Comparative performance of the protocol of plasma rich in growth factors - universal 1 (PRGF-U1) for obtaining platelet rich plasma.

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Abstract: Objective: To compare the platelet concentration obtained after application of the protocol of plasma rich in growth factors - universal 1 (PRGF-U1) and the protocol of Anitua and Andia (PRP-A) for obtaining platelet rich plasma. Material and Method: A descriptive, cross-sectional and comparative study was carried out with a simple random probabilistic sample consisting of 16 patients who attended the Periodontics service of the Unit of Second Specialization in Stomatology of the National University of Trujillo. Five blood samples were obtained from each patient, after applying a health questionnaire to rule out any disease or drug consumption, in order to obtain the baseline platelet concentration and that obtained after PRGF-U1 and PRP-A. To compare the platelet concentrations of the two protocols, Student's t-test was used considering a significance level of $p<0.05$. RESULTS: The baseline platelet concentration was 371,250±68,203 platelets/µL, for PRGF-U1 it was 747,875±121,645 platelets/µL and for PRP-A it was 595,000±129,202 platelets/µL. A statistically significant difference ($p<0.001$) was found between both protocols. Conclusion: The PRGF-U1 protocol yielded a higher platelet concentration compared to the Anitúa and Andía protocol.

Keywords: platelet rich plasma, platelet-derived growth factors, platelet count, regenerative medicine.

INTRODUCTION.

The concept of stimulation and maximization of healing mechanisms has gained prominence over the last years. In order to minimize the aftermath of invasive surgical treatments several methods have appeared. One of them is the platelet rich plasma (PRP) or plasma rich in growth factors (PRGF) that is a rich source of growth factors (GF).[1,2] PRP is a gel containing autologous human platelets concentrate (at concentrations higher than plasma levels) in a small volume of plasma. PRP is a source of easy access to GF, which are secreted by the platelets when the healing process begins, allowing the acceleration and improving the processes of healing and tissue regeneration.[1,2]

The techniques for obtaining the PRP vary in the number of centrifugation steps the speed of centrifugation and the use of calcium chloride or other substances for their activation.[1,3,4] One of the most used protocols and that is considered the gold standard for obtaining PRP is that proposed by Anitua and Andia, which has shown good

clinical results in improving the healing of hard and soft tissues.[1,3-5] This protocol obtains the PRP by means of a single centrifugation at 1800 revolutions per minute (rpm) for 8 minutes producing 280g (relative centrifugal force or RCF).[1-3,6]

Marx *et al.,*[7] claim that the PRP obtained with a single centrifugation does not produce a true therapeutic concentrate because the red cells interfere with the separation of the platelets. However, other authors do not recommend a two-step centrifugation because there is a risk of excessive manipulation, which leads to contamination or premature activation of platelets and loss of GF.[1-3,6]

Currently there is a problem with the commercial kits because they include centrifuges already calibrated, having as main disadvantage their high cost, without the possibility of using other centrifuges. The aim of this study was to compare the platelet concentration obtained by using the plasma protocol rich in growth factors-universal 1 (PRGF-U1) and by the protocol of Anitua and Andia (PRP- A) for the production of platelet rich plasma.

MATERIALS AND METHODS.

This is a cross-sectional study carried out in the Unit of Second Specialization in Stomatology (USSS) and in the Laboratory of University Welfare of the Universidad Nacional de Trujillo (UNT). It was approved by the Committee of Permanent Bioethics Research of the Faculty of Medicine of the UNT. The sample consists of 16 patients who attended the Periodontics service of the USSS.

Selection criteria

Patients older than 18 years who had a good periodontal and general health status (evaluated by a resident of the USSS, previously calibrated by a periodontist, and through a health questionnaire) were included. Those who consumed medication, had blood parameters outside the normal range, pregnant women, and those who had any mental condition that made communication impossible were excluded.

Blood sample

Five peripheral blood samples were drawn from each fasting patient by a trained laboratory technician using the vacutainer system: One sample in EDTA test tubes (basal sample), two samples in test tubes with 3.8% sodium citrate (PRP-A), and two samples in test tubes with 3.2% sodium citrate (PRGF-U1), each with a capacity of approximately 4.5ml.

Protocols

The baseline sample was immediately analyzed and the other samples were processed according to each protocol. The formula $(RCF=1.12xRadiusx (rpm/1000)^2)$ was used to calculate the rpm corresponding to 280g for the centrifuge (Heraeous Labofuge 200r, Daigger, USA) with fixed rotor at 45° and a radius of 7.7cm, resulting in 1800rpm for 8 minutes for the PRP-A, which was carried out at room temperature. (Heraeous Labofuge 200r, Daigger, USA) with a radius of 7.7cm.[1-3,6] For the PRGF-U1 a centrifugation of 1600rpm for 7 minutes was carried out at room temperature in a centrifuge with a rotor radius of 9.8cm (Heraeous Labofuge 200r, Daigger, USA).

The PRP was extracted as follows: The topmost 0.5ml (PPP) were removed first, floowed by the middle 0.5ml (plasma under normal conditions), and finally 0.3ml were removed from the bottom of the tube (PRP), using an automatic micropipette.

Then 0.1ml of the PRP sample for each protocol were taken to a Newbauer Chamber and Thomas pipette to obtain the platelet concentration. Each micropipette step was performed by a single person calibrated for this purpose. The remaining PRP sample was processed for use in scheduled surgery.

Statistical analysis

Data were processed in the SPSS 23.0 statistical package (IBM, SA). To compare platelet concentrates, t test was used with a significance level of $p<0.05$.

RESULTS.

The baseline platelet concentration was 371,250± 68,203 platelets/μL, for PRGF-U1 it was 747,875±121,645 platelets/μL and for PRP-A it was 595,000±129,202 platelets/μL.

A statistically significant difference ($p<0.001$) was found between both protocols.

Table 1. Platelet concentrations obtained by PRP protocols.

Authors	Procedure	First Centrifugation / Second Centrifugation (rpm/RCF)	Time (minutes)	Platelet concentration (platelet/µL±SD)
Mazzocca *et al.,* (2012)[7]	Arthrex ACP Double Syringe System	1500 rpm	5	378,300±58,640
	Manual	1500 rpm	5	447,700±183,750
		6300 rpm	20	
Anitua *et al.,* (2011)[8]	PRGF - Endoret	580 g	8	663,000
Sundman *et al.,* (2011)[7]	Arthrex ACP Double Syringe System	1500 rpm	5	361,000±87,000
	Biomet GPS III Mini Platelet Concentrate Separation Kit	3200 rpm	15	701,000±473,000
Castillo *et al.,* (2010)[7]	MTF Cascade	1100 g	6	443,800±24,700
	Biomet GPS III Mini Platelet Concentrate Separation Kit	1100 g	15	566,200±292,600
Mazzucco *et al.,* (2009)[7]	Regen PRP - Kit	1500 g	10	430,000±109,000
Mazzucco *et al.,* (2008)[7]	Plateltex	180 g	10	338,700±73,569
		1000 g	10	
Sánchez *et al.,* (2007)[9]	PRGF System II	460 g	8	634,000±217,000
Everts *et al.,* (2006)[7]	Biomet GPS The Gravitational Platelet Sequestration System	3200 rpm	12	569,000±247,000
Leitner *et al.,* (2006)[7]	Fibrinet System Cascade	1000 g	15	399,000±26,627
De Obarrio *et al.,* (2000)[10]	Manual	5600 rpm	6	12,500

rpm= revolutions per minute; **RCF** = Relative centrifugal force; **SD** = standard deviation

DISCUSSION.

The results of this research show that higher platelet concentrations are obtained with the PRGF-U1 protocol and these are in turn higher than those found by other authors, as shown in Table 1.[7-10] Thus, we believe that this new protocol could lead to clinical improvements since having a higher concentration of platelets should result in a greater clinical benefit.

Despite the limitations of the manual method used in this protocol, the previous calibration of the centrifuge and the use of a lower concentration of sodium citrate are factors that could improve the platelet concentration in PRP. Furthermore, any centrifuge may be employed during the protocol by using the mathematical formula to calculate rpm, without the need of using a commercial kit.

However, more studies are needed to compare this protocol with others, verifying the quality of the product and its effectiveness in laboratory and clinical settings.

CONCLUSION.

The PRGF-U1 protocol resulted in a higher platelet concentration compared to Anitúa and Andía protocol.

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