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Evaluation of Perioral Skin Rejuvenation with Platelet – Rich Plasma

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ABSTRACT

Introduction: Using Platelet- Rich Plasma (PRP)in aesthetic dermatology has been recently considered and its safety and effectiveness have been widely reported. We conducted this study to evaluate the technique and degree of benefit.
Methods: Fifteen (15) patients with different ages, sexes, and facial perioral wrinkles have participated in the current research and received a session of monthly PRP injections (three courses), targeting line and wrinkles in the perioral area. In this study, we have prepared the PRP by using a special test tube and then centrifuge it with relative centrifugal force (RCF) of 1700G for 5 minutes, followed by two minutes of soft centrifugation at room temperature.
Results: Mean age ± SD was 40 ± 1.36 years. 44.7% were female. 84.5% of patients are considered adherent (≥ 95% of doses were consumed). Married patients had better adherence (p = 0.04). Age, sex, addiction, imprisonment history, intravenous drug usage were not associated with adherence (p> 0.05). We also extracted previews history of treatment cessation and regimen changes. Efavirenz containing regimens had a higher rate of failure (p =0.001).
Conclusion: Wrinkles in perioral area management by PRP injection is an easy technique, with high- quality result and high patient satisfaction, no serious side effects, high patient toleration, and short downtime. Platelet- Rich Plasma can be a competitive substitute for other fillers and revitalization methods in lips and perioral rejuvenation.

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Introduction

With the increase of aesthetic demands minimally invasive cosmetic procedures on the lip and perioral region have been increased (1). Skin aging is associated with pigmentation, wrinkles, epidermal and dermal atrophy, rough skin, and skin looseness (2).

The main causes of an ageing face are the loss of skin elasticity due to diminished collagen, and volume loss through the loss of fatty tissue. Facial muscles sag, cheeks and lips lose their youthful fullness (3). Actinic damage may accelerate the reduction in the overall collagen (4).

Platelet-rich plasma (PRP) is a supraphysiological concentration of human platelets in a small amount of plasma (5,6). PRP has been used over the last several decades for its proposed ability to improve wound healing (7).

Facial rejuvenation utilizing autologous platelet growth factors is a natural approach to restoring dermal regeneration of aging face (8). It is safe, cost-effective product, readily available, provides antibacterial protection, and eliminates the concern of immunogenic reactions (9).

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It is a bio-stimulator, creates an early, long lasting effect with high level of patient satisfaction (10).

The platelet derived angiogenesis factor (PDAF), epithelial cell growth factor (ECGF), transforming growth factor TGF-b (including β 1 and β 2 isomers), epidermal growth factor (EGF), interleukin -1, platelet factor 4 (PF4), thrombospondin vascular endothelial growth factor (VEGF), osteocalcin, platelet-derived endothelial growth factor (PDEGF), vitronectin, insulin-like growth factor (IGF), osteonectin, fibronectin, and fibrinogen are proteins of the platelets (11).

PDGF stimulated cellular replication (mitogenesis). It can increase the rate of healing cells, such as mesenchymal stem cells and osteoprogenitor cells that construct the connective tissue-bone healing cellular complex (12).

The lips are in close connection with the perioral skin, exert pressure on the teeth that contribute to their adaptation, closing the oral cavity, protecting it against penetration of external agents and preserve its hygrometry and temperature (13).

Wrinkles are depressions in the skin of a uniform depth, while rhytides irregular aggregations of lines formed in the epidermis (14).

However; both terms are usually unchangingly used. Perioral ageing is one of the significant contributors to an overall aged appearance; it results from the dynamic effects of muscle movement and characterized by formation of rhytides, changes in lip shape or fullness, deepening of nasolabial folds, and ptosis of the chin (15).

Shallow and deep wrinkles are two types of wrinkles. The former is resulted from the distortion of the epidermis due to water-loss, whereas the latter is resulted from the distortion of the dermis because of elasticity loss due to a reduction in collagen and elastin fibers (16).

The most superficial kind of crease are the lines, which form in the epidermis, and is considered as an atrophied rhytides (14,17).

As individual ages, lengthening of the upper lip, drooping the oral commissures, and thinning the vermilion and philtral columns occur. Also, thinning of the lower lip and rolling inward is observed and marionette lines are more noticeable (18).

The nasolabial fold shows a progressive transformation into a nasal line, making a bitterness fold that can extend and form the anterior side of the jowl. Dewlaps, double chin, shriveled skin and jowls, change the face oval contour and a well-defined chin and neck (19).

Radial surface lines can be observed because of the interaction between skin and muscle as well as muscle bed atrophy leading to lip contouring (20).

Marionette lines form and deepen due to hyperactivity of the depressor anguli oris muscle and loss of skin structure and bony support, resulting in unhappy expression (15).

The chin loses its delineated look and begins to sag. The skin of the chin often becomes dimpled from the muscular contraction (15).

Usage of PRP in aesthetic dermatology has been recently (20); however, limited number of studies on PRP are available (21). Its safety and effectiveness have been widely reported, but it is essential to standardize the techniques to prepare and implement PRP protocols for more advancements (22).

The importance of this study is to statistically justify this non-invasive method of perioral rejuvenation that could be more satisfactory by the patient than other cosmetic procedures, as it is a natural autologous product, cost-effective, and relatively painless and participates in improving patient's quality of life.

Methods

Fifteen patients with different ages, sexes, and perioral wrinkles have participated in the current research and had received a session of injections with PRP monthly (three courses). Following (8), after a thorough history taking and clinical examination, blood investigation and viral screen tests were requested (23).

Exclusion Criteria: In this study, it was mandatory to exclude all medically compromised patients or patient with previous scars caused by a skin disease or trauma.

The researcher then discussed the advantageous and probable adverse effects of the treatment in convenient interview with the patient, evaluated the demand of the patients, their attitude, motivation, and expectation. in this step the researcher collected the patient`s singed consent form.

For the sake of classification Fitzpatrick skin type classification was adopted (24).

For evaluating the type of the skin for each patient, some curtail lines and Wrinkles in the perioral region for injecting the PRP were selected:Upper radial lip lines, Lower radial lip lines,Corner of mouth lines, Marionette line , Nasolabial folds ,Cheek lines and Labio-mental crease (chin crease).

Platelet rich-Plasma Preparation:

The procedure of PRP preparation started by aspirating venous blood (27 ml) using venipuncture of the Median Cubital forearm vein by the 21 g butterfly needle , followed by using a special sterile tube for collecting the blood (neogenesis

kit) that contain three milliliters of anticoagulant ACD-A. Next, by centrifugation, the plasma that contains 'Buffy coat' (white blood cells (WBCs) and platelets) can be isolated from the red blood cells (RBCs).

Blood Centrifugation begins with a RCF of 1700G for five minutes then we added two minutes soft centrifugation at room temperature. , here the researcher used buffy coat method of PRP preparation (25).

After completion of the centrifugation, the collection of the PRP starts by gently pushing out the platelet-poor plasma to inhibit being mixed with the PRP. It is easy to collect the remaining PRP by subsequently pushing it up from the test tube and preparing it to being activated by adding CaCl2 (0.1 per 0.9 ml of PRP) to reach a concentration of activated PRP. By this method we approximately, produced 3 ml of PRP, for injecting it intradermally through a 30 G needle. Prior to injection, the targeted skin surface is cleaned completely using alcohol pads. It was useful to use topical Eutectic Mixture of Local Anesthetics (EMLA) cream (2.5 % lidocaine plus 2.5 % prilocaine) as a compound for inducing topical aneasthesia (26).

The preferable timing for application is one hour before injection. It significantly decreases the pain of injections and increases patient comfort (27).

Injection technique:

To proceed for injection , we have used different injection techniques according to the injection location, (linear retrograde and fanning) for the nasolabial fold, (linear retrograde) for marionette line, corner of the mouth, upper lip lines, lower lip lines, and chin crease, and (serial puncture) for cheek lines. After each session of PRP injection, instructing the patient by the following instruction was mandatory:

♦Ice packs application immediately after treatment to reduce swelling.

♦Avoiding the touch or scrubbing the injection areas for 24 hours following injection.

♦Sleeping on back and head elevation.

♦Avoiding high heat directly (blow dryer, being exposed to sun, sauna, steam room, hot baths, hot yoga, strenuous exercise, etc.) 24 hours following therapy.

♦Face cosmetics are avoided for at least 24 hours after the treatment

Evaluation:

All along the study, we were documenting the results photographically for subsequent evaluation. For unbiased calibration of the outcome ,we use PSS (patient's satisfaction score) and a CLSS (clinician's satisfaction score) and the DGS (definitive graduated score) according to Redaelli et al (28) .The patients reported their satisfaction by selecting a score from " 1 to 8" , expressing their degree of satisfaction by the outcome .

According to CLSS, and PPS , " no result" stands for a score of (≥ 0 and < 2) , and a score of (≥ 2 and < 4) is for " fair result " , a score of (≥4 and < 6) means " good result" , and a score of ≥ 6 and< 8 means "very good " , the result is going to be "excellent" if the patient or the researcher selected the score 8 . The definitive graduated score (DGS) is the summing of the total value of every parameter of the CLSS and PPS as: (≥ 0 and < 4 = none), (≥ 4 and < 8 = moderate), (≥ 8 and < 12 = good), (≥ 12 and< 16 = very good), and (16 = excellent) following Redaelli et al (28).

In the evaluation of severity of wrinkles, the researcher followed the facial wrinkle severity scale regarding objective assessment designed by Day et al (29): (Type 1) Absent , means the lack of visible fold , continual skin lines, (Type 2) Mild ,means shallow and visible fold characterized by a small indentation , small facial features, (Type 3) Moderate , stands for moderate deep folds, obvious facial features detectable in normal appearance; however undetectable while stretching, (Type 4) Severe , was to describe the very long and deep wrinkles and considerable facial features, < 2 mm detectable wrinkles while stretching. Finally (Type 5) Extreme, was to express extensively deep and long wrinkles, disruptive for appearance , 2 to 4 mm wrinkles with V shape wrinkles while stretching (29).

All cases were subjected to follow up at least for three months following the last session. At the end of collecting the whole information, an expert statistician tabulated and analyzed the data by using statistical program for social science (IBM SPSS version 19).

The statistician used the Student t-test, Chi-square test, and Pearson correlation coefficient (r) to test significance of the results , the obtained findings is statistically significant when P≤0.05, and is highly significant at P≤ 0.01.

Result

This research included fifteen cases (14 female and 1 male; average age: 34 ± 9.9 years). Based on Fitzpatrick skin type classification (28), 8 cases (53.3%) had skin type III, and 7 cases (46.7%) had skin type IV.

Following The Wrinkle Severity Rating Scale (WSRS) score related to the nasolabial folds, 53.3% of patients were of type I (had no visible folds, only continuous skin lines), 6.7% were of type II with shallow but obvious fold characterized by a small indentation, 33.3% represented

type III with moderately deep folds, and 6.7% had type IV skin with very long and deep folds.

The mean value of whole blood platelet count was ≃ 272000/mm3 and the mean of PRP platelet count was ≃ 1229000 /mm3. This means that by following this technique of PRP preparation have achieved 4.5 ± 0.46 fold increases in platelet concentration over baseline. Assessing the level of texture and homogeneity improvement , tonicity and elasticity, acne scars of facial skin was the rule in all follow-up visits.

WSRS

The mean ± SD of WSRS score for the nasolabial folds before PRP injection was 1.9333 ±1.o99, whereas after injection it was 1.2 ± 1.264 with an average percentage of improvement of 38%, which was statistically significant (P=0.00).

Patient's Satisfaction Score (PSS)

The patients expressed an overall appreciation and satisfaction by for the ease of use, safety of the technique and the result obtained. A final PSS measurement was 4.4 which is consistent with (good) result (Table 1).

Table 1: PSS (Patients' satisfaction score)

*Final PSS	Patients' No.	Mean	Std. Deviation
PSS	15	4.40	1.183

Clinician's Satisfaction Score(CLSS):

Photographs assessment as well as the patient examination were the method for obtaining the Clinician's satisfaction score CLSS. The degree of the final CLSS was nearly the same degree of the patient satisfaction score PSS and the average result was good (4.33) (Table 2),(Figure 1).

Table 2: CLSS (Clinician's satisfaction score)

Final CLSS	Patients' No.	Mean	Std. Deviation
CLSS	15	4.33	1.175

Figure 1: A 30 years old female who had skin grade III and WSRS grade 1. (A) prior to therapy, (B) following one session therapy, (C) after two sessions treatment, (D) after three sessions , with homogeneity and texture improvements and increase in volume.

Definitive Graduated Score (DGS):

The degree of DGS was determined through summing both PSS, and the CLSS. The DGS average result was 8.73 which is consistent with (good) (Table 3).

Table 3: DGS (Definitive graduated score)

	Patients' No.	Mean	Std. Deviation
DGS	15	8.73	1.981

Comparison between First and Final Session Results:

The study reviled a higher degree of patients and clinician satisfaction after completing third session ,the mean of improvement after the first session obtained from patient's satisfaction score (PSS) was 2.9 , whereas the mean of improvement after the third session was 4.4.

The degree of difference between them was highly significant, (P value = 0.01) (Figure 2).

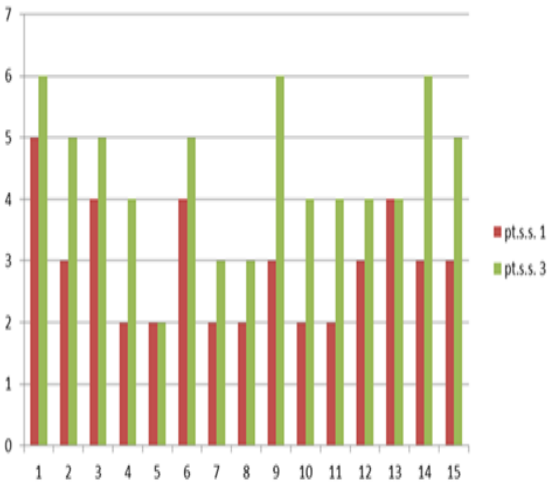

Figure 2. Comparison of improvement between PSS (Patient satisfaction score) in the first session and third session.

In addition, statically, the mean improvement of first session according to Clinician's satisfaction score was 3.2, whereas the mean of improvement after the third session was 4.33. The difference between them was significant, (P value =0.03).

Correlation between Whole Blood Platelet and PRP Platelet Count and Definitive Graduated Score:

By analyzing the platelets count, the platelet concentration in PRP was significantly correlated with platelet count in whole blood p (P< 0.01) (Figure 3).

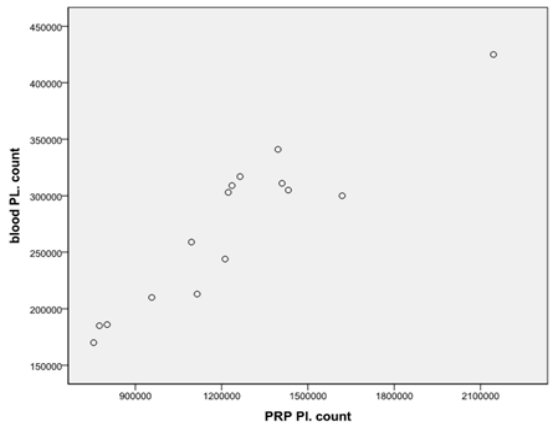

Figure 3. Correlation between blood platelet count and PRP platelet count or concentration

Moreover, PRP platelet concentration was statically significantly correlated with the definitive graduated score DGS, (P = 0.03), correlation coefficient (r = 559) (Figure 4).

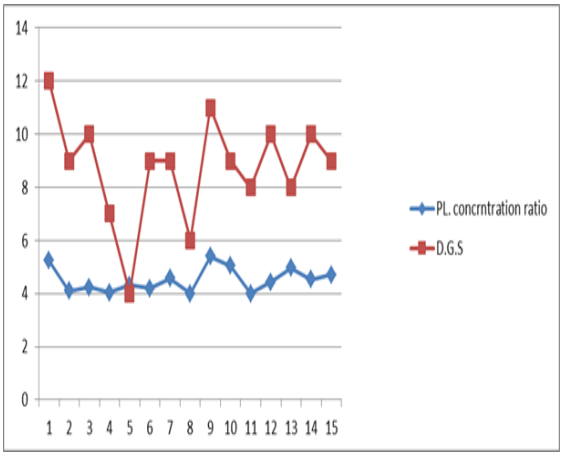

Figure 4. Correlation between platelet concentration and definitive graduated score (DGS)

Correlation between Age of the Patients and Definitive Graduated Score:

The study reviled no statistical relationship between the age of the patients and the definitive graduated score.

Effect of Skin Type on the DGS(Definitive Graduated Score):

The study showed that there is no significant difference between skin type III and type IV in the final DGS.

Side Effects:

Patient didn't report any serious or persistent side effects. There was an observation of mild and transient adverse events e.g. 26.7% of cases reported redness ecchymosis tolerated well. 40.0% of cases expressed burning sensation after injection, 50% of patients reported minimal swelling, and 6.7% developed headache for few hours.

All these complications were transient and didn't necessitate any treatment. None of the patients had reported any serious cases of infection or hematoma.

Discussion

The facial rejuvenation is not an easy process. We aimed at restoring harmonious volumetric facial ratios via obtaining the cheekbone contours and decreasing jaw lines and nasolabial wrinkles (30).

This process is classified as face lift procedure and non-operative, minimally invasive surgery, like laser abrasion, fat grafts, fillers, injecting botulinum toxin, and thread lifts. Plastic surgeons are needed to develop the skills for performing different rejuvenation methods (31).

Mesotherapy gained its popularity due to painless nature, but, till date, the mode of action of many products is either doubtful or unknown and, there is insufficient data evaluating the safety of the technique and the medicines used. Thus, its use is debatable (32).

Facial rejuvenation is not an easy process, in which facial harmony is restored (30). Minimally invasive cosmetic procedures (MICPs) for lip and perioral region have been recently considered (1).

In this current study the patients experienced a significant improvement in the nasolabial folds appearance; they also expressed an improvement in acne scars, texture, homogeneity, tonicity and elasticity of perioral skin after PRP injection. Our findings are in line with similar studies (28).

To avoid bias in the evaluation, we used the patient satisfaction score PSS for the evaluation. The Patients were satisfied in a high degree by the improvement in their skin texture and appearance measured by PSS. The results were consistent with the results of Redaelli (28), who treated patients with one session of injections with PRP monthly with good result, and the results of Mikhael and El-Esawy study (8), which yields satisfactory results after treating twenty healthy females by monthly PRP injections, After the first session, our patients reported a noticeable improvement for both PSS, and CLSS. Elnehrawy et al (6), also observed a significant correction of wrinkles, without a need for downtime. This current study shows that the improvement in PSS and in CLSS, between the first session and the last session was significant. This result does not go along with Mikhael and El-Esawy (8) and Elnehrawy et al (6), who obtained nearly the same results of improvement after the first and the last session.

The classical PRP method for platelets rich plas-

ma preparation: is a primary centrifugation for separating RBCs by constant acceleration leading to separating the whole blood into 3 layers: the upper layer containing platelets and WBCs, the intermediate one (buffy coat) containing WBCs, and the bottom layer consisting of RBCs. The upper and layer and intermediate layers are then transferred into the sterile tube. The second centrifugation step is then performed which is needed to be appropriate to form soft pellets (erythrocyte-platelet) at the bottom of the tube.

Removal of the upper portion including platelet-poor plasma is done, and the lower third will be the PRP suspending in the smallest final volume. The buffy coat technique is done as follows: centrifugation of the whole blood (high speed) followed by collecting the buffy coat containing a high levels of leucocytes, then separation of this thin buffy coat layer including WBCs and platelets, using the underlying RBC layer. This can be used as PRP and WBCs, or Centrifugation at low speed for separating WBCs or using leucocyte filtration (25).

In this study we adopted the buffy coat method. Unlike the PRP method, buffy coat method does not involve close cell contact during preparation ,this contact will cause early activation which affect platelet metabolism, platelet function and morphology of platelets (33).

Metcalfe et al, showed that the extent of this activation was remarkably higher in PRP platelets compared with buffy coat method (34). Buffy coat method is an automatic process with a high and reproducible platelet recovery and associated with increased platelet yield and quality, reduced bacterial contamination rates (35).

In the current study, we used a special sterile tube (Neogenesis PRP kit) to collect the blood, making PRP separation faster and easier. Using CaCl2 (0.1 ml per 0.9 ml of PRP) was the method of activating the PRP and releasing their components of presynthesized growth factors that take place in 7-10 minutes after activation. According to Langer C, Mahajan V (36), the release of packed GFs starts within 10 minutes of clot initiation. So, PRP must be applied within 10 minutes of activation.

The platelets can be activated endogenously by coagulation factors. Following their attachment to on the cell surfaces, activation of some intracellular processes facilitates extracellular matrix accumulation and improves cell proliferation and differentiation .Tissue regeneration is a result of cell proliferation, angiogenesis and cell migration (37).

Injecting PRP in the region of the nasolabial fold needs experience and special care. The needle tract in the nasolabial folds near the facial vein (37).

The PRP regenerative effectiveness is associated with the concentrations of secretory proteins secreted on activation, the processing technique and the completeness of activation (38). The increased number of platelets delivers a higher number of growth factors to the surgical area (39).

The current study confirmed the high correlation between the baseline (donor whole blood) and end (PRP) thrombocyte counts. This is consistent with the result of Weibrich et al (40), and may suggest the whole blood platelet count as a rough estimate of the PRP platelet count likely to be produced.

There is controversy about the therapeutic level of platelet concentration. The present work shows a reasonable correlation between platelet count of PRP and DGS (the final definitive graduated score). A higher platelet number can result in more growth factors and more favorable clinical outcomes. This does not go with the definition of therapeutic PRP level by Graziani et al (41), and Mazzucco et al (42), who stated that the platelet levels of nearly 2.5 folds more than native blood is the positive effector, and that an adverse events will be observed at higher dosages.

In the literature however, it has been reported that PRP is needed to achieve 3 to 5-fold increase in platelet level over baseline to have a good effect (38,43).

According to the protocol of this study, we have extracted a PRP with platelet concentration of 1229.066x10 3 /mL which is 4.5 ± 0.46 times the base line. This result goes with the Marx's requirement for obtaining PRP platelet concentration of 1000x103 /mL to be considered as therapeutic (44). A higher platelet number results in more growth factors and more favorable clinical results (45).

Platelets- Rich Plasma injection for peri oral skin is an easy technique, and the procedure has virtually no permanent side-effects .In the present study, mild and transient side effects, like tolerable headache, mild burning sensation, swelling, redness were the observations that resolved without treatment. Many studies had reported the same side effects (8,28).

After PRP, bruising and discoloration are developed because of capillary disruption as well as thrombosis during injection and can remain for few days. Induration at injection site can be explained by fat necrosis and capillary thrombosis. Such consequences can occur following using fillers, as well (37).

Conclusion

PRP is a safe, natural, cost-effective substitute to other lip and perioral rejuvenation methods. It is a form of biostimulation that has an early effect

with natural looking results. High patient's satisfaction was reported and results became more significant after the third session.

Clinical Significance

The study importance lies in that it can be considered as cost –effectiveness study using a readily available, safe material to rejuvenate the perioral area, in addition, calibrating the results of the therapy using patient satisfaction scores and definitive graduated score was reliable.

Conflict of interest

The authors declare no conflicts of interest.

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