Research Article ISSN 2690-537X

Dermatology Research

Efficacy and Safety of Autologous Platelets Concentrated in the Treatment of Perioral Skin Aging

Israel Alfonso-Trujillo[1], Yetter Cruz-Leon[1], Melissa Johana Espitia-Cordero[1], Eduardo Jesús Núñez-Jordán[2], Yaquelin Luciana Morales Novo[3], Jesus Lazaro Diego-De la Campa[4], Angela Rosa Gutierrez-Rojas[5], Julio Antonio López-Silva[6] and Daymi Serpa-Almaguer[7]

[1]*Dermatology Specialist, Doctor of Medical Sciences, Professor and Senior Researcher*

[2]*Dermatology Resident, Clinical Surgical Hospital, Hermanos Ameijeiras. Havana Cuba.*

[3]*Graduate in Health Technology, Assistant Professor, Clinical Surgical Hospital: Hermanos Ameijeiras. Havana Cuba.*

[4]*Specialist in Hematology, Assistant Professor, Clinical Surgical Hospital: Hermanos Ameijeiras. Havana Cuba.*

[5]*Specialist in Biostatistics, Assistant Professor, Clinical Surgical Hospital: Hermanos Ameijeiras. Havana Cuba.*

[6]*Hematology Specialist, Assistant Professor, Clinical Surgical Hospital: Hermanos Ameijeiras. Havana Cuba.*

[7]*Immunology Specialist, Instructor Professor, Clinical Surgical Hospital: Hermanos Ameijeiras. Havana Cuba.*

***Correspondence:**

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

Received: 02 May 2021; **Published:** 25 May 2021

Citation: Alfonso-Trujillo I, Cruz-Leon Y, Espitia-Cordero MJ, et al. Efficacy and Safety of Autologous Platelets Concentrated in the Treatment of Perioral Skin Aging. Dermatol Res. 2021; 3(1): 1-6.

ABSTRACT

Introduction: *The rejuvenation of the perioral region has a certain degree of difficulty. The search for painless and minimally invasive alternatives is a challenge in the field of aesthetic medicine.*

Objective: *To evaluate the efficacy and safety of intradermal microinjection of autologous platelet concentrate (APC) in the treatment of perioral skin aging signs.*

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 = 0.028), in the Global Aesthetic Improvement Scale (P <0.032) and in the Evaluative Scale of the severity range of the nasolabial fold (P = 0.031). The adverse events found were pain, inflammation and ecchymosis. The degree of satisfaction reported by the patients was good (16.7%) and very good (83.8%) (P <0.038).*

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

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

Introduction
The rejuvenation of the perioral region must take into account the three processes that accompany aging: loss of volume, ptosis (fall) and skin changes (wrinkles, atrophy, flaccidity, and dehydration), and processes that are closely related to each other. The loss of volume can be corrected with fillers (hyaluronic acid and autologous fat). Ptosis can be improved with tensioning threads or lifting [1,2]. Skin changes have traditionally been treated with lasers, peels, mesotherapy (vitamins, minerals, and hyaluronic acid) and recently with regenerative therapy (platelet-rich plasma) [3,4].

Taking into account the pathophysiology of skin aging and the properties of platelets and their growth factors (GF), [5] its use to improve skin changes would be justified, however, few studies objectively evaluate its efficacy and safety, which motivated the realization of the present investigation.

Goals
The primary objective was: to determine the efficacy and safety of the microinjection of autologous platelet concentrate (APC) in the treatment of perioral skin aging signs and the secondary objectives were: 1) to evaluate the clinical response to treatment, 2) to evaluate 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.

The treatment was applied monthly for 12 months. Three months after the end of the treatment, the response to it (final evaluation) was evaluated, comparing the current state of the lesions (barcode, lips, Cupid's bow, marionette wrinkles, nasolabial fold, pigmentations, lentigos and querotosis) 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 (Glogau classification), [6] nasolabial fold (NGS) grade 2 to 5 (evaluative scale of severity range of the nasolabial fold), [7] normal complementary examinations, with signed informed consent.

Exclusion criteria

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.

Treatment
Once the patients gave informed consent, the included subject's registry template and the investigator's internal registry were filled out. The blood was extracted (500 milliliters), then the CPA was obtained with the Rotixa centrifuge (221 mm radius) according to international standards [8]. To obtain the CPA, a first light centrifugation of the whole blood was carried out 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 were obtained. Platelet rich plasma (PRP); 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, 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 CPA was performed, distributed among the NGS, the entire facial area, V of the décolleté, neck and the back of the hands. Subsequently, with a 25G × 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, backtrace and nappage).

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), [6] evaluative scale of the severity range of the nasolabial fold (WSRS) (Table 3) [7] and the global aesthetic improvement scale (GAIS) (Table 4) [9].

Table 2: Classification of photoaging according to Glogau [6].

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: Evaluative scale of the severity range of the nasolabial fold (WSRS) [7].

Grade		Characteristics
5	**Extreme**	Extremely large and deep furrows. Detrimental facial appearance. The skin stretch shows a 2-4 mm deep V-shaped fold. It is unlikely to achieve a satisfactory correction with an injectable implant.
4	**Severe**	Large deep furrows. Prominent facial manifestation. When the skin is stretched the fold is visible less than 2mm deep. Significant improvement is expected with injectable implant.
3	**Moderate**	Moderately deep furrows. Clear and visible facial manifestation. The crease disappears when the skin is stretched. Excellent correction is expected with injectable implant.
2	**Slight**	Visible but superficial groove, like a slight footprint. Minor facial manifestation. With an injectable implant, a slight improvement in appearance is expected.
1	**Absent**	No visible furrow, continuous skin line.

Table 4: Global aesthetic improvement scale (GAIS) [9].

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).

Adverse Events

Adverse events reported in the reviewed literature are pain, edema, and ecchymosis at the microinjection site [4,5].

Classification of adverse events (Table 5) [10]

Table 5: Intensity scale of adverse events[10].

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.

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) [11].

Table 6: Scale of the degree of patient satisfaction [11].

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.

Bioethical considerations

The protocol was submitted to 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 subjected to 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 gathered, a Microsoft Office version XP database in Excel format was created, 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 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 affectation of the severity of the nasolabial fold and degree of satisfaction), the absolute numbers and percentages were calculated, before and after the treatment, which were compared using the test. Pearson's Chi-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; [12] 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).

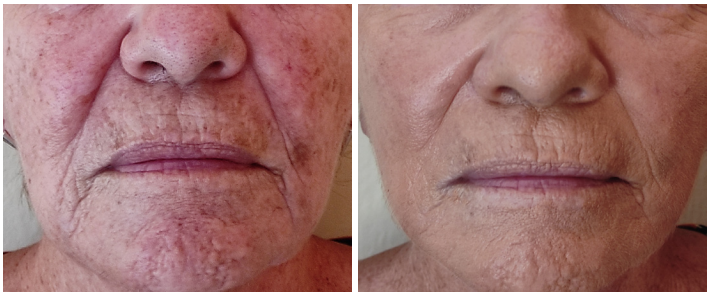
Table 7: Epidemiological and clinical characteristics of the subjects.

Age	Mean (SD)	45.6 (± 4.3)	
	(Minimum; Maximum)	(27; 58)	
		N	%
	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 phototype	II	24	40.0
	III	33	55.0
	IV	3	5.0
Glogau	II	9	15.0
	III	51	85.0
WSRS	22 2	8	13.3
	3	32	53.3
	4	12	20.0
	E 5	8	13.3

Regarding the Glogau Photodamage Scale, 51 patients were classified as grade III, and 9 as grade II before the start of the study. After treatment, 36/51 (70.6%) patients who were classified as grade III were reclassified as grade II and 5/9 (55.5%) patients who were classified as grade II were reclassified as grade I (p = 0.028); the rest of the patients remained in the same grade assigned before treatment.

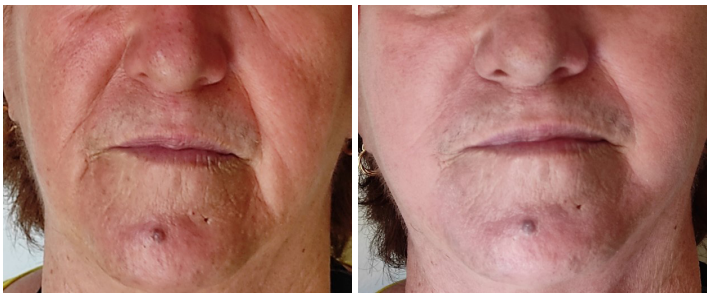
According to the Global Aesthetic Improvement Scale, there were significant changes after treatment (p <0.032); 8/60 (13.3%) patients achieved a total response, 32/60 (53.3%) patients achieved a marked partial response, and 20/60 (33.3%) patients achieved a slight partial response.

Regarding the evaluative scale of the severity range of the nasolabial fold (WSRS), 8 patients were classified as grade 5, 12 as grade 4, 32 as grade 3, 10 as grade 2 and 8 as grade 1, before the start of the study. After treatment, 4/8 (50.0%) patients who were classified as grade 5 were reclassified as grade 4, 17/30 (56.6%) patients who were classified as grade 4 were reclassified as grade 3, 9 / 14 (64.3%) patients who were classified as grade 3 were reclassified as grade 2 and 5/10 (50.0%) patients who were classified as grade 2 were reclassified as grade 1 (p = 0.031); the rest of the patients remained in the same grade assigned before treatment (Figures 1 and 2).



A B

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



A B

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

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 (83.3%) lasted 2 to 3 days and the ecchymosis at the puncture sites (66.6%) were of short duration (five to seven days in duration) (Table 8).

Table 8: Adverse events.

		APC N = 60 N %	
Adverse events	Pain	60	100.0
	Inflammation	50	83.3
	Equimosis	40	66.6
Duration	Less than 7 days	60	100.0
intensity	Light	60	100.0
Attitude	No changes	60	100.0
Result	Resolved	60	100.0

Of the 60 patients treated with CPA, 10/60 patients (16.7%) reported a good level of satisfaction and 50/60 patients (83.8%) reported a very good level of satisfaction, due to the fact that they achieved evident improvement with respect to their condition initial (Table 9).

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

Satisfaction	APC N = 60		p
	N	%	
Regular	0	0	<0.038 (χ2)
Good	10	16,7	
Very good	50	83,3	

Discussion

The typical changes in the appearance of facial aging are secondary to the progressive effect of gravity on skin with less elastic properties, thinner and drier. This is reflected in vertical wrinkles on the skin of the lips known as "barcodes", in the loss of lip volume and the definition of the linea alba and cupid's bow. The corners of the lips and the tissues of the cheeks descend, deepening the nasolabial folds and generating the marionette lines. Another important factor is bone resorption and adipose tissue ptosis, which causes natural facial furrows to deepen. The activity of the sebaceous glands decreases over the years causing drier skin. Thinning of the skin causes the contraction of the underlying muscle to move to the surface in the form of wrinkles [13,14].

PRP is rich in multiple growth factors secreted by pla-granules of activated platelets, such as platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF) and insulin-like growth factor (IGF). It is believed that these can regulate processes of cell migration, proliferation and differentiation of fibroblasts, increase the production of collagen, elastin and hyaluronic acid, and promote the accumulation of extracellular matrix, which would provide a skin with more shine, better texture, and hydration and elasticity [15,16].

Elnehrawy NY et al. conducted a study in 20 subjects to evaluate the efficacy and safety of a single intradermal injection of autologous PRP for the treatment of the nasolabial fold (GNS). Results were clinically assessed (before and 8 weeks after treatment) using the Wrinkle Severity Rating Scale (WSRS), the Aesthetic Improvement Scale, and the Subject Satisfaction Scale. The most significant results were in the youngest subjects with mild and moderate NGS. The mean WSRS value decreased from 2.90 ± 0.91 (before treatment) to 2.10 ± 0.79 (8 weeks after treatment). Fourteen out of seventeen subjects showed more than 25% improvement in their appearance. The degree of satisfaction of the subjects was good. Treatment side effects were minimal to mild and with excellent tolerability [17].

Aloosi S et al. Published a study evaluating the effectiveness of PRP injections (monthly for 3 months) in the treatment of wrinkles in the perioral area. They included 15 patients with a mean age of 40 ± 1.36 years. The results showed that the WSRS score for the SNG was 1.9 ± 1.099 (before treatment) and 1.2 ± 1.264 (after treatment), the WSRS reached an average percentage improvement of 38% (P = 0.00). The degree of patient satisfaction (PSS) was good (4.4). The degree of satisfaction of the physicians (CLSS) was good (4.33). The final graded score (DGS) that is, the sum of PSS + CLSS was good (8.73) (P = 0.01) [18].

Kamakura T et al. Evaluated the efficacy of PRP plus basic fibroblast growth factor (bFGF) in the treatment of wrinkles and depressed areas of the skin of the face. Of the 2005 patients treated, 1,889 were women and 116 men. The mean age was 48.2 years. The treated areas included 1461 nasolabial folds, 437 marionette lines, 1413 nasolabial folds, 253 mid-cheek folds. The results indicated that the patient satisfaction level was 97.3% and the investigator satisfaction level was 98.4%. The period for the effectiveness of the therapy to become apparent was an average of 65.4 days. PRP plus bFGF therapy resulted in a better WSRS grade. The average WSRS improvement was 0.55 degrees in the light grade, 1.13 degrees in the moderate grade, 1.82 degrees in the severe grade, and 2.23 degrees in the extreme grade [19].

In our study, 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 = 0.028), in the Global Aesthetic Improvement Scale (P <0.032) and in the Evaluative Scale of the severity range of the nasolabial fold (P = 0.031). The adverse events found were pain, inflammation and ecchymosis. The degree of satisfaction reported by the patients was good (16.7%) and very good (83.8%) (P < 0.038), which is why it is concluded that autologous platelet concentrate is effective and safe in reducing signs of aging of the perioral region, associated with a high degree of patient satisfaction.

References

1. Iblher N, Stark GB, Penna V. The aging perioral region -- Do we really know what is happening? J Nutr Health Aging. 2012; 16: 581-585.
2. Penna V, Stark GB, Voigt M, et al. Classification of the Aging Lips: A Foundation for an Integrated Approach to Perioral Rejuvenation. Aesthetic Plast Surg. 2015; 39: 1-7.
3. Wollina U. Perioral rejuvenation: restoration of attractiveness in aging females by minimally invasive procedures. Clin Interv Aging. 2013; 8: 1149-1155.
4. Sclafani AP. Platelet-rich fibrin matrix for improvement of deep nasolabial folds. J Cosmet Dermatol. 2010; 9: 66-71.
5. Fernández Tresguerres CA, Alfageme Roldán F, Burón Álvarez I, et al. Bioestimulación cutánea con plasma rico en plaquetas autólogo. Estudio controlado con ecografía. Piel. 2013; 28: 69-74.
6. Glogau RG. Aesthetic and anatomic analysis of the aging skin. Semin Cutan Med Surg. 1996; 15: 134-138.
7. Day D, Day DJ, Littler CM, et al. The Wrinkle Severity Rating Scale: a validation study. Am. J Clin Dermatol. 2004; 5: 49-52.
8. Nester T, AuBuchon JP. Decisiones en hemoterapia y sus resultados. En: American Association of Banks Blood AABB. Manual Técnico de la AABB. 17a Ed. Buenos Aires: Asoc. Argentina Hemoterapia e Inmunohemat. 2013.
9. Savoia A, Accardo C, Vannini F, et al. Outcomes in thread lift for facial rejuvenation: a study performed with happy lift revitalizing. Dermatol Ther (Heidelb). 2014; 4: 103-104.

10. Moher D, Hopewell S, Schulz KF, et al. CONSORT 2010 Explanation and Elaboration: updated guidelines for reporting parallel group randomised trials. BMJ. 2010; 340: c869.
11. Larson L, Rovers J, Mackeigan L. Patient satisfaction with pharmaceutical care: update of a validated instrument. J Am Pharm Assoc. 2002; 42: 44-50.
12. C4-Study Design Pack (C4- SDP) para el cálculo de tamaño de muestra (CTM). Versión 1.1®. Desarrollado por: Departamento de Biometría de Glaxo Wellcome, S.A. Disponible en: http://www.e-biometria.com/ebiometria/c4-sdp/c4-sdp.htm
13. Wollina U. Perioral rejuvenation: restoration of attractiveness in aging females by minimally invasive procedures. Clin Interv Aging. 2013; 8: 1149-1155.
14. Bierenbroodspot F, Schouten HJ, Schepers RH, et al. Periorale behandelingsmogelijkheden in de cosmetische aangezichtschirurgie [Perioral treatment options in cosmetic facial surgery]. Ned Tijdschr Tandheelkd. 2017; 124: 325-332.
15. Lubkowska A, Dolegowska B, Banfi G. Growth factor content in PRP and their applicability in medicine. J Biol Regul Homeost Agents. 2012; 26: 3S-22S.
16. Anitua E, Sánchez M, Orive G. Potential of endogenous regenerative technology for in situ regenerative medicine. Adv Drug Deliv Rev. 2010; 62: 741-752.
17. Elnehrawy NY, Ibrahim ZA, Eltoukhy AM, et al. Assessment of the efficacy and safety of single platelet-rich plasma injection on different types and grades of facial wrinkles. J Cosmet Dermatol. 2017; 16: 103-111.
18. Aloosi S, Ahmed B. Evaluation of Perioral Skin Rejuvenation with Platelet – Rich Plasma. Reviews in Clinical Medicine. 2020; 7: 168-175.
19. Kamakura T, Kataoka J, Maeda K, Teramachi H, Mihara H, Miyata K, Ooi K, et al. Platelet-Rich Plasma with Basic Fibroblast Growth Factor for Treatment of Wrinkles and Depressed Areas of the Skin. Plast Reconstr Surg. 2015; 136: 931-939.