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Communication

Platelet yield and recovery following the PRGF-U1 protocol in a Peruvian population.

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Abstract: Objective: To determine the platelet recovery and yield of the PRGF-U1 protocol in a Peruvian population. Methods: An observational, descriptive and cross-sectional study was conducted with a simple random probability sample of 32 patients who attended the Laboratorio Scalab in Trujillo, Peru. A blood sample was taken from each patient in order to obtain the concentration of platelets and before and after the PRGF-U1 protocol, in order to determine platelet recovery and yield. To compare basal platelet concentrations and platelet recovery and yield with gender and age, the chi-square test, Student's t-distribution and Pearson's correlation coefficient were used considering a significance level of $p<0.05$. Results: Platelet yield was less than 2.2 in two patients and greater than or equal to 2.2 in 30 patients; platelet recovery was equal to 0.4 in 30 patients and greater than 0.4 in two patients. A statistically significant relationship ($p<0.05$) between basal platelet count and age was found when comparing basal platelet count, platelet recovery and platelet yield according to gender and age, but no significant relationship between the other variables ($p>0.05$). Conclusion: The PRGF-U1 protocol presents optimal platelet yield and minimal expected platelet recovery.

Keywords: Plasma rich in growth factors; platelet count; platelet-rich plasma; regenerative medicine.

INTRODUCTION.

The field of medicine and dentistry is rapidly advancing toward the development of non-invasive procedures and accelerated treatments that can result in a good functional recovery of patients, in order to improve their quality of life. In recent years, the concept of stimulation and maximization of healing mechanisms has gained prominence and generated simple and cost-effective procedures that have had a potential impact by reducing the cost of treatments.[1-3]

One of these simple and cost-effective procedures, which minimizes the consequences of invasive surgical treatments, is platelet rich plasma (PRP) or plasma rich in growth factors (PRGF), which is a rich source of growth factors (GFs). PRP is an autologous biological gel derived from the patient's blood that contains a higher platelet concentration than circulating blood. PRP is an easily accessible source of GFs secreted by platelets when the healing process begins, allowing for the acceleration and improvement of the healing processes and tissue regeneration.[1-4]

Techniques for obtaining PRP vary regarding the centrifugation, number of steps and the speed of centrifugation, and the use of calcium chloride or other substances for its activation.[1,36] One of the most widely

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used protocols and considered the gold standard for obtaining PRP was proposed by Anitua and Andia, and has shown good clinical results in improving the healing of hard and soft tissues.[1,7] This protocol obtains PRP by means of a single centrifugation at 1800 revolutions per minute (rpm) during 8 minutes producing 280g (relative centrifugal force, RCF).[1,2,8]

Currently there is a problem with commercial kits because they require the use of already costly calibrated centrifuges without the possibility of using other centrifuges. Therefore, a new protocol was proposed (plasma rich in universal growth factors 1 or PRGF-U1[1]) in a Latin American population (Peru) to allow obtaining PRP using a conventional centrifuge, thus providing wider access to the use of GF and to its advantages in dental surgery. Consequently, the aim of this study was to determine the platelet recovery and yield of the PRGF-U1 protocol in a Peruvian population.

MATERIALS AND METHODS.

This is an observational, descriptive and cross-sectional study conducted at the Universidad Nacional de Trujillo (UNT) and at Laboratorio Scalab in Trujillo, Peru. It was approved by the bioethics committee of the postgraduate school of medical sciences of the UNT. The sample consisted of 32 blood samples from 32 patients who attended the Laboratorio Scalab. Selection of patients was done by simple randomized sampling.

Selection criteria

Blood samples were included from patients over 18 years of age, who had a normal basal platelet count (150000-400000 platelets/µL) that required some type of hematological analysis and without distinction of gender. Subjects who reported consumption of medication that could cause a variation in blood parameters, those who had blood parameters outside the normal range, and those who did not sign the informed consent were excluded from the study.

Blood sample

A peripheral blood sample was collected from each fasting patient by a trained laboratory technician using the vacutainer system. All of the samples were taken into 3.2% sodium citrate test tubes, each with a capacity of approximately 4.5ml. Patients signed an informed consent form prior to obtaining the blood sample.

Protocol

A 0.5ml sample was extracted to corroborate if the basal platelet count was within the normal range to meet the inclusion and exclusion criteria. The rest of the sample was processed following the PRGF-U1[1] protocol

using a centrifugation of 1600 revolutions per minute (rpm) on a rotor with a radius of 97.6mm, for 8 minutes (280g), and then extracting the PRP and platelet-poor plasma (PPP).

For the platelet count, an automatic XS-1000i Roche cell counter was used and estimating counts per high power field on a Wright's-stained blood smear.

Platelet recovery and yield

Platelet yield was obtained by dividing the platelet count in the PRP by the basal platelet count to determine how many times more platelets the PRP had in relation to basal platelets and then classify it as normal (<2.2) or optimal (≥2.2). Platelet recovery was obtained by dividing the platelet count in the PPP by the basal platelet count to determine how many times more platelets the PPP had in relation to the amount of basal platelets and then classify it as low (<0.4), minimal (0.4), or optimal (>0.4).

Statistical analysis

The statistical package SPSS 23.0 (IBM, USA) was used to process the data. Pearson's statistical correlation test was used to compare baseline platelet counts with age. Student's t-test was used to compare basal platelet count with gender, platelet yield and recovery with age. The chi-square test was used to compare platelet yield and recovery with gender, all these statistical tests considered a significance level of $p<0.05$.

RESULTS.

Platelet yield was less than 2.2 in 2 patients and greater than or equal to 2.2 in 30 patients; platelet recovery was equal to 0.4 in 30 patients and greater than 0.4 in 2 patients.

Comparing basal platelet count, platelet yield and recovery with gender and age, it was found that there is a statistically significant relationship ($p<0.05$) between basal platelet count and age, and there is no significant relationship between the other variables ($p>0.05$) (Table 1 and Table 2).

Table 1. Basal platelet count, per microliter, according to gender and age.

Variable		n	Mean	Standard Deviation	*p*-value
Gender	Male	15	276000	88309	0.131*
	Female	17	234412	62520	
Age	<40 years	16	287750	83073	0.005** ($r^2 = -0.89$)
	≥40 years	16	220062	54973	

*: Student´s t-test. **: Pearson Correlation.

Table 2. Platelet yield and recovery according to gender and age.

	Variable		n	Normal / Min. expected	Optimal	*p*-value
Platelet yield	Gender	Male	15	2	13	0.212*
		Female	17	0	17	
	age	<40 years	16	2	14	0.059**
		≥40 years	16	0	16	
Platelet recovery	Gender	Male	15	13	2	0.212*
		Female	17	17	0	
	Age	<40 years	16	15	1	0.709**

*: Chi square. **:Student´s t-test. **Min:** Minimum.

DISCUSSION.

The results of the present study show that the platelet yield and recovery of PRGF-U1, when the centrifuge is previously calibrated to obtain 280g forces, is optimal.

In addition, it shows that the highest concentrations of basal platelets are found in people less than 40 years of age and that they are lower as age progresses. These results are similar to those found by Biino *et al.,*[9] Troussard *et al.,*[10] and Jones,[11] who state that platelet count decreases with age because as age progresses the haematopoietic stem cell reserve decreases.

It also shows that the concentration of basal platelets does not vary with gender. These results are different from those found by Biino *et al.,*[9] who reported that differences exist and that women have a higher number of basal platelets than men. In addition, the results obtained here show that there is no difference between PRP performance and recovery with age and gender. No study has reported similar results using the same methodology and comparing these two variables.

It should be noted that this study has two limitations: the selection of the patients' health condition and the size of the sample. It was assumed the patient's health condition was normal when the platelet count was within the normal parameters.

This means that the subjects who participated in the present study may not necessarily have been healthy, since for example, diabetes like other systemic diseases, does not affect platelet counts.

However, despite this limitation, which may influence results and introduce bias, it is observed this way of obtaining PRP could lead to clinical improvements taking into consideration that the basal platelet count is within normal parameters.

In addition, it is helpful that any centrifuge may be used for the PRGF-U1 protocol using the following mathematical formula to calculate rpm, without the need for a commercial kit: RCF=1.12 x Radius x (rpm/1000).[2]

CONCLUSION.

The PRGF-U1 protocol presents optimal platelet yield and minimal expected platelet recovery.

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