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Research Article

A New Technique for Obtaining Platelet Rich in Growth Factors (PRP). A Descriptive Study in 25 Patients to Repair Alveolar Dent and Comparison with Others Procedures Published in Literature

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Abstract

Introduction: The diversity of procedures for obtaining platelet and plasmatic growth factors, the absence of control in most of them and the growing field of clinical application, makes them necessary methods adequately structured, documented, controlled and tested, playable by any author. The present series of clinical cases aims to introduce and test a specific technique for obtaining PRP, with precise characteristics both production and final composition of compound got, in 25 hematological healthy patients, derived by dentists to repair alveolar dent, comparing our results with those obtained by other procedures scientifically tested.

Material and Methods: 25 caucasian patients were selected, 13 male and 12 female with age range between 15 and 65, healthy haematologically. The procedure for obtaining the PRP, consisted of a single centrifugation of the blood sample for 30 minutes at 3500 rpm in a angular shaft of 16 tubes centrifuge serie (CEMCON 2) and micropipetting the protein fraction rich in platelet and plasmatic growth factors and cell through open technique under aseptic conditions in horizontal laminar flow hood Grade A at a temperature of 22 ° C, with the use of leuco-platelet or Buffy-coat layer (PRP rich in leukocytes).

Results: No correlation between the amount of concentrated platelets and the amount of growth factors finally obtained was observed. The protocol set forth concentrated levels of platelets and leukocytes approximately 3 to 5 times higher than baseline levels with a predominance of mononuclear. Levels of growth factors from 7-10 times greater than the patient's baseline levels, with little variation in them. The growth factor levels were stable in the blood of each patient within 24 h of treatment between 7 and 9 times higher compared to the previous baseline. Compared with other procedures discussed in the literature; This method achieves concentration between 1.5 and 3 times more platelets in the final product, with a purification of growth factors overall type VEGF and TGF-B clearly superior.

Conclusion: the technique disclosed is more effective since concentrate achieves greater amount of platelets and growth factors and efficient since it maintains a serum protein in these stable sera of patients after 24 hours of administration thereof.

Keywords: *Platelet Rich Plasma; Growth Factors; Centrifugation; Buffy-Coat*

Introduction

Use of plasma growth factors, commonly known as Platelet-rich plasma (PRP), is a technique considered medication since 2013, with several medical. However there is a strong controversy and debate as to the usefulness of the PRP in the recovery of dental alveolar bed

with lyophilized bone plasty, objectifying alveolar increased, improving the healing of the soft tissues and facilitating greater cohesiveness particulate graft, which would useful in dental implantology This led to think that the higher the concentration of these factors would be more effective regeneration, promoting the use of systems that got a higher concentration of growth factors, systems that were approved and used without thinking of the concentration obtained product end It is the closest thing to the elaboration of a sheet of the concentrate produced in order to guarantee maximum efficiency and safety for the patient [1, 2]. The present work, series of clinical cases, aims to introduce and test a specific technique for obtaining PRP, with precise characteristics both production and final composition of compound got, in 25 hematological healthy patients, comparing our results with those obtained by other procedures scientifically tested.

Materials and Methods

25 caucasian patients were selected, 13 male and 12 female with age range between 15 and 65, healthy haematologically following analytical standards autologous inclusion of the Spanish Society of Hematology and Hemotherapy regarding biochemical, hematological and serological before obtaining samples of whole blood controls. A closed system blood by Vacutainer tube connected to 9 ml EDTA was utilized. A 20-gauge needle forearm venous access was used. The procedure for obtaining the PRP, consisted of a single centrifugation of the blood sample for 30 minutes at 3500 rpm in a angular shaft of 16 tubes CEMCON 2 centrifuge series and selecting the protein fraction rich both leukocyte and plasmatic growth factors through open technique under aseptic conditions in a grade A horizontal laminar flow hood Telstar type at 22°C of constant temperature, with the use of leuco-platelet or Buffy-coat layer (PRP rich in leukocytes). In the final product, we proceeded to cell counting by hemocytometer Coulter (Beckman), platelets, leukocytes, granulocytes, monocytes and CD 34 + / mm3 and the following platelet growth factors: growth factor derived from platelets (PDGF), transforming B factor (TGF-B), Insulin like-1 growth factor (IGF-1) and vascular endothelial growth factor (VEGF) using specific kits of enzyme-linked immuno-assay (ELISA). Measurements were made at baseline, before treatment, in final PRPs obtained and in patients 24 h after administration. The route of injection was local in alveolar dent by odontologist. Most studiated techniques in literature for obtaining PRP (Anitua., *et al,* De Obarrio., *et al,* Okuda., *et al*, Garcia., *et al* and Sierra Perez Lorente., *et al*) were revised and utilized to compared their performance with ours: [3,4]. Descriptive statistical settings (Maximum, Minimum and Average) were used to interpretation of analytical variables in each case.

Results

In Tables 1, 2 and 3 you can see the results for the 25 patients in terms of cell count and levels of growth factors obtained with the maximum and minimum values collected, and the average parametral achieved both at baseline as in the PRPs and blood at 24h of treatment for each patient. There was not correlation between the amount both leukocytes and platelets concentrated and levels of growth factors finally obtained. The protocol set forth concentrated levels of platelets and leukocytes approximately between 3 to 5 times higher than basal levels in patients, predominating mononuclear cells (75 - 90% of the white cellularity), up to 1 - 3% of them were positive for CD34+ marker without changes in cell counts at 24 hr after treatment, except for a increase between 150 and 300 times higher in the determination of CD 34 cells with respect baseline count. This fact could be seen in Figures 2, 3 and 4 respectively. Levels in growth factors at PRPs obtained were between 7-10 times greater than the patient's baseline levels, with little variation in them. Growth factor levels were stable in blood at 24h after treatment between 7 and 9 times higher compared to the previous baseline, visible in Figure 1, Compared with other procedures discussed in the literature, this method achieves concentration between 1.5 and 3 times more platelets, as we can see in Figure 5, with a purification in type VEGF and TGF-B growth factors clearly superior, visible in Figure 6.

	PDGF-AB (10-50 pg/ml)	TGF-B1 (10-70 pg/ml)	IGF-1 (0,5- 19,5 pg/ml)	VEGF (15-85 pg/ml)	Platelets (150.000-350.000/ mm^3)	Leukocytes (3.200- 9000/ mm^3)	Granulocytes/mm^3	Mononuclears /mm^3	CD 34 +/mm^3
Patient 1	45	60	18	80	210000	7500	4875	1275	0.9
Patient 2	40	25	10	45	210000	6500	3575	1625	0.3
Patient 3	43	55	17	80	190000	6230	3738	1246	0.4
Patient 4	43	67	15	75	170000	7500	4500	1125	0.5
Patient 5	15	25	7	30	180000	8900	5340	1335	0.3
Patient 6	35	24	12	40	175000	8900	5340	1956	0.2
Patient 7	20	15	7	30	260000	7200	4320	1440	0.2
Patient 8	30	20	7	35	176000	7430	4458	1114	0.4
Patient 9	91	60	16	75	350000	7430	4086	1337	0.7
Patient 10	45	55	18	70	195000	9500	5700	1425	0.7
Patient 11	35	20	15	40	205000	8300	4980	1909	0.2
Patient 12	12	15	4	25	250000	8500	5100	1890	0.1
Patient 13	45	60	17	75	240000	8700	5481	1131	0.7
Patient 14	43	55	17	70	300000	7600	4560	1140	0.4
Patient 15	15	55	18	70	214907	7500	4500	1500	0.2
Patient 16	42	67	18	87	220659	7590	4109	1221	0.8
Patient 17	41	21	10	44	215401	6201	3600	1624	0.5
Patient 18	44	53	17	88	195793	6013	3490	1276	0.8
Patient 19	40	66	15	74	181098	7901	4501	1112	0.2
Patient 20	17	22	7	32	191209	8587	5354	1309	0.6
Patient 21	37	23	12	43	175397	8401	5176	1966	0.6
Patient 22	25	17	7	31	262981	7010	4012	1490	0.6
Patient 23	33	22	7	33	169127	7091	4301	1830	0.5
Patient 24	99	68	16	77	339129	7178	4912	1900	0.9
Patient 25	41	52	18	76	184091	9280	5769	1421	0.8
Maximum	99	69	18	89	350000	9500	5911	1966	0,9
Minimum	11	13	4	22	169127	613	3123	1012	0,1
Average	32,75	36,09	12,14	53,03	259563	7709	4619	1405	0,5

Table 1: *Rheological characteristics of patients at baseline.*

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	PDGF-AB (10-50 pg/ml)	TGF-B1 (10-70 pg/ml)	IGF-1 (0,5-19,5 pg/ml	VEGF (15-85 pg/ml)	Platelets (150.000-350.000/ mm^3)	Leukocytes (3.200-9000/ mm^3)	Granulocyte / mm^3	Mononuclears / mm^3	CD 34+/ mm^3
Patient 1	296	450	250	575	500000	21000	3150	18270	240
Patient 2	270	300	150	545	600000	22000	4400	16500	180
Patient 3	190	370	200	590	500000	21000	3150	16800	270
Patient 4	250	480	190	540	400000	20000	4000	17400	210
Patient 5	150	365	110	460	600000	21000	4200	14700	170
Patient 6	160	370	160	530	500000	24000	6000	19200	175
Patient 7	200	390	120	470	400000	21500	4515	15910	170
Patient 8	150	350	105	390	700000	21500	3440	12900	120
Patient 9	253	520	277	590	600000	21500	4085	18705	215
Patient 10	220	470	210	590	700000	24000	3600	20400	200
Patient 11	150	370	160	480	690000	23000	4600	17940	150
Patient 12	190	350	190	320	500000	20000	4000	12000	70
Patient 13	280	420	230	570	710000	22000	3300	18700	200
Patient 14	250	420	199	570	650000	22000	4400	16500	185
Patient 15	245	430	190	590	570000	23000	4370	19550	200
Patient 16	280	459	253	590	620000	21000	3100	18500	240
Patient 17	270	380	153	580	660000	22600	4500	16700	180
Patient 18	250	390	290	570	710000	27000	3000	16500	270
Patient 19	230	490	170	590	630000	20900	4900	17900	210
Patient 20	220	390	100	499	700000	21530	4100	14500	259
Patient 21	130	380	190	580	610000	24070	6400	19900	300
Patient 22	210	290	100	489	730000	26700	4400	15900	350
Patient 23	230	400	103	391	630000	25000	3500	12480	270
Patient 24	240	590	240	570	730000	22700	4001	18400	200
Patient 25	200	490	200	510	670000	23000	3670	20900	290
Maximum	296	590	290	590	730000	27000	6400	20900	350
Minimum	130	290	100	320	400000	20000	3000	12000	70
Average	215,39	407,19	172,82	521,32	604147	22411	4038	16913	203

Table 2: *Rheological characteristics of PRPs obtained by the tested procedure.*

	PDGF-AB (10-50 pg/ml)	TGF-B1 (10-70 pg/ml)	IGF-1 (0,5-19,5 pg/ml	VEGF (15-85 pg/ml)	Platelets (150.000-350.000/ mm^3)	Leukocytes (3.200-9000/ mm^3)	Granulocytes/mm^3	Mononuclears/mm^3	CD 34+/ mm^3
Patient 1	200	420	220	530	200436	8120	4210	1932	0.4
Patient 2	230	270	130	510	251465	8401	5300	1890	0.2
Patient 3	120	320	180	520	201154	8598	5900	1900	0.5
Patient 4	200	440	150	500	330012	7901	4390	1140	0.4
Patient 5	110	325	100	420	260123	7689	4211	1500	0.5
Patient 6	110	340	140	510	218013	7211	4600	1275	0.8
Patient 7	160	360	100	430	219032	6480	3410	1625	0.3
Patient 8	110	320	99	350	191913	6219	3800	1219	0.7
Patient 9	203	500	250	530	171934	7500	4212	1119	0.6
Patient 10	190	420	200	560	187091	8967	5012	1321	0.8
Patient 11	110	350	130	440	175708	8941	5900	1780	0.6
Patient 12	120	300	170	300	260000	7212	4150	1503	0.9
Patient 13	220	400	200	520	174814	7012	4800	1145	0.5
Patient 14	210	410	180	550	255060	7432	4120	1903	0.6
Patient 15	215	400	170	540	195000	9019	5500	1093	0.5
Patient 16	220	409	240	540	260124	8190	4123	1012	0.4
Patient 17	220	350	130	530	255098	8122	5450	1701	0.5
Patient 18	230	360	250	530	243981	8000	5911	1016	0.8
Patient 19	200	450	140	540	317321	7801	4012	1045	0.5
Patient 20	190	350	90	445	217877	7546	4560	1501	0.3
Patient 21	100	330	150	550	212066	7209	4900	1200	0.8
Patient 22	180	270	98	440	217912	6109	3123	1601	0.4
Patient 23	190	350	99	340	190543	6320	3800	1222	0.5
Patient 24	190	520	210	530	178913	7591	4911	1125	0.4
Patient 25	170	430	180	500	188912	8011	5901	1333	0.5
Maximum	230	450	200	505	290000	8900	4000	2000	20
Minimum	100	120	55	200	190000	3950	3100	1600	5
Average	169,74	272,7	127,3	415,45	223602	7638	3629	1768	11

***Table 3**: Blood serum features in patients at 24h of treatment.*

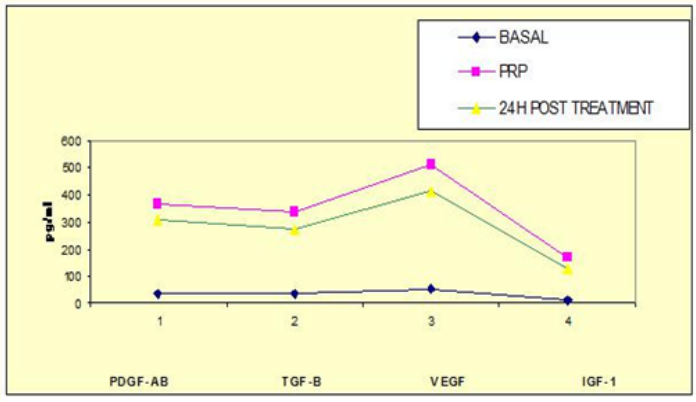

Figure 1: *Average levels of growth factors in Alcaraz, Oliver and col technique measured at baseline, PRP and 24 h after the treatment. Growth factor levels were stable in the blood of each patient within 24 h of treatment between 7 and 9 times higher compared to the previous baseline.*

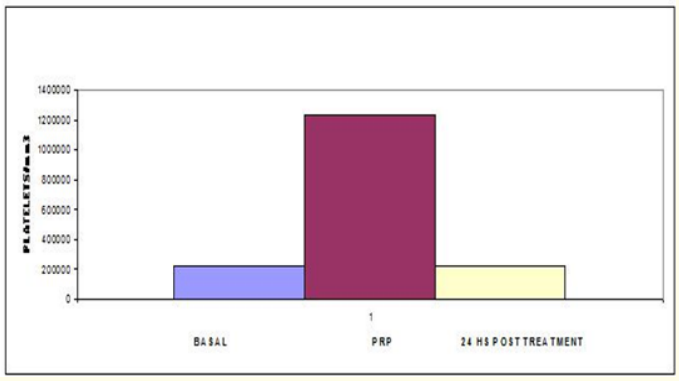

Figure 2: *Half platelet counting in Alcaraz, Oliver et al technique at baseline, PRP and 24 h after treatment. Method concentrated levels of platelets approximately 3 to 5 time higher than baseline.*

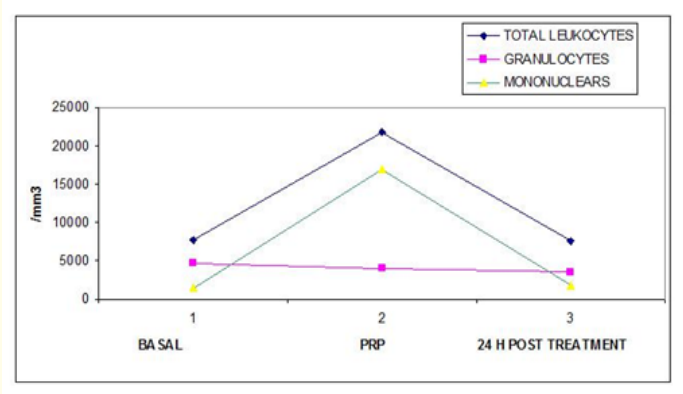

Figure 3: *Half leukocitary (granulocitary and mononuclear) counting in Alcaraz, Oliver and col technique at baseline, PRP and 24 h after treatment.*

Protocol of treatment obtains levels of leukocytes between 3 and 5 times higher than baseline levels with a predominance of mononuclears.

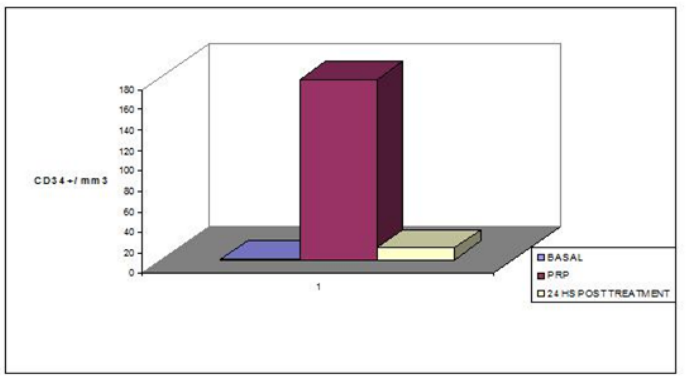

Figure 4: *Half CD34⁺ counting in Alcaraz. Oliver and col technique at baseline, PRP and 24 h after treatment. Changes in cell counts practically safe increase between 150 and 300 times higher in the determination of CD 34⁺ at 24 hr post administration of PRP baseline count.*

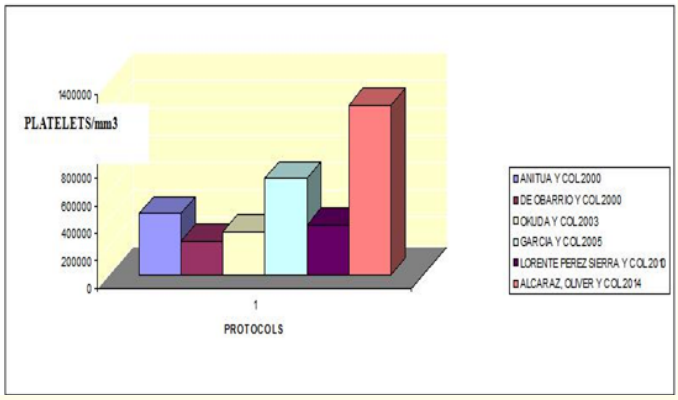

Figure 5: *Average count of platelets in the PRPs of 6 procedures examined. Compared with other procedures discussed in the literature; This method achieves concentration between 1.5 and 3 times more platelets in the final product.*

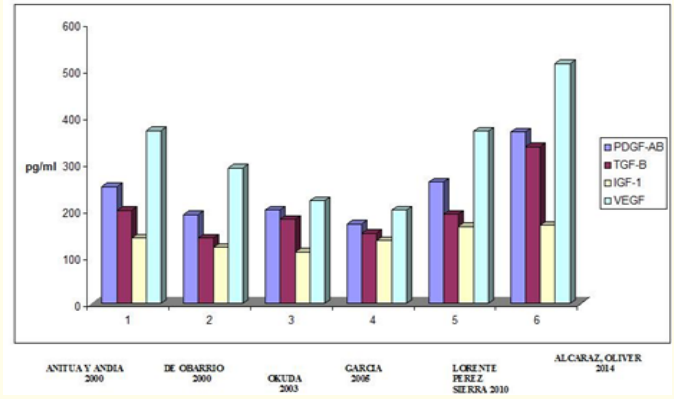

Figure 6: *Average concentration of growth factors in the PRPs of 6 procedures examined. Compared with other procedures discussed in the literature; This method achieves purification of growth factors overall type VEGF and TGF-B clearly superior.*

Discussion

Although size of sample in this case series type study was small, the proposed technique concentrates a greater amount of platelets compared with other treatment protocols revised, without correlation at levels in growth factors obtained regardless of the age and sex of patient [3,4], coinciding with affirmed by other authors previously. It was noteworthy that in our PRPs rich in leukocytes there was greater amount of platelets and growth factors overcoat TGF-B and VEGF, compared to other procedures in which leukocyte-Buffy coat fraction was depleted in final product obtained, as already proposed in previous studies, that could be explained by the presence at most platelets in the border area of leuco-platelet spinning [5,6]. Another fact to note was the presence of blood levels both platelets and plasma growth factors stable at 24 h post-treatment very similar to the corresponding PRPs focused on patients, which could be explained by the great capacity of diffusion through different tissues having these proteins, regardless of the means used to manage [3]. Similarly, review the mobilization capacity that have on CD 34+, detecting blood higher levels at 24 h post-application compared to baseline count, figures [4,5]. Adequate studies in translational medicine are still needed to corroborate these results and correlate clinically with those medical applications where it really be useful.

Conclusion

Compared to other methods of obtaining platelet and plasma factors growth reviewed in the literature, the technique disclosed is more effective since concentrate achieves greater amount of platelets and growth factors and efficient since it maintains a serum protein in these stable sera of patients after 24 hours of administration thereof.

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