

# **EFFECT OF CENTRIFUGATION SPEED AND CENTRIFUGATION DURATION ON PLATELET RICH PLASMA (PRP) PLATELET LEVELS IN BLOOD IN PATIENTS WITH HYPERCHOLESTEROLEMIA**

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**Objective:** 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..

**Methods** 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 were 5 different methods of preparing PRP: Method 1: with two-step centrifugation, start with 800 rpm for 15 minutes, then continued with 2000 rpm for 10 minutes. Method 2: with two-step centrifugation, start with 3000 rpm for 3 minutes 45 second, then continued with 3000 rpm for 15 minutes. Method 3: with two-step centrifugation, start with 150g for 10 minutes, then continued with 450g for 10 minutes. Control: patient without hypercholesterolemia

**Result :**

**Conclusion :**

**Keyword:** centrifugation rate, centrifugation duration, platelet level

## INTRODUCTION

The association of periodontal disease with various systemic diseases has been widely reported with various studies, one of which is the excess fat in the blood, namely hypercholesterolemia. High LDL (low density lipoprotein) cholesterol levels and low HDL (high density lipoprotein) cholesterol levels in the blood are thought to cause cholesterol buildup in blood vessel walls resulting in the formation of atherosclerotic or atheroma lesions.<sup>1,2</sup>

Periodontal disease is an inflammatory disease in the supporting tissues of the teeth caused by subgingival specific bacteria. Periodontal disease can be divided into two, namely gingivitis and periodontitis. Periodontal disease will cause damage to periodontal tissue and alveolar bone.<sup>3</sup>

Given the adverse effects of periodontal disease, it raises the need for periodontal disease treatment. Regeneration of periodontal tissue is a process to heal and grow periodontal ligaments, cementum and alveolar bone through complex processes involving certain techniques with the use of certain materials.<sup>4,5</sup>

Platelets also secrete fibrin, fibronectin, and vitronectin, which acts as a matrix of connective tissue and adhesion molecules for cell migration more efficiently. This led to the idea of using platelets as a therapeutic tool to improve tissue repair especially in healing periodontal wounds.<sup>6</sup>

Platelet rich plasma (PRP) is a blood concentrate that has a platelet concentration several times higher than the normal concentration of platelets in human blood. PRP is used because platelet concentrations are at least four times the normal platelet concentration, or 1,000,000 platelets / Ml.

## **Literature Review**

### **Hypercholesterolemia**

Cholesterol is one of the lipid fractions, which is transported by lipoprotein compounds to various organs of the body through blood circulation. Lipoprotein which has a major role in the transport and lipid metabolism of plasma, namely kilomycron: very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL).<sup>7</sup>

Hypercholesterolemia is defined as high plasma cholesterol levels, with normal plasma triglycerides, with an increase in low density lipoprotein (LDL). Hypercholesterolemia also causes HDL levels to decrease and increases LDL levels in the blood.<sup>7,8</sup>

Research on the relationship between serum cholesterol and the index of erythrocytes or platelets in large human populations has not been widely reported. However, several studies have shown a positive correlation between serum cholesterol and both hematocrit or hemoglobin.<sup>9</sup>

### **Platelets**

Blood is a special form of connective tissue consisting of three main cell types, namely: erythrocytes (red blood cells), leukocytes (white blood cells) and platelets. These cells, also called blood formed element, circulate in a liquid medium, namely plasma. Blood cells carry gas, nutrients, waste products, hormone antibodies, various chemicals, ions, and other substances in the plasma to and from the cells of the body.<sup>10</sup>

Blood cells are all cells contained in the blood, which are divided into red blood cells (erythrocytes), white blood cells (leukocytes), and platelets (platelets). Platelets or platelets are a discoid component of peripheral blood and play a role in various processes of hemostasis and natural human defense. Platelets or platelets have a rounded character, 2-4  $\mu\text{M}$  in diameter, do not have a nucleus but have many vesicles and granules and normal levels of 150,000-400,000 cells /  $\mu\text{L}$ . Platelets are formed in the bone marrow in a larger form called megakariocytes (cells with large nuclei), then mature into platelets that do not have another cell nucleus and circulate

in the bloodstream. The life span of platelets in the blood circulation is approximately 7-10 days. In platelets there are 3 granules: alpha- $\alpha$ , dense, and lysosomal. A Granules are the most granules and contain more than 300 different proteins, and are synthesized by megacaryocytes. Elements of blood cells namely leukocytes, erythrocytes and platelets that are suspended in plasma. Normal total circulating blood volume of about 8% body weight (5600 mL in 70 kg men). About 55% of the volume is blood plasma.<sup>12</sup>

Platelets are small granulated bodies in a 2-4  $\mu\text{m}$  diameter with around 300,000 per microliter of circulating blood and normally have a half-life of about 4 days. Platelet is one of the blood components, has a small size, does not have a cell nucleus, is produced by megakaryocytes, spherical, has mitochondria, microtubules, cytoskeleton complex, glycogen granule, golgi element, several enzymes for aerobic and anaerobic metabolism, consisting of two region is the central region dense and peripheral light region of hyalomere. Central region densities called granules, which consist of alpha granules, gamma granules, lambda granules, peroxidase, and peripheral light region of hyalomere which function to form platelet morphology and hemostasis processes.<sup>13</sup>

Growth factors are excreted from alpha granule, which plays a role in the healing process. A $\alpha$  granule (alpha granule) has more than 30 bioactive proteins that play a role in blood cessation and tissue repair. Formed in the bone marrow. Platelets do not have a nucleus, but organelles such as: mitochondria, microtubules and granules ( $\alpha$ ,  $\gamma$ ,  $\delta$ ). Normal platelet counts between 150,000-300,000 /  $\mu\text{L}$ . According to Marx, platelets that are damaged or considered nonviable, will not release bioactive growth factors, so the resulting PRP is disappointing. The PRP used for the treatment application requires around 1,000,000 platelets /  $\mu\text{L}$ . If whole blood contains  $200,000 \pm 75,000$  /  $\mu\text{L}$ , then PRP for the application of treatment must have an average percentage increase of around 400% of the initial platelet count.<sup>13</sup>

### **Platelet Rich Plasma**

*Platelet rich plasma* (PRP) is an autologous product produced from whole blood through a centrifugation process so as to produce high platelet concentrations in low plasma

volumes. Many techniques for making PRPs vary depending on the number, speed and length of the rotation.<sup>14-17</sup>

With the many growth factors contained in it, PRP serves to accelerate regeneration of endothelium, epithelium and epidermal, stimulates angiogenesis, stimulates collagen synthesis, accelerates soft tissue healing, decreases scar tissue on the skin, accelerates homeostatic response to injury, thereby stimulating wound healing, and reverse the inhibition of wound healing caused by glucocorticoids. It is also an adhesive fibrin with hemostatic function. Because it is an autologous material, so it is a biocompatible material, safe and effective. The high leukocyte concentration in PRP adds to the anti-microbial effect.<sup>16,18-21</sup>

Platelet rich plasma (PRP) is used in various clinical applications and is considered to trigger tissue regeneration caused by the cytokine content and growth factors possessed by PRP. In various previous studies, PRP has been used both in applications for hard and soft tissue.<sup>21,22,23</sup> The therapeutic use of PRP is pioneered in dentistry<sup>14</sup>, whereas recently, its clinical application has been extended to other fields, such as cardiac surgery, ophthalmology, oral and maxillofacial surgery, orthopedic surgery, plastic surgery therapy in the field of sports and medicines for cosmetic purposes.<sup>22, 23-32</sup>

From a certain point of view, PRP has the potential for a natural healing process, releasing several growth factors in a biologically determined ratio. Autologous PRP is also considered safer and does not provide concern about infectious diseases, such as human immunodeficiency virus, hepatitis, and is not immunogenic to the host. The use of PRP is also considered more economical than other therapeutic approaches. The various advantages offered by PRP make PRP a choice of treatments that are currently chosen in the biological wound healing process. In addition, this is also a reference for researchers in this study to explore further about PRP levels in hypercholesterolemic patients by modifying the PRP centrifugation method in terms of speed and duration.<sup>33-35</sup>