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RESEARCH ARTICLE

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Treatment of Cutaneous Photoaging of Glabella with Autologous Platelet Concentrate

Israel Alfonso Trujillo*, Yetter Cruz Leon, Yudy Alvear Carvajal, Melissa Johana Espitia Cordero, Yaquelin Luciana Morales Novo, Angela Rosa Gutierrez Rojas, Jesus Lazaro Diego De la Campa, Julio Antonio López Silva, Daymi Serpa Almaguer

Clinical Surgical Hospital, Hermanos Ameijeiras, Havana Cuba.

ABSTRACT

Introduction: Platelet rich plasma (PRP) is an autologous blood product obtained after centrifugation of the blood. Contains high concentrations of platelets and growth factors. It is currently a very popular therapy in dermatology; however, few studies objectively evaluate its efficacy.

Objective: To evaluate the efficacy and safety of intradermal microinjection of autologous platelet concentrate (APC) in the treatment of glabella rejuvenation.

Method: An observational, analytical and longitudinal study was carried out in 60 patients from the Hospital Clínico Quirúrgico: "Hermanos Ameijeiras", in the period between March 1, 2017 and March 31, 2020. The treatment was applied monthly. for 1 year. The final evaluation was carried out 3 months after the end of the treatment.

Results: 60 women with an average age of 45 ± 4.3 years were treated. After treatment, there were significant changes in the Glogau Photo Damage Scale (P = 0.012), in the Photo numeric Scale for evaluating the severity of glabella wrinkles (P = 0.031) and in the Global Aesthetic Improvement Scale (P = 0.012). The adverse events found were pain, inflammation and ecchymosis. The degree of satisfaction reported by the patients was good (25.0%) and very good (75.0%) (P = 0.027).

Conclusions: The autologous platelet concentrate proved to be effective and safe in reducing the signs of skin aging in glabella, associated with a high degree of patient satisfaction.

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KEYWORDS

Platelet-rich plasma, Glabella rejuvenation, Glabella skin photoaging, Autologous platelet concentrate.

Introduction

Platelet-rich plasma (PRP) is an autologous blood product, obtained after centrifugation of the blood. It contains high concentrations of platelets and is rich in growth factors that promote tissue repair, alter angiogenesis, and possess versatile immunomodulatory effects [1,2].

These qualities have made it a very popular therapy, yet few studies evaluate objectively its effectiveness, which motivated the realization of the present investigation.

Goals

The primary objective was to determine the efficacy and safety of autologous platelet concentrate (APC) microinjection in the treatment of glabella photoaging and the secondary objectives were: 1) to evaluate the clinical response to treatment, 2) to evaluate the type and intensity of adverse events that occur and 3) describe the degree of patient satisfaction.

Method

An observational, analytical, longitudinal study was carried out in 60 patients at the Hospital Clínico Quirúrgico: "Hermanos Ameijeiras", in the period between March 1, 2017 and March 31, 2020.

Treatment with APC was applied monthly for 12 months. Three months after the end of the treatment, the response to it was evaluated (final evaluation), comparing the current state with the initial state; For this, the patient had to attend the scheduled consultation. Throughout the study there was a rigorous control of adverse reactions. Before and after the procedure, the platelets were quantified to determine the quality of the applied product (the average degree of concentration of the platelets after the procedure increased 10.8 times its initial value). Microbiological culture of the extracted plasma was performed to guarantee that a sterile germ product was administered.

Inclusion criteria

Patients between 20 and 60 years old, of any sex and skin phototype, skin photoaging grade II to IV according to Glogau's classification [3] grade 1 to 5 according to the photo numeric scale for the evaluation of the severity of the glabella wrinkles [4] normal complementary tests (hemogram with differential, coagulogram, blood chemistry and serology for HIV, hepatitis B and C), with signed informed consent.

Contact Israel Alfonso-Trujillo ✉ Managua Road # 1133 between Cayman and Quemados, Las Guásimas, Arroyo Naranjo, Havana, Cuba, CP: 19320.

Exclusion criteria (Table 1).

Table 1: Exclusion criteria and their relationship with the time limits to perform the procedure.

Criteria	Time limits
Congenital or acquired coagulation disorders.	Prior and simultaneous to the procedure.
Bone marrow aplasia.	Prior and simultaneous to the procedure.
Prone to forming keloids.	Before the procedure.
Cardiovascular or pacemaker, neurological, liver, kidney, endocrine or immunological diseases, decompensated.	Simultaneous to the procedure.
Severe psychiatric disorder or other limitation that prevents the patient from giving his informed consent or makes his evaluation difficult.	Simultaneous to the procedure.
Pregnancy or breastfeeding	Simultaneous to the procedure.
Treatment with anticoagulants, antifibrinolytics, macrolides, terfenadine, cimetidine, amiodarone, fluoxetine, NSAIDs, or corticosteroids.	One month prior to the procedure.
Application of topical retinoids, aesthetic treatments in the region to be treated, including lasers, intense pulsed light, chemical peels, mesotherapy, carboxytherapy or others.	Three months prior to the procedure.
Fillers in the region to be treated.	One year prior to the procedure.
Active neoplastic diseases or during the follow-up period	Five years post-healing prior to the procedure.

Elimination criteria

Patients who wish to abandon the study, presence of an adverse event and / or complication that prevents continuing with the treatment or patients who have missed a treatment session.

Procedures

Once the patients gave informed consent, the included subjects registry template and the investigator's internal registry were filled out. All information on the included patients was compiled in the data collection notebook. The blood was extracted (500 milliliters), then the APC was obtained with the Rotixa centrifuge (221 mm radius) according to international standards [5]. To obtain the APC, a first light centrifugation of the blood was performed total in the plastic bag for 3 minutes at 2800 rpm at 22°C, with a centrifugation force of 2000 g, in this way 250 ml of red blood cells and 250 ml of PRP were obtained; then a second weighted centrifugation was performed on the PRP in the plastic bag for 5 minutes at 4500 rpm at 22°C, with a centrifugation force of 5000 g. Once the heavy centrifugation had been carried out, the supernatant plasma was transferred through the tubes that have the plastic bags for blood collection and only 10 ml were left and it is in said volume that by shaking the platelets that were deposited in the cell were resuspended. bottom of the bag as results of the centrifugation procedure. Subsequently, the red blood cells were returned to the patients and finally a microinjection of 10 milliliters of the APC was performed, distributed among the glabella, crow's feet, forehead, other facial areas, neck, V of the neckline, and back of the hands. Glabella asepsis and antisepsis were performed. Subsequently, with a 27G × 16 mm hypodermic needle and 1 ml syringes, intradermal injections of approximately 1.5 ml were administered at a distance of 1.5 to 2 mm between each application area (point-to-point, fan, back trace and nap page).

Variables related to the response to treatment

The response to treatment was evaluated taking into account the clinical examination of the patient, using the Glogau photodamage scale (Table 2) [3] the photo numeric scale for the evaluation of the severity of the glabella wrinkles (Table 3) [4] and the global aesthetic improvement scale (GAIS) (Table 4) [6].

Adverse events

The adverse events reported in the reviewed literature are pain, edema, and ecchymosis at the microinjection site [1,2].

Classification of adverse events (Table 5) [7]

Degree of satisfaction of patients to treatment

The degree of satisfaction (PSSS) of the patients with the treatment was evaluated taking into account what was reported by the patient according to the scale (Table 6) [8].

Bioethical considerations

The protocol was submitted for the consideration and approval of a Review and Ethics Committee for Clinical Research created for this purpose, which evaluated it from an ethical point of view. Additionally, this protocol was submitted for scientific and methodological review and approval by the Institutional Scientific Council of the Hospital Clínico Quirúrgico "Hermanos Ameijeiras".

Statistical methods used

The medical records of the patients included in the study were stored in the Department's file. With the information collected, a Microsoft Office version XP database in Excel format was made, which was exported to the SPSS version 21.0 system for analysis. To summarize the information of the study sample, the arithmetic mean, standard deviation and minimum and

Table 2: Classification of photoaging according to Glogau [3].

Type	Characterization
Type I "No wrinkles"	Early photoaging: slight pigmentary changes, no keratosis, minimal wrinkles, no scars, young patient, generally 28-35 years of age, no or minimal makeup.
Type II "Movement wrinkles"	Early to moderate photoaging: visible early senile lentigo, early actinic keratosis, slight signs of scars, wrinkles and parallel smile lines begin to appear, patient age: late 30s or 40s, usually she wears some makeup.
Type III "Wrinkles at rest"	Advanced photoaging: obvious dyschromia and telangiectasias, visible keratoses, neoplasms (+), wrinkles even when not moving, patient age: fifty years or older, always wears a lot of makeup.
Type IV "Wrinkles only"	Intense photoaging: grayish-yellow skin, cutaneous neoplasms (+++), all wrinkled skin, no normal skin, age of patient: sixties or seventies, cannot wear makeup, "hard and cracked".

Table 3: Photo numeric scale for the evaluation of the severity of the glabella wrinkles [4].

Grade	Characteristics
0	Without wrinkles.
1	Very fine wrinkles, hardly noticeable.
2	Fine and superficial wrinkles.
3	Moderately deep wrinkles.
4	Deep wrinkles, with well-defined edges.
5	Very deep wrinkles, redundant crease.

Table 4: Global aesthetic improvement scale (GAIS) [6].

Evaluation		Degree of improvement
1	Total answer.	**Patient with exceptional or much better improvement** (excellent corrective result, total disappearance of the lesions).
2	Marked partial response.	**Patient greatly improved or considerably better** (marked improvement in appearance, but not completely optimal, reduction of lesions by ≥ 50% and <100%).
3	Slight partial response.	**Improved or somewhat better patient** (appearance slightly better than initial condition, but needs more treatments, <50% lesions decrease).
4	Non-response	**No change (**the same number and size of lesions as at the start of treatment).
5	Progression.	**Worse (**increased number or size of lesions).

Table 5: Intensity scale of adverse events [7].

Intensity	Characteristics
Mild	if the adverse event subsided without treatment.
Moderate	if treatment was required, but the adverse event subsided with it.
Serious	if he required hospitalization or did not yield to treatment.
Very serious	if it endangered the life of the patient, if it caused sequelae or disability.

Table 6: Scale of the degree of patient satisfaction [8].

Evaluation		Degree of satisfaction
1	Very bad.	I did not get any improvement and the treatment caused me multiple discomforts (inflammation, bruising and pain).
2	Bad.	I did not get any improvement, but the treatment did not cause me any discomfort.
3	Regular.	The improvement was little.
4	Good.	The improvement was noticeable, but not total.
5	Very good	The improvement was complete with minimal discomfort.

Table 7: Epidemiological and clinical characteristics of the subjects.

	Mean (SD)	45.6 (± 4.3)	
	(Minimum; Maximum)	(27; 58)	
		N	%
Age	**20-29**	15	25.0
	30-39	12	20.0
	40-49	27	45.0
	50-60	6	10.0
Sex	**Female**	60	100.0
Skin photo type	**II**	24	40.0
	III	33	55.0
	IV	3	5.0
Glogau	**II**	9	15.0
	III	51	85.0
Degree of the glabella wrinkles.	**22 1**	8	13.3
	2 2	10	16.6
	3	14	23.3
	E 4	28	46.6

Table 8: Adverse events.

		APC N = 60 N	%
Adverse events	**Pain**	60	100.0
	Inflammation	10	16.6
	Equimosis	2	3.3
Duration	**Less than 7 days**	60	100.0
intensity	**Light**	60	100.0
Attitude	**No changes**	60	100.0
Result	**Resolved**	60	100.0

Table 9: Degree of satisfaction, according to the patients' own satisfaction scale (PSSS).

Satisfaction	APC N = 60		p
	N	%	
Regular	0	0	
Good	15	25,0	0,027 (χ2)
Very good	45	75,0	

Figure 1: Images showing the improvement of the skin on the glabella of a patient (A) before and (B) three months after treatment with APC.

Figure 2: Images showing the improvement of the skin on the glabella of another patient (A) before and (B) three months after treatment with APC.

maximum values were used. For all quantitative variables, the student's t test was used. For all qualitative variables (degree of photodamage, degree of aesthetic improvement, degree of severity of glabella wrinkles and degree of satisfaction), absolute numbers and percentages before and after treatment were calculated, which were compared using the Chi test Pearson square. In all hypothesis tests carried out, a significance level $\alpha = 0.05$ was used.

Sample's size calculation

The sample size was calculated using the C4-Study Design Pack computerized program. (C4- SDP) for sample size calculation (CTM). Version 1.1 ® Glaxo Wellcome. SA; [9] considering the following values: percentage of success reported in the literature 70%, percentage of success in the current study of 80%. With an alpha error of 0.05, a power of 80% and covering a loss of 5% of the patients, it was necessary to have 60 subjects in total.

Results

The study sample consisted of 60 women with skin phototypes between II and IV. The average age ranged around 45 ± 4.3 years (Table 7).

Regarding the Glogau Photo Damage Scale, 51 patients were classified as grade III, and 9 as grade II before the start of the study. After treatment, 28/51 (54.9%) patients who were classified as grade III were reclassified as grade II and 7/9 (77.7%)

patients who were classified as grade II were reclassified as grade I (p = 0.012); the rest of the patients remained in the same grade assigned before treatment.

Con relación a la escala fotonumérica para evaluación de la severidad de las arrugas de la glabela, 28 pacientes fueron clasificados como grado IV, 14 como grado III, 10 como grado II y 8 como grado I ante del inicio del estudio. Posteriormente al tratamiento, 10/28 (35,7%) pacientes que se clasificaron como grado IV fueron reclasificadas como grado III, 12/14 (85,7%) pacientes que se clasificaron como grado III fueron reclasificadas como grado II, 8 / 10 (80,0%) pacientes que se clasificaron como grado II fueron reclasificadas como grado I y 7/8 (87,5%) pacientes que se clasificaron como grado I fueron reclasificadas como grado 0 (p = 0,031); el resto de los pacientes se mantuvieron en el mismo grado asignado antes del tratamiento.

De acuerdo con la Escala global de mejoría estética, hubo cambios significativos tras el tratamiento (p = 0,012); 6/60 (10,0%) pacientes lograron respuesta total, 42/60 (70,0%) pacientes lograron respuesta parcial marcada y 12/60 (20,0%) pacientes lograron respuesta parcial ligera (Figure 1,2).

All the patients reported some adverse event (pain, inflammation and ecchymosis), which were of slight intensity, did not imply changes before the intervention and were completely resolved. The pain occurred during the procedure and disappeared immediately after the completion of the procedure (100%), the inflammation (16.6%) lasted 2 to 3 days and the ecchymoses at the puncture sites (3.3%) were infrequent and of short duration (five to seven days in duration) (Table 8).

Of the 60 patients treated with CPA, 15/60 patients (25.0%) reported a good degree of satisfaction and 45/60 patients (75.0%) reported a very good degree of satisfaction, because they achieved evident improvement with respect to their condition initial (Table 9).

Discussion

PRP contains more than 20 natural growth factors (GFs), including platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and insulin-like growth factor 1 (IGF 1). Various types of GF bind to their receptors on the cell surface and activate cell signaling pathways, resulting in gene expression and synthesis of various needs necessary for mitogenesis, increasing cell numbers, and angiogenesis, and stimulating the vascular growth [10].

Motosko CC et al conducted a systematic review of articles published between 2006 and 2015. All clinical studies and case reports that addressed platelet-rich plasma alone and / or in combination with fat grafts for facial rejuvenation, scars were included. acne or androgenic alopecia. Of the 22 articles included in the analysis, seven studies used platelet-rich plasma only for facial rejuvenation. Most of the studies where positive results are used for skin rejuvenation, but the procedure is limited by the lack of a standardized method for its preparation and application (number and frequency). It is not entirely clear to what extent significant variability in preparation and / or application methods can affect clinical outcomes [11].

Kim DH et al conducted a study where they investigated the effects of activated platelet-rich plasma (aPRP) and activated platelet-poor plasma (aPPP) on the remodeling of the extracellular matrix, a process that requires the activation of dermal fibroblasts, essential for the rejuvenation of aged skin. Platelet rich plasma (PRP) and platelet poor plasma (PPP) were prepared using a double spin method and then activated with thrombin and calcium chloride. The proliferative effects of aPRP and aPPP were measured by the [3H] thymidine incorporation assay, and their effects on matrix protein synthesis were evaluated by quantifying the levels of carboxy-terminal peptide (PIP) of type I procollagen using enzyme-linked immunosorbent assay (ELISA). The production of collagen and matrix metalloproteinases (MMP) was studied by Western blotting and reverse transcriptase polymerase chain reaction. The results show that the number of platelets in the PRP increased to 9.4 times the initial values. Both aPRP and aPPP stimulated cell proliferation, and maximum proliferation occurred in cells grown in 5% aPRP. PIP levels were higher in cells grown in the presence of 5% aPRP. Furthermore, aPRP and aPPP increased the expression of type I collagen, MMP-1 protein, and mRNA in human dermal fibroblasts [12].

Lee ZH et al conducted a study where they evaluated the efficacy and satisfaction of 31 patients with a single PRP treatment. They injected four milliliters of PRP at 6 standardized spots on each side of the face. Results were evaluated by a research independent dermatologist using pre and post treatment photographs using the Wrinkle Severity Rating Scale (WSRS) and the Global Aesthetic Improvement Scale (GAIS). In addition, the results reported by the patients were evaluated through their scores of the degree of satisfaction achieved according to the FACE-Q survey. Post-treatment WSRS scores improved in only 1 patient; GAIS scores for 14 patients indicated cosmetic improvement. Analysis of the FACE-Q scores revealed statistically significant increases in participants' satisfaction with overall facial appearance and cheeks. The most frequently reported adverse effects were pain 7/31 (23.4%), facial tightness 6/31 (20.0%); 6 of 31) and inflammation 6/31 (20.0%) [13].

In order to evaluate the efficacy and safety of dermal injections of autologous PRP in the rejuvenation of facial skin, Cameli N et al conducted an investigation involving 12 patients, who underwent 3 sessions of PRP injection at 1-month intervals. The clinical and instrumental results were evaluated before and 1 month after the end of the treatment by trans epidermal water loss, craniometry, cutometer, Visio scan and Visio face. One month after completing the treatment, the clinical and patient evaluation showed an improvement in skin texture. Gross skin elasticity, skin smoothness parameters, skin barrier function, and capacitance were significantly improved (p <0.05) [14].

In our study, there was clinical improvement in the Glogau photodamage scale (p = 0.012), in the photo numeric scale for evaluating the severity of glabella wrinkles (p = 0.031) and in the global scale of aesthetic improvement (p = 0.012), associated with a high degree of patient satisfaction (p = 0.027).

Conclusions

The application of autologous platelet concentrate proved to be effective and safe in reducing the signs of skin aging in

glabella, associated with a high degree of patient satisfaction.

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