflammatory healing phases, cellular proliferation, and subsequent tissue remodeling. PRP may also encourage neovascularization, which could improve the blood flow and nutrition required for cells to repair damaged tissue, bring new cells into the area, and clear debris from it[3,10]. In osteoarthritis of the knee, a metaanalysis comparing PRP to other controls, such as placebo, hyaluronic acid, corticosteroid injections, oral drugs, and homeopathic therapies, was conducted by Shen et al. on 14 randomized clinical trials (RCTs) involving 1423 patients. At the 3-, 6-, and 12-month follow-ups, the meta-analysis revealed a substantial improvement in the Western Ontario and McMaster Universities Osteoarthitis Index (WOMAC) scores (=0.02, 0.04, < 0.001, respectively). Patients with mild to moderate OA have been found to benefit more with PRP, according to subgroup analyses looking at the treatment's effectiveness based on knee OA severity[11-15].

However, different PRP preparation methods also have different impacts on patients; some recover over a short period of time, while others take a lot longer. Additionally, it is not entirely known how PRP injections work. The lack of consistency in the preparation is one of PRP's drawbacks. Platelets, leukocytes, growth factors, and other cell types may therefore differ in volume, concentration, color, and other characteristics from one preparation to the next, which might result in unfavorable clinical outcomes [16]. Drawing whole blood into tubes containing anticoagulants, centrifuging the samples to get a higher concentration of platelets, and then repeating the process is how manual PRP preparation is done. Thus, in order to optimalize the first single spin method, this study aims to standardize the PRP preparation to yield optimum concentration of PDGF and TGF-β1.

Methods

Blood Collection: We took blood samples from five healthy volunteers who gave their informed consent to take part in the trial and had no history of comorbid illnesses or recent medication use. We used a vacutainer to collect 250 ml of peripheral venous blood from the donors while using sterile and aseptic precautions. We also used a 3.2% sodium citrate anticoagulant solution. In accordance with the protocol examined, we used 3 ml Vacutube 3.2% sodium citrate blood collection tubes. In order to prevent reporting and sample bias, each protocol was examined with technical and biological doubles. The values' average was chosen for analysis.

Blood routine test. Each donor's blood was subjected to a normal blood test. Blood chemistry tests, complete blood counts, red blood cell indices (MCV, MCH, MCHC), SGOT and SGPT tests, and other basic blood tests were all performed on the red blood cells layer at the bottom of the blood sample. The SYSMEX XN-3000 automatic five classifications hematology analyzer measured a blood routine test. (SYSMEX, Kobe, Japan).

PRP Preparation: Samples were centrifuged at incremental acceleration rates ranging from 600 to 1200 rpm (600, 800, 1000, and 1200 rpm) for 8, 10, and 12 minutes in a laboratory centrifuge Combi R515 (Hanil, Korea) with swing-out S750-4 rotors, a round bucket, and an adaptor tr15c-14 in order to standardize the procedure for spinning 10 ml of whole blood from 20 standardization. As can be seen in Table 1, there were a total of 16 samples in the model. To make the process applicable for analysis, the centrifuges were not chilled, and the centrifugation was done at room temperature (22 ^{0}C). Red blood cells are located at the bottom, buffy coats are in the center, and plasma is at the top of the total blood. Using a pipette, we removed the top plasma layer without disturbing the middle or bottom layers.

The examination of PDGF and TGF-β1 concentration : After centrifugation, PRP layers were collected and subjected to an enzyme-linked immunosorbent assay (ELISA) test with a 450 nm absorbance. The PRP has been used to investigate the effects of PDGF and TGF- β1.

Descriptive statistics. To summarize the findings of the parameter analyses, a descriptive test was performed. The Kolmogorov-Smirnov test was used to determine whether the variables' distributions were normal, and all of the variables that were examined had normal distributions ($p > 0.05$ for all variables). We used the mean and standard deviation to encapsulate the continuous variables. To compare the average of all PDGF and TGF-I concentrations, we performed a one-way anova. For statistical analysis, we used IBM SPSS Version 25 (Chicago, USA). A p value of 0.05 or lower was deemed significant.

Results

Blood routine test analysis: The results of routine blood tests of donors are shown in table 1, in which A, B, C, D, E are labels for the five donors. The values of blood routine tests for all blood donors were within the normal range.

Table 1. The results of descriptive statistics

Variable	A	B	C	D	E	Mean ± SD
Hemoglobin (HGB)	13,3	13,1	14,0	12,2	12,7	13.060 ± 0.6731
White blood cell (WBC)	9,47	9,76	4,67	6,87	12,65	8.684 ± 3.037
Hematocrit (HCT)	42	40	42	38	40	40.40 ± 1.673
Platelet count (PLT)	315	303	278	406	259	312.20 ± 56.769
MCV	87	87	84	83	86	85.40 ± 1.817
MCH	28	29	28	27	28	28.00 ± 0.707
MCHC	32	33	33	32	32	32.40 ± 0.548
RDW-CV	13,0	12,6	12,0	14,4	12,7	12.940 ± 0.8933
SGOT	16	19	16	20	16	17.40 ± 1.949
SGPT	13	15	6	10	13	11.40 ± 3.507
Ureua	19	20	15	14	15	16.60 ± 2.702
Creatinine	0,6	0,6	1,0	0,7	0,8	0.740 ± 0.1673
Random glucose test	94	90	78	86	88	87.20 ± 5.933

The examination of PDGF concentration: In this work, the ELISA technique with a 450 nm absorbance was used for the PDGF analysis. In the study, which involved five participants, one donor's blood was centrifuged using sixteen different techniques.

Table 2. Statistic Analysis of PDGF concentrations

Centrifugation methods	N (donor)	Mean (pg/ml)	Std. Deviation	p-value (Kolmogorov Smirnov)	p-value (Oneway Anova)
8 min 600 rpm	5	473.909	175.683	0.129	0,001
8 min 800 rpm	5	406.883	96.196	0.058	
8 min 1000 rpm	5	183.136	75.782	0.200	
8 min 1200 rpm	5	170.478	66.481	0.200	
10 min 600 rpm	5	317.396	91.734	0.200	
10 min 800 rpm	5	377.712	204.417	0.034	
10 min 1000 rpm	5	154.471	84.034	0.200	
10 min 1200 rpm	5	64.086	37.713	0.073	
12 min 600 rpm	5	355.761	303.919	0.200	
12 min 800 rpm	5	183.79	119.052	0.200	
12 min 1000 rpm	5	183.600	82.199	0.200	
12 min 1200 rpm	5	139.666	28.432	0.200	
14 min 600 rpm	5	215.075	96.562	0.200	
14 min 800 rpm	5	238.466	78.977	0.200	

Centrifugation methods	N (donor)	Mean (pg/ml)	Std. Deviation	p-value (Kolmogorov Smirnov)	p-value (Oneway Anova)
14 min 1000 rpm	5	68.042	37.875	0.200	
14 min 1200 rpm	5	50.296	42.223	0.200	

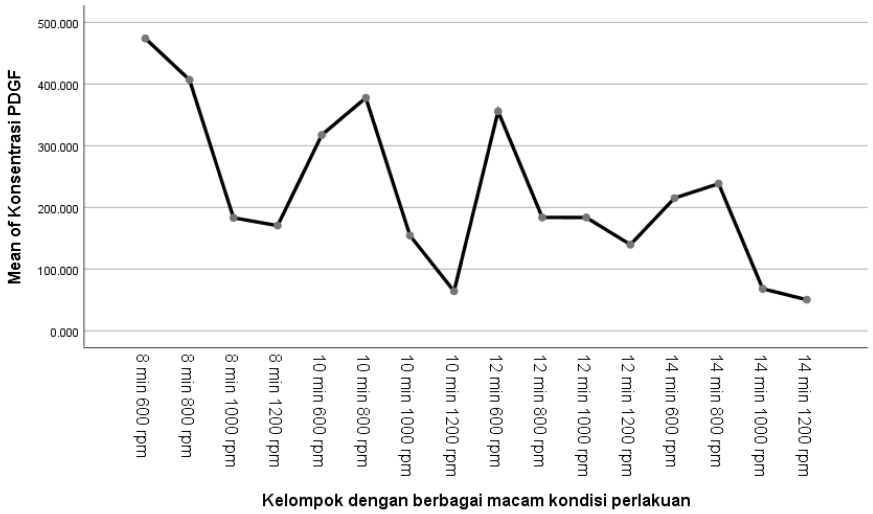

Figure 1. Graph of average PDGF concentrations

The p-value was determined for all treatment conditions to be greater than 0.05 based on the Kolmogorov-Smirnov normality test in Table 1. Thus, it may be said that the distribution of PDGF concentration across all treatment scenarios is typical. Therefore, the one-way ANOVA test is employed to determine the average difference in PDGF concentrations between 16 experimental conditions. According to the one-way Anova test, the PDGF concentration variance is different since the p value for the Levene test's test of homogeneity of variances is 0.001.

The p-value for the average PDGF concentration is 0.001, meaning that H0 is rejected and indicates that there is a significant difference in the average PDGF concentration where the p-value meets the significance of $p<0.05$. The highest average PDGF concentration in table 1 was at 8 minutes 600 rpm, which was 473.909 pg/ml.

The Examination of TGF-β1 concentration. The TGF-1 test was conducted using the ELISA technique and a 450 nm absorbance. The study consisted of 5 people in which blood from 1 donor was subjected to 16 centrifugation methods.

Table 3. Statistic Analysis of TGF-β1 concentrations

Centrifugation methods	N (donor)	Mean (ng/mL)	Std. Deviation	p-value (Kolmogorov Smirnov)	p-value (Oneway Anova)
8 min 600 rpm	5	7.127	2.278	0.200	0.001
8 min 800 rpm	5	5.232	1.886	0.200	
8 min 1000 rpm	5	3.911	0.915	0.200	
8 min 1200 rpm	5	2.538	1.079	0.152	
10 min 600 rpm	5	6.322	1.061	0.200	
10 min 800 rpm	5	3.755	2.223	0.200	
10 min 1000 rpm	5	2.925	1.449	0.200	
10 min 1200 rpm	5	1.149	0.591	0.200	
12 min 600 rpm	5	3.023	2.721	0.148	

Centrifugation methods	N (donor)	Mean (ng/mL)	Std. Deviation	p-value (Kolmogorov Smirnov)	p-value (Oneway Anova)
12 min 800 rpm	5	2.248	1.497	0.200	
12 min 1000 rpm	5	2.118	1.214	0.128	
12 min 1200 rpm	5	1.387	1.219	0.200	
14 min 600 rpm	5	4.954	1.153	0.200	
14 min 800 rpm	5	4.196	0.574	0.200	
14 min 1000 rpm	5	1.477	0.846	0.200	
14 min 1200 rpm	5	0.558	0.279	0.021	

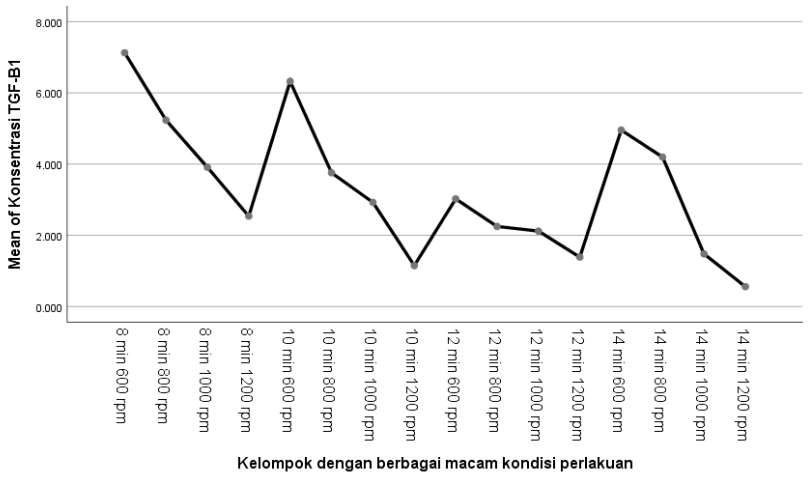

Figure 2. Graph of average TGF-β1 concentrations

Based on the Kolmogorov-Smirnov normality test. the p value was obtained for all treatment conditions> 0.05. Thus it can be concluded that the concentration of TGF-β1 for all treatment conditions is normally distributed. Therefore. the test used to see the average difference in TGF-β1 concentrations from 16 experimental conditions is the Oneway Anova test. Based on the Oneway Anova test. the p value for the test of homogeneity of variances on the Levene test is 0.007. so the PDGF concentration variance is different.

The p value for the average TGF-β1 concentration is 0.001. meaning that H0 is rejected and indicates that there is a significant difference in the average TGF-β1 concentration where the p value fulfills a significance of p<0.05. The highest average concentration of TGF-β1 in table 1 is at 8 minutes 600 rpm. which is 7.127 ng/mL.

Discussion

For hospitalized patients. blood RT is a routine procedure that measures 24 important indicators. named white blood cell count (WBC). neutrophil percentage (NE.%). lymphocyte percentage (LY.%). eosinophil percentage (EO.%). basophil percentage (BA.%). monocyte percentage (MO.%). neutrophil count (NE.#). lymphocyte count (LY.#). eosinophil count (EO.#). basophil count (BA.#). monocyte count (MO.#). red blood cell count (RBC). hemoglobin (HGB). hematocrit (HCT). mean corpuscular volume (MCV). mean corpuscular hemoglobin (MCH). mean corpuscular hemoglobin concentration (MCHC). coefficient variation of the distribution width of the red blood cell (RDW-CV). standard deviation of the distribution width of the red blood cell (RDW-SD). platelet count (PLT#). thrombocytopenia (PCT). the proportion of large platelets (PLCR). mean platelet volume (MPV). and platelet distribution width (PDW) [17]. In this study. the values of blood routine tests for all blood donors were within the normal range.

Red blood cells. the "buffy coat" of white blood cells. and plasma. which contains platelets concentrated on average between 2 and 5 times that of whole blood. are separated and concentrated by single-spin

centrifugation. Single-spin PRP preparations have been shown to have anabolic and stabilizing effects on cartilage's extracellular matrix [18].

The 8-minute. 600 rpm treatment produced the highest levels of PDGF and TGF-1 (p 0.05). Among the sixteen methods. this one has the shortest centrifugation period and the slowest centrifugation speed. This investigation is connected to work done by Fantini et al. in 2021[19]. Six Catalan jennies' entire blood was used by the researchers to create a quick. manual. and affordable PRP procedure. They contrast the centrifugation times of tubes 1 and 2. with tube 1 being spun for 10 minutes and tube 2 for 15 minutes. According to the study. the technique with a quicker centrifugation time had a higher quantity of TGF-1. A larger concentration of live. non-activated platelets in this protocol compared to the other protocol may be the cause of the higher TGF-β1 concentration in the shortest time procedure. The amount of live non-activated platelets is wanted in order to have a longer influence on growth factor release over time. according to some authors. but this has not yet been confirmed[19]; therefore. more research on platelet activation is required.

Leukocyte platelet-rich plasma (L-PRP) injection with concentrated plasmatic proteins was utilized by Schiavone et al. to treat male- and female-pattern baldness clinically (single spin centrifugation at baseline and double spin centrifugation at three months). At 6-month follow-up. patients who had PRP almost always displayed some degree of clinical improvement. and around 2 out of 5 patients (40.6%) attained at least a substantial level of improvement. which was regarded as clinically meaningful. All of the biological effects of PRP are caused by PDGFs[19].

Human dermal fibroblasts were cultured using a medium supplemented with either fetal bovine serum (FBS) or PRP. and Berndt et al compared the results. the PRP produced by a single. 5-minute. 1.500 x g. soft spin centrifugation. Additionally. it was the quickest centrifugation time to produce PRP. In comparison to FBS. PRP increased cell proliferation by 7.7 times. and PRP therapy activates fibroblasts[19].

Conclusion

The highest concentration of PDGF and TGF-β1 were obtained from centrifugation process at 600 rpm for 8 minutes. Meanwhile. the lowest concentration of PDGF and TGF-β1 were obtained from centrifugation process at 14 min with 1200 rpm.

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Ethical Requirements and Consent to Participant

This research was conducted after obtaining ethical consideration and approval from the Research Ethics Commission of M Djamil General Hospital Number (LB.01.02/XVI.1.3.2/1441/IX/2022). All patients were provided written informed consent.

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The authors declare that there is no source of funding in this study.

Conflict of Interest

There is no conflict of interest in this study.

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