Effect of Hypercholesterolemia in Platelet Rich Plasma (PRP)

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Abstract

The procedure to obtain the Platelet Rich Plasma (PRP) is very essential in the success of periodontal treatment. Such a way is crucial in producing a good quality and quantity of platelets in PRP. PRP-making protocols in different literatures vary from centrifugation speed, as well as the centrifugation duration. Hypercholesterolemia affects the amount of erythrocytes and platelets in the affected venules and arterioles.

The study was conducted with pure laboratory experiments using several centrifugation methods in PRP making so as to obtain optimal platelet levels. In this study there was 1 method of preparing PRP with two-step centrifugation, start with 800 rpm for 15 minutes, then continued with 2000 rpm for 10 minutes.

There are differences in Platelet Rich Plasma levels before and after centrifugation.

Platelet Rich Plasma is influenced by several factors including viscosity in the blood, especially in people with hypercholesterolemia.

Introduction

Periodontal disease is a chronic inflammatory disease of the teeth supporting tissues which initiated and perpetuated by subgingival specific bacteria. Nevertheless, the activation of host-mediated dectructive processes can be developing an indirect mechanism of secondary periodontal damage which trigger more severe conditions.[1,2]

The link between periodontal disease and various systemic diseases has been widely reported with various studies, one of which is the excess of blood fat, hypercholesterolemia. Hypercholesterolemia affects the number of red blood cell components. Basically cholesterol is a substance that is useful for the body to regulate chemical processes such as building cell membranes, producing vitamin D, and forming steroid hormones.[3] High levels of LDL (low density lipoprotein) cholesterol and low levels of HDL (high density lipoprotein) cholesterol in the blood are thought to cause cholesterol cumulation in blood vessel walls resulting in the formation of atherosclerotic or atheroma lesions.[3,4]

Meanwhile, in severe periodontal disease cases it can cause damage to periodontal tissue and alveolar bone.[5] Mostly in those cases, natural hard and soft tissue self-healing could not be completely achieved.[6,7,8] Moreover, a delay healing can be aggravated if the disturbance of systemic health occurred.[9,10]

The healing of periodontal tissues requires a sequence of interactions between epithelial cells, gingival fibroblasts, periodontal ligament cells, and osteoblasts. Disruption of vascularization during wound healing results in fibrin formation, platelet aggregation, and the release of several growth factors into the tissue from platelets through molecular signals primarily mediated by cytokines and growth factors. There is evidence that the content of growth factors and cytokines in platelets play an important role in inflammation and wound healing.[11]

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Platelets also secrete fibrin, fibronectin, and vitronectin, which act as a matrix for connective tissue and molecular adhesion for more efficient cell migration. This led to the idea of using platelets as a therapeutic tool to improve tissue repair especially in periodontal wound healing.[11] The utilized growth factors which was gained from different platelets components due to systemic conditions of each host can be affecting the regeneration of periodontal tissue.[12,13]

Hypercholesterolemia

Cholesterol is one of the lipid fractions, which are transported by lipoprotein compounds to various organs of the body through blood circulation. Lipoproteins that have a major role in the transport and metabolism of lipids to plasma are kilomycrons, i.e: very low-density lipoprotein (VLDL), low density lipoprotein (LDL), and high-density lipoprotein (HDL).[14]

Hypercholesterolemia has been defined as high plasma cholesterol level, with normal plasma triglyceride, and increased of low-density lipoprotein (LDL).[15] Hypercholesterolemia also causes HDL levels to decrease and increases LDL levels in the blood.[14]

Research on the relationship between serum cholesterol and the index of erythrocytes or platelets in large human populations has not been widely reported. But several studies have shown a positive correlation between serum cholesterol and either hematocrit or hemoglobin. Given that hypercholesterolemia, erythrocytosis and thrombocytosis, and membrane cholesterol content, both erythrocytes and platelets are all risk factors for cardiovascular disease, providing an understanding of the fundamental relationship between serum cholesterol and erythrocytes and platelet lineages in humans.[16]

Platelets

Blood cells are all cells in the blood, which are divided into red blood cells (erythrocytes), white blood cells (leukocytes), and platelets (platelets). Platelets are a component of discoid-shaped peripheral blood and play a role in various processes of human hemostasis and natural defense. Platelets have a round shape, 2-4 µM in diameter, have no nucleus but have many vesicles and granules and normal levels of 150,000-400,000 cells/µL. Platelets are formed in the bone marrow in a larger form called megakaryocytes (cells with large nuclei), then mature into platelets that no longer have cell nuclei and circulate in the bloodstream. The lifetime of platelets in the blood circulation is approximately 7-10 days. In platelets there are 3 granules: alpha-α, dense, and lysosomal. Granule α is the largest granule and contains more than 300 different proteins and is synthesized by megakaryocytes.[17]

The normal number of platelets is between 150,000-300,000/µL. According to Marx, platelets that are damaged or considered nonviable will not release bioactive growth factors, so that the resulting PRP is disappointing. The PRP used for the treatment is about 1,000,000 platelets/µL. If whole blood contains 200,000±75,000/µL, then the PRP for the application of treatment must have an increasing average percentage about 400% of the initial platelet count.[17]

Platelet Rich Plasma

Platelet rich plasma (PRP) is an autologous product produced from whole blood through a centrifugation process resulting in high platelet concentrations in low plasma volume. Many techniques for making PRP vary depending on the number, speed and duration of the rotation.[18-21] With so many growth factors contained in it, PRP functions to accelerate endothelial, epithelial and epidermal regeneration, stimulate angiogenesis, stimulate collagen synthesis, accelerate soft tissue healing, reduce scarring on the skin, accelerate homeostasis response to injury, thereby stimulating the healing process of wounds, and reversing the inhibition of wound healing caused by glucocorticoids. It is also a fibrin adhesive with hemostatic function. Because it is an autologous material, so it is a biocompatible, safe and effective material. The high concentration of leukocytes in PRP adds to the anti-microbial effect.[20,22-25]

Recently, the PRP uses is a popular therapy in periodontal disease. The treatment is oriented to biological improvement by releasing growth factors the surrounding tissues. PRP play important role in homeostasis, coagulation, tissue repair, bone remineralization, and matrix synthesis of the tissues.[26-28]

However, behind it polarity, there are still contradictions related to the use of PRP in some cases in the literature.[29-31] Several studies show that the use of PRP with different levels showed an excellent therapy effects in some clinical

cases.[32-35] The differences in PRP levels can also be influenced by various factors like hosts, the method of manufacture includes the speed and duration of centrifuges.[36,37] The protocol for PRP preparations is very diverse, and there is not enough evidence regarding the best method to become a standard protocol that can be used in PRP preparations.[16,33,36]

High plasma cholesterol levels have a potential influence in the PRP levels which produced from the blood of hypercholesterolemic patients. This is as already mentioned in some literature about the relationship between cholesterol serum, erythrocytes and platelets.[38-41] The lack of literature explaining the relationship between PRP and hypercholesterolemia encourages researchers in this study to explore further about the effects of hypercholesterolemia on the produced PRP levels.

Materials and methods

The research method was carried out by laboratory pure experimental using centrifugation method in making PRP so as to obtain optimal platelet levels. The method of making PRP in this study with two centrifugations, the first 800 rpm for 15 minutes, then continued with a second centrifugation of 2000 rpm for 10 minutes.

Control: blood of patients who are not hypercholesterolemic.

Results

Based on data from research conducted on 40 samples, the data presentation can be seen in the following tables and charts. The results of the platelet count before and after centrifugation in the study sample and control groups can be seen in Table 1 and Figure 1.

Groups		Before	After	p value
Research sample	Mean	241.15	290.75	0.037*
	SD	56.00	137.36	
Control	Mean	257.80	306.75	0.029**
	SD	31.48	109.69	

* Paired t test
** Wilcoxon test

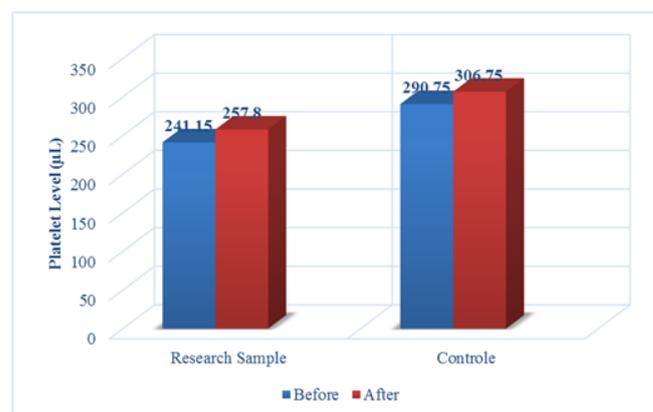**Table 1.** Platelet count before and after centrifugation.

Figure 1. Platelet count before and after centrifugation.

Results of comparison of platelet rich plasma platelet levels after centrifugation in the study sample and control groups can be seen in Table 2, Figure 2.

Groups		Change
Research Sample	Mean	49.60
	SD	98.68
Control	Mean	48.95
	SD	99.24
p-value		0.984

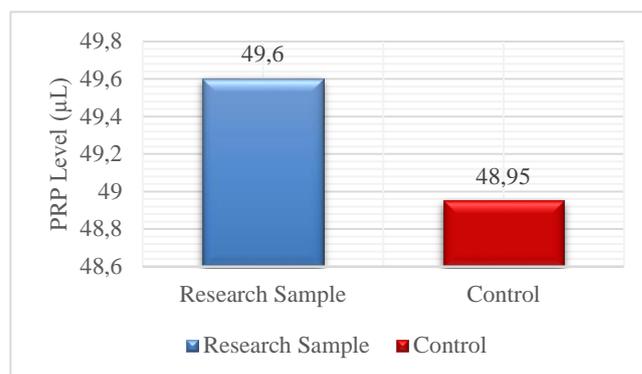* Independent t test

Table 2. PRP levels.

Figure 2. PRP levels.

Discussion

Research has been conducted to see the effect of hypercholesterolemia on platelet rich plasma on 40 samples of men and women aged 20-45 years, the subjects were from blood tests at Wahidin Sudirohusodo Hospital in Makassar. Subjects who were taken did not smoke, did not suffer from systemic diseases other than cholesterol, did not suffer from malignancy/

cancer, did not take drugs, were never hospitalized at least 1 week before taking blood, not during menstruation and menopause, and laboratory results routine blood clinical pathology and blood lipids.

Table 1 shows the differences before and after centrifugation. In the average prior research group of 241.15 and after 290.75, the results of the statistical test obtained p value (0.037) <0.05 which means that there is a difference between before and after centrifugation. Whereas in the average control group before amounting to 257. 80 and after amounting to 306.75, the results of statistical tests obtained p value (0.029) <0.05 which means there is a difference between before and after centrifugation.

Table 2 shows the differences in changes that occur between the sample group and the control group. The results of the analysis showed that the highest change occurred in the sample group of 49.60 while in the control group was 48.95. Statistical test results obtained p value (0.984)> 0.05, which means that there is no difference in mean changes between the sample group and the control group.

The results of this study differed from Utomo and Rofi'i's research[42] in normal patients with an increase of 436%. In the test results obtained an increase in platelet levels that were not significant before and after the sample group centrifugation of 17.05% and for the control group of 15.95%.

Conclusions

The speed and duration of centrifugation affect PRP platelet levels in the blood in patients with Hypercholesterolemia. It can be seen by an increase in platelet levels before and after centrifugation in both the sample group and the control of the research data although the results were not significant. This is because platelet levels in the blood are influenced by several factors, one of which is influenced by the viscosity factor in the blood of hypercholesterolemia patients.

Declaration of Interest

The authors report no conflict of interest.

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