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Trace Element Analysis in Platelet Rich Fibrin Obtained from Titanium and Glass Tubes

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ABSTRACT

This study aims to analyze whether the number of trace elements in the Platelet-Rich Fibrin (PRF) substance obtained from rat blood changes with the different tubes used. Trace elements in blood samples were measured using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) method. Levels of trace elements Aluminum (Al), Titanium (Ti), Zinc (Zn), Copper (Cu), Manganese (Mg), Selenium (Se), Silver (Ag), Nickel (Ni), Cobalt (Co), and Cadmium (Cd) were measured. Trace element levels of Titanium-Platelet-Rich Fibrin (T-PRF) group 2 were higher than the other two groups. Zn, Mg, and Ag levels of this group were significantly different from the other two groups ($p<0.05$). The group1 had significantly higher Al levels than the sham group and T-PRF had ($p<0.05$), whereas the Co and Cd levels were not significant. The higher amount of serum trace elements obtained by using titanium tube compared to the other two groups showed that titanium is more biocompatible.

Keywords: Platelet-rich fibrin, Trace element, ICP-MS, Titanium tube, Glass tube

INTRODUCTION

Platelet-Rich fibrin (PRF) is a second-generation platelet concentrate [1]. PRF is a fibrin clot rich in platelets without the addition of thrombin during preparation [2]. Prepared from centrifuged blood, first developed by Choukroun, et al. as an autologous leukocyte and PRF biomaterial; it appears to improve and ease the use of this technique. It has been reported to have stimulant potential on cell chemotaxis and proliferation [3-5].

Platelet-rich fibrin which is fully recovered from recovery and healing. Several studies have been performed in which platelet-rich fibrin has been used in different areas such as implants, extraction sockets, and bone grafts. Most of them resulted in accelerating physiological recovery [6,7].

Platelet-rich fibrin concentrates almost all of the blood platelets and growth factors. What makes it different from other platelet concentrates is that it is a strong natural fibrin matrix with unique mechanical properties. PRF provides wound healing and regeneration. Many studies have shown that wound healing is faster with the use of PRF without it [8,9].

ICP-MS (Inductively Coupled Plasma-Mass Spectrometer) is a high-tech analysis technique that allows quantitative, quantitative, or semi-quantitative measurement of a large number of elements in solid and liquid samples quickly, cheaply, precisely, and accurately [10]. ICP-MS has many advantages. One of the most important of these is that it provides the opportunity to receive a large number of data and allows us to make more than one measurement while performing an analysis [10]. The fact that isotope ratios can be directly measured in ICP-MS is one of the unique features of this detector [11]. Simsek, et al. investigated the systemic toxic effects of Bioaggregate (BA), Biodentin (BD), and Micro Mega Mineral Trioxide Aggregate (MM-MTA) dental materials on the liver, brain, and kidney tissues of rats using the ICP-MS method [12]. They calculated the accumulation of trace elements such as Chromium (Cr), Magnesium (Mg), Calcium (Ca), Aluminum (Al), Lead (Pb), and Arsenic (As) in different organs using the ICP-MS analysis method. They found that although some element values were found at high levels in the liver, brain, and kidney, these values were below toxic levels. BD, MM-MTA, and BA found that they were not toxic according to the levels of trace elements in the liver, kidney, and brain samples of rats.

Elements in a sample at the concentration level of mg L^{-1} or µg L^{-1} are called trace elements. Determination of components smaller than 0.01% in a solid sample can be defined as trace analysis [13]. Trace elements can enter the human body in a variety of ways. The way these elements enter the human body and their proportion in the body is very important. For example, elements such as iron, copper, molybdenum, zinc, vanadium, manganese, cobalt, chromium, selenium, and fluorine are vital elements for the human body. However, even small amounts of elements such as mercury, cadmium, arsenic, and lead can have toxic effects. For this, analysis of trace elements is extremely important [14].

Trace elements may have a role important for life. For example, Zinc (Zn) is an important trace element that has a role in the protection of homeostasis which is the basis for living. The immune system is essential for oxidative stress responses, neurocognitive function, and growth and development [15].

The proper functioning of metabolic functions depends on at least nine trace elements (iodine, iron, zinc, manganese, copper, chromium, selenium, cobalt, and molybdenum). These elements important in a role-play of a variety of functions including catalytic, structural, and regulatory activities in which they interact with macromolecules such as pre-secretory granules, biological membranes, pro-hormones, and enzymes [16].

The platelet-rich fibrin preparation technique depends on the transfer to the centrifuge and the speed of blood collection. Without anticoagulant, blood samples immediately begin to coagulate as soon as the tube contacts the glass, and by centrifugation for just a few minutes, the fibrinogen concentrates in the upper and middle of the tube [17]. There is a potential health hazard in blood collection tubes drained with glass with a silica activator. O'Connell described the silica contact. Tunali, et al. developed a new product called T-PRF (Thrombocyte-Rich Fibrin) prepared with titanium [18,19]. In Chouckroun's work, they hypothesize that Leukocyte and Platelet-Rich Fibrin (L-PRF) methods may be more effective than silica activators used with glass tubes, which are among the methods used to activate platelets. Although the silica particles in the tube are dense enough to be precipitated by red blood cells, they are small enough for a fraction to remain suspended as a colloid in fibrin, thin white layer, and platelet-poor plasmal layers; therefore, these particles can reach the patient when the product is used for treatment. Titanium is a biocompatible material. This material has tried to dispel negative thoughts about glass or glass-coated plastic silica tubes [19,20].

Trace elements in blood samples were measured using the ICP-MS (Inductively Coupled Plasma-Mass Spectrometry) method. In this study, we aimed to define a comparative analysis of the effects of platelet-rich fibrin on trace element level with titanium and glass tubes on rat serum.

MATERIALS AND METHODS

PRF membrane, which is prepared in glass tubes, has been used successfully in many clinics. However, the disadvantages of this are present in some sources. Although successful clinical results have been obtained regarding thrombocyte-rich fibrin obtained in a glass tube; O 'Connell, et al. stated that the blood in the glass tube affects fibrin formation and this interaction is inevitable and activated by silica [18]. During centrifugation, blood samples in glass tubes can hit the tube walls and interfere with them. As a result, the silica particles separated from the walls can hang in the fibrin matrix and pass to the patient during the treatment. Depending on use, glass or glass-coated plastic tubes may cause side effects. TT-PRFs have been developed to eliminate these side effects in the long term [18]. In an animal study conducted by Tunali, et al., it was shown that the platelet accumulation formed in the titanium tube was similar to the glass tube and the clot formed gave clinical results similar to the clot produced in the glass tube. They concluded that T-PRF is more compatible with tissues when titanium activation and silica particles that activate platelets are compared [20].

A total of 22 Wistar albino rats weighing 200 g-220 g, aged 12-14 weeks old were used in this study. The animals were obtained from Ataturk University Animal Laboratory (Erzurum, Turkey). The animals were randomly placed in separate cages under controlled room temperature 25 ± 2°C with a 12/12 h light/dark cycle and were fed food and water. 6 ml of blood taken from each Wistar albino rat were divided into three groups of 2 ml and then PRF was formed. Twenty-two Wistar albino rats were subjected to glass tube PRF (Group 1), T-PRF (Group 2), Sham control (Group 3). Levels of trace elements, Aluminium (Al), Titanium (Ti), Zinc (Zn), Copper (Cu), Manganese (Mg), Selenium (Se), Silver (Ag), Nickel (Ni), Cobalt (Co), and Cadmium (Cd) were measured. Trace elements in blood samples were measured using the ICP-MS method.

Preparation of PRF's

6 ml of blood taken from each Wistar albino rat were divided into three groups of 2 ml and then PRF was formed. Wistar albino female rats were anesthetized using Thiopental sodium. Following the anesthesia of the rats, the thorax of each rat was opened and 6 cc of blood was obtained directly from their hearts with a 10 cc injector. The blood obtained was then transferred to a 2 cc glass tube, 2 cc titanium tube, and 2 cc into a biochemistry tube to obtain serum. The blood in glass and titanium tubes was immediately centrifuged at 3000 rpm for 10 minutes, and the blood in biochemistry tubes at 3500 rpm for 10 minutes. Platelet-rich fibrins obtained in glass and titanium tubes and serums obtained from biochemistry tubes were separated into Eppendorf tubes and quickly analyzed by the Inductively Coupled Plasma Mass Spectrometry (ICP-MS) method. ICP-MS method was used for trace element analysis.

ICP-MS Analysis

Standard solutions of the elements (Cr, As, Al, Se, Co, Ni, Cu, Ag, Zn, Cd, Pb, and Sb) to be analyzed in increasing concentrations were prepared using a 2% nitric acid solution. During the analysis, germanium bismuth, scandium and indium were used as internal standards, and calibration curves were drawn to correct for deviations in the calibration curve. Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Agilent 7700) was used for analysis. PRF and serum samples were weighed placed in screw-capped (15 ml) tubes. 100 µL hydrogen peroxide and 400 µL nitric acid solutions were added to them. It was burned in a microwave device (Ethos Easy, Milestone) for 120 minutes at 100°C. 9.5 ml of deionized water was added to the samples and vortexed to make them ready for analysis. The ICP-MS system was operated at 1550 W radio frequency power and as method conditions 4.3 mL/min He flows rate, 15 L/min argon (Ar) plasma gas flow rate; and Ar auxiliary and carrier gas flow rates of 1 L/min and 0.99 L/min, respectively, were determined and analyzed.

Statistical Analysis

The data were analyzed using SPSS version 18 statistical software (SPSS Inc., Chicago, IL, USA). Paired t-test and t-test were used to compare continuous variables. p-values<0.05 were considered statistically significant.

RESULT

Although trace element levels of T-PRF group 2 are higher than the other two groups; statistically, Zn, Mg, and Ag levels were significantly different from the other two groups ($p<0.05$). The group1 had significantly higher Al levels than the sham group and T-PRF had ($p<0.05$), whereas the Co and Cd levels were not significant. Group 1 and Group 2 when compared with Group 3; Cu, Zn, Ti, Mn, Se levels increased, whereas Ni levels decreased. Co and Cd levels were not statistically significant in all three groups. The main findings of our study, titanium tubes are having increase platelet-rich fibrin formation as well as trace element levels compared to glass tubes (Table 1).

Table 1 Platelet-Rich fibrin (PRF) values obtained glass tube, T-PRF, and control (mean ± SD)

Trace Element	Glass tube-PRF (Group1) (n=22)	T-PRF (Group2) (n=22)	Control (Group3) (n=22)	p-values*
Al	12.92 ± 5.08	19.54 ± 6.77	19.05 ± 2.56	0.002
Ti	18.71 ± 6.54	21.54 ± 6.10	9.19 ± 2.57	0.000
Mn	1.31 ± 0.42	1.98 ± 0.63	0.47 ± 0.18	0.000
Co	0.11 ± 0.03	0.12 ± 0.04	0.10 ± 0.05	0.283
Ni	2.15 ± 0.64	3.62 ± 1.69	3.84 ± 0.52	0.000
Cu	169.98 ± 42.49	169.56 ± 39.78	107.77 ± 11.16	0.003
Zn	186.71 ± 42.32	269.44 ± 46.87	121.41 ± 14.85	0.000
Se	44.29 ± 6.13	46.98 ± 6.20	39.90 ± 6.24	0.031
Ag	0.62 ± 0.19	0.80 ± 0.19	0.09 ± 0.06	0.000
Cd	0.05 ± 0.02	0.05 ± 0.02	0.04 ± 0.04	0.521
*p-values for comparison between glass tube-PRF and Titanium tube-PRF and Control- PRF. respectively				

DISCUSSION

PRF was first developed in France in 2001 by Choukroun, et al. [3]. PRF is a thrombocyte concentration that contains all blood components and plays a role in wound healing and immunity [19,20]. T-PRF provides a tighter fibrin network structure than silica in platelet activation [20]. There are studies on T-PRF showing better results in bone and soft tissue healing [20]. Tunali, et al. Placed the PRF obtained from the blood taken into titanium tubes under the mucoperiosteal flap in rabbits, and on certain (3^{rd}, 5^{th}, 10^{th}, $15^{th,}$ and 30^{th}) days, they stained and examined the tissue samples taken from this area with hematoxylin-eosin dye. They stated that although T-PRF started to resorb in the tissue on the 5^{th} day, it was not completely resorbed until the 10^{th} day, which is the time required for the onset of new bone formation [20]. Lee, et al., on the other hand, filled the peri-implant defects in the test group with T-PRF after placing the implant in the defects they had formed in the rabbit tibia and left the control group to heal on their own. Histological examination revealed that peri-implant defects healed completely at 8 weeks. Thus, it has been stated that T-PRF can be applied in bone healing [21].

In our study, the higher levels of trace elements compared to glass tubes are similar to those studies. We can say that it may be more beneficial to use titanium tubes instead of glass tubes used in the routine. T-PRF is fibrin rich in platelets and leukocytes. Titanium-induced platelet creates distinctive activation [22]. Arabaci, et al. showed that T-PRF is more successful than traditional methods in periodontal healing [23].

Titanium exhibits very good biocompatibility due to its non-corrosive properties [24]. Titanium; suitable for many biomedical uses such as spinal fusion surgery, medical devices, implants, and artificial heart valves [25,26]. Although authors are discussing the bio- and hemocompatibility of pure titanium; titanium is a good material with promise in the future [27-31].

Trace elements are extremely important for the normal growth and tissue metabolism of living organisms [32]. Zn is essential for RNA transcription, cell division, DNA synthesis, and cell activation [33]. An important component of the respiratory enzyme complex cytochrome c oxidase is Cu. Cu and Zn are essential factors in protecting against oxidation, which act as cofactors for antioxidant enzymes such as superoxide catalase and dismutase [34]. Se is antioxidant enzymes containing thioredoxin reductase and glutathione peroxidase.

Mn, together with Vitamin K, supports blood coagulation. Also, cellular energy is vital for bone growth, regulation of blood sugars, and immune system functioning, defense against free radicals [35]. In the literature, there is no publication investigating how PRF, obtained by comparing glass tubes and/or titanium tubes, affects trace element levels in rats or humans. This reveals how original our work is. The surface of the titanium tubes is often covered by TiOx and/or TiO_2 layers [36]. The effect of titanium tubes on the growth factor is the presence of nanobubbles. One of the factors regarding the tubes having different materials is their hydrophilicity/hydrophobicity [37].

CONCLUSION

In this study, we believe that the titanium tube increased platelet-rich fibrin formation as well as trace element levels compared to the glass tube. And as a result, it will be useful for wound healing, tissue repair, and antioxidants. Since our study is the first study to investigate the trace element level in PRFs, new studies are needed to create a standard protocol in the future. We can say that this study as a method will be useful in the future.

DECLARATIONS

Conflicts of Interest

The authors declared no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

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