Original Contribution

Journal of Cosmetic Dermatology, **0**, 1–10

Combined autologous platelet-rich plasma with microneedling verses microneedling with distilled water in the treatment of atrophic acne scars: a concurrent split-face study

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Summary

Background Acne scarring causes cosmetic discomfort, depression, low self-esteem and reduced quality of life. Microneedling is an established treatment for scars, although the efficacy of platelet-rich plasma (PRP) has not been explored much.
Objective The objective of this study was to evaluate the efficacy and safety of platelet-rich plasma (PRP) combined with microneedling for the treatment of atrophic acne scars.
Methods Fifty patients of 17–32 years of age with atrophic acne scars were enrolled. Microneedling was performed on both halves of the face. Intradermal injections as well as topical application of PRP was given on right half of the face, while the left half of the face was treated with intradermal administration of distilled water. Three treatment sessions were given at an interval of 1 month consecutively. Goodman's Quantitative scale and Quantitative scale were used for the final evaluation of results.
Results Right and left halves showed 62.20% and 45.84% improvement, respectively, on Goodman's Quantitative scale. Goodman's Qualitative scale showed excellent response in 20 (40%) patients and good response in 30 (60%) patients over right half of the face, while the left half of the face showed excellent response in 5 (10%) patients, good response in 42 (6%) patients and poor response in three patients.
Conclusion We conclude that PRP has efficacy in the management of atrophic acne scars. It can be combined with microneedling to enhance the final clinical outcomes in comparison with microneedling alone.

Keywords: microneedling, calcium chloride, atrophic acne scars, roller, wound healing, platelet-rich plasma, collagen

Introduction

Atrophic acne scarring is a consequence of abnormal resolution or wound healing following acne inflammation. They are broadly classified as macular, atrophic and hypertrophic or keloidal scars. Atrophic acne scars are further divided into three types: icepick, boxcar, and rolling scars.[1] The lesions can be assessed using Goodman's Qualitative scale and Quantitative scale[2,3] (Tables 1 and 2). The Qualitative scale is based on the type of lesion, visibility of lesion, and depth of the scar and categorized into four grades of severity (1–4). The Quantitative scale is a further modification of the above based on the individual lesion count and scores them between 0 and a maximum of 84.

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Accepted for publication December 13, 2015

Table 1 Goodman's qualitative scarring grading system. Adaptive from (2)

Grades of post acne scarring	Level of disease	Clinical features
1	Macular	These scars can be erythematous, hyper- or hypopigmented flat marks. They do not represent a problem of contour like other scar grades but of color
2	Mild	Mild atrophy or hypertrophy of scars that may not be obvious at social distances of 50 cm or greater and may be covered adequately by makeup or the normal shadow of shaved beard hair in men or normal body hair if extrafacial
3	Moderate	Moderate atrophic or hypertrophic scarring that is obvious at social distances of 50 cm or greater and is not covered easily by makeup or the normal shadow of shaved beard hair in men or body hair if extrafacial, but is still able to be flattened by manual stretching of the skin (if atrophic)
4	Severe	Severe atrophic or hypertrophic scarring that is evident at social distances greater than 50 cm and is not covered easily by makeup or the normal shadow of shaved beard hair in men or body hair if extrafacial and is not able to be flattened by manual stretching of the skin

Table 2 Goodman's quantitative global acne scarring grading system. Adaptive from (3)

Grade or type	Number of lesions 1 (1–10)	Number of lesions 2 (11–20)	Number of lesions 3 (>20)
(A) Milder scarring (1 point each): Macular erythematous pigmented Mildly atrophic dish-like	1 point	2 points	3 points
(B) Moderate scarring (2 points each): Moderately atrophic, dish-like Punched out with shallow bases small cars (<5 mm) Shallow but broad atrophic areas	2 points	4 points	6 points
(C) Severe scarring (3 points each): Punched out with deep but normal bases, small scars (<5 mm) Punched out with deep but abnormal bases, small scars (<5 mm) Linear or troughed dermal scarring Deep, broad atrophic areas	3 points	6 points	9 points
(D) Hyperplastic: Papular scars Keloidal/hypertrophic scars	2 points Area < 5 mm 6 points	4 points Area 5–20 cm^2 12 points	6 points Area > 20 cm^2 18 points

Microneedling has become an important treatment modality for acne scars. The principle of using microneedling is to initiate collagen synthesis. This is achieved by causing minute injury to the dermis with the use of microneedles. The needles are so fine and thin that tissue damage is almost negligible.[4–6] The "nerve stimulus," transmitted by electrical signals, triggers the cascade of the healing process, releasing growth signals to undifferentiated cells and phase 1 inflammation starts immediately after the injury. Fibroblasts migrate to the point of intrusion for wound closure and stimulate endothelial cells resulting in neoangiogenesis. This natural tissue remodeling continues for 8 weeks to 1 year.[5]

Autologous platelet-rich plasma (PRP) contains high concentrations of platelet growth factors. The optimal PRP platelet concentration should be more than 10 lakhs platelets/μL having 300–700% enrichment.[7] Higher amount of platelet-derived growth factors makes it suitable to use in several dermatological indications such as treatment of androgenic alopecia,[8] graft survival in hair transplantation,[8] acne scar,[9] skin rejuvenation[10,11] and other cosmetic procedures. Autologous PRP eliminates the chances of cross transmission of HBsAg, HIV or any other blood-borne infections. Platelets on activation release several growth factors and cytokines, like platelet-derived growth factors (PDGF), transforming growth factors (TGF), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), epidermal growth factor (EGF), and interleukin (IL)-1. PDGF improves dermal regeneration and acts locally to promote protein and collagen synthesis, causes endothelial migration or angiogenesis, and induces the expression of TGF-beta.[12,13] TGF-

beta activates fibroblasts causing it to undergo cell division and produce collagen. This collagen deposition is responsible for reducing the scars.

Calcium chloride is used for the activation of platelets. To activate PRP, first 10% calcium chloride should be mixed with small amount of PRP in a ratio of 1:5. A clot will be formed in a few minutes with a supernatant fluid. This supernatant fluid, autologous thrombin, is then added to the remaining PRP in a ratio of 1:2 for platelet gel preparation.[14] Collagen is a natural platelet activator and does not require thrombin or calcium chloride for platelet activation.[15]

A number of treatments are available to reduce the appearance of scars: chemical peels, dermabrasion/microdermabrasion, laser treatment, punch techniques, dermal grafting, needling and combined therapies for atrophic scars. All of them are effective to a variable extent. PRP has recently got worldwide attention in acne scar treatment due to its ability in modifying wound healing and collagen synthesis. This study was done to assess the efficacy of PRP for the management of atrophic acne scars.

Material and method

The study was conducted in the outpatient department of skin and V.D, from October 2013 to February 2015. It was a prospective time-bound study and included all patients suffering from atrophic acne scars who fulfilled the inclusion criteria. The study conducted was approved by the scientific and medical ethics committee of the hospital.

Inclusion criteria

- Patients with grade 2 to grade 4 acne scars, classified on the basis of Goodman's Qualitative classification.
- Patient with equal Goodman's Quantitative and Qualitative scores on both halves of the face.
- No active acne lesions.
- Patients with atrophic scars only.

Exclusion criteria

- Positive history of keloidal tendency.
- Positive history of bleeding or platelet disorder.
- Positive history of major surgery in past 6 months.
- Presence of any acute infection on face like, herpes, folliculitis.
- Patients of HIV, HBsAg, or any chronic illness.
- Pregnancy.

Patient's information sheet was given to each patient. Informed consent was taken and case record form was filled for each patient. All patients were asked for history of bleeding disorder, anticoagulant drugs use, collagen injections, and injectable fillers in the previous 6 months, and personal and familial history of hypertrophic and keloidal scars. Baseline investigations were performed on each patient. All patients were screened for human immunodeficiency virus (HIV), hepatitis B (HBsAg), total leukocyte count (TLC), haemoglobin (Hb), platelet count (PC), bleeding time (BT), clotting time (CT), prothrombin time (PT) and activated partial thromboplastin time (APTT).

Photographic evaluation

Prior to each session, digital photographs were captured at a distance of 50 and 10 cm of both halves of the face. Later, these photographs were assessed by an independent dermatologist for the final evaluation of acne scars.

Procedure

During each session, topical anesthesia (eutectic mixture of lidocaine and prilocaine) was applied over the area of interest on face and removed after 2 h. Any adverse effects associated with anesthetic creams were noted.

Platelet-rich plasma was prepared by double-spin method for each session. Seventeen milliliters of blood was withdrawn in a 20-mL syringe prefilled with 3 mL of acid-citrate-dextrose anticoagulant. First centrifugation was performed at 293.88 *g* for 5 min (soft spin). Both buffy coat and plasma layer were taken for further centrifugation and red cell sediments were discarded. Second centrifugation was performed at 690.94 *g* for 17 min (hard spin) resulting in the formation of platelet-poor plasma above and platelet-rich zone at the bottom. Platelet-poor plasma (PPP) was removed and discarded leaving behind a solution of 2 mL PRP. Platelet counts were assessed using an automated analyzer machine, at base line and then PRP solution for each session.

Microneedling was performed on all patients using a standard roller device (CE approved) with 192 needles, each of 1.5 mm in size. Patients were placed in supine position with head stable, and face was divided into four quadrants. Rolling was performed six times in four different directions which were perpendicular and diagonal to each other with to-and-fro motion. Pinpoint bleeding was taken as endpoint of microneedling

which was performed in similar manner on both halves of face. Uniform and firm pressure was applied to the roller, and the performing physician remained the same throughout the study. A new roller device was used for each session.

Of the 2 mL of PRP, 1 mL was first mixed with 0.1 mL of 10% calcium chloride and then injected intradermally, 0.1 mL/cm^2 in the right half of face targeting acne scars. The remaining 1 mL of PRP was again mixed with 0.1 mL of 10% calcium chloride and allowed to form a platelet gel. The supernatant fluid and gel were applied over the same half of face. On the left half, distilled water was injected intradermally (0.1 mL/cm^2).

The face was cleaned with distilled water after 1 h and any bruise or other adverse effects were noted if any. Patients were followed up on the 3rd and 7th days, and any signs of inflammation or adverse effects were noted. A total of three sessions of microneedling were performed at monthly intervals. Each patient was followed up for a period of 3 months after the final session regarding persistence of any adverse effect.

All patients were instructed to assess themselves on a visual analogue scale of 0 (no response) to 10 (maximum) during each session. A questionnaire including reduction in visibility, and roughness of scars was given to each patient for assessment. If required, self scores from previous sessions were also shown to each patient and thus allowing assessment of the degree of improvement to make further changes in the scores. Patients were also assessed by an independent dermatologist for clinical improvement and scored on a scale of 0 (no improvement) to 10 (maximum) during each monthly visit. Goodman's Quantitative and Qualitative scoring was performed before the first session and a month after the final session.

Pre- and post-treatment Goodman's Qualitative and Quantitative scores, independent dermatologist score, and patient satisfaction scores were timely updated on an excel sheet for each patient. SPSS 16 software was used for statistical analysis.

Final evaluation

- Pre- and post-treatment Goodman's Qualitative grades (zero and 3rd month) were evaluated for both the halves. Reduction in two grades was taken as an "excellent response", reduction in one grade as a "good response" and zero or no reduction as a "poor response".
- Mean scores of pre- and post-treatment Goodman's Quantitative scores (zero and 3rd month) were calculated for right and left halves and mean percentage of improvement was calculated for both the halves independently. "Paired *t*-test" was applied among pre- and post-treatment scores of right and left halves separately. "Unpaired *t*-test" was applied among final scores of both right and left halves.
- Final independent dermatologist scores were calculated for right and left halves of each patient. Scoring: 0 was taken as no response, 1–3 poor response, 4–5 fair response, 6–7 good response, and 8–10 excellent response. "Unpaired *t*-test" was applied among final scores of both the halves.
- Final patient satisfaction scores were calculated for right and left halves of each patient. Scoring: 0 was taken as no response, 1–3 poor response, 4–5 fair response, 6–7 good response, and 8–10 excellent response. "Unpaired *t*-test" was applied among the final scores of both the halves.

Results

All the fifty patients completed the treatment. There were no dropout and each patient attended regular follow-up visits. The mean age of patients was 25.72 years. The youngest patient was a 17-year-old male and the eldest was a 32-year-old male. In this study, there were 25 male and 25 female patients. All patients were of III to V Fitzpatrick skin type (Table 3).

All patients had tolerated the procedure well, yet a few adverse effects were noted; two patients showed acne flare-up, four patients showed postinflammatory hyperpigmentation (PIH), one patient showed milia, one patient showed persistent erythema, and two patient developed bruising. Topical 4% hydroquinone was given to the patient suffering from PIH. Tacrolimus 0.03% was given for persistent erythema. Milia were removed with electrofulguration. Topical antibiotics

Table 3 Distribution of enrolled patients according to sex, age and skin type

Characteristics of patients		Number (n = 50)	Percentage (%)
Sex	Male	25	50
	Female	25	50
Fitzpatrick's skin type	V	19	38
	VI	25	50
	III	6	12
Age group (in years)	<18	1	2
	18–24	17	34
	25–30	28	56
	>30	4	8

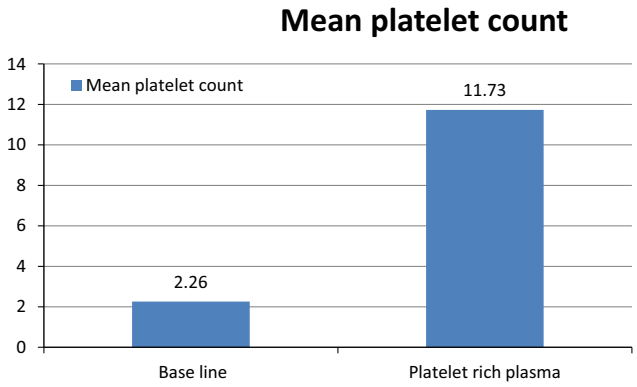

Figure 1 Mean platelet count of all sessions at base line and PRP.

were given for flare-up of acne, and bruising. All the patients were followed till 3 months post-treatment and no residual side effects observed. Erythema with little swelling was noted among two patients following application of topical anesthetic cream which subsided spontaneously. Postprocedure erythema and peeling of skin was resolved spontaneously by the 4th–5th day on the left half and a little earlier on the right half of face. All the patients were allowed to resume their daily activities following the procedure.

Mean platelet count for baseline and PRP was 2.26 and 11.73 lakhs/μL, respectively, and showing nearly five fold increment that is 519% enrichment (Fig. 1).

On evaluation of Goodman's Qualitative scores, the right half of the face showed reduction of two grades in 20 (40%) patients and reduction of one grade in 30 (60%) patients. The left half of the face showed reduction of two grades in 5 (10%) patients, reduction of one grade in 42 (84%) patients and no reduction of grade in 3 (6%) patients (Fig. 2).

Goodman's Quantitative scores of right half and left half of the face had equal mean scores of 19.24 before treatment. After treatment, mean score of right half of the face was 7.08 with 62.20% improvement in acne scars. Left half of the patients had a mean score of 10.42 with 45.84% improvement of acne scars following treatment (Fig. 3). "Paired *t*-test" showed significant improvement on both right (t −13.783933, P value < 0.00001) and left haves (t −12.195866, P value < 0.00001). "Unpaired *t*-test" showed significant difference in both the halves (t 4.729529, P value < 0.00001).

On evaluation of independent dermatologist scores, right half of the face showed excellent response in eight patients, good response in 31 patients, fair response in 10 patients and poor response in one patient. Left half of the face showed excellent response

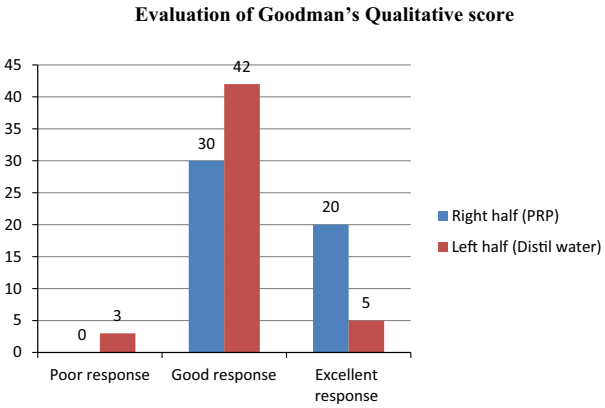

Figure 2 Final response on Goodman Qualitative scale for right and left halves of the face.

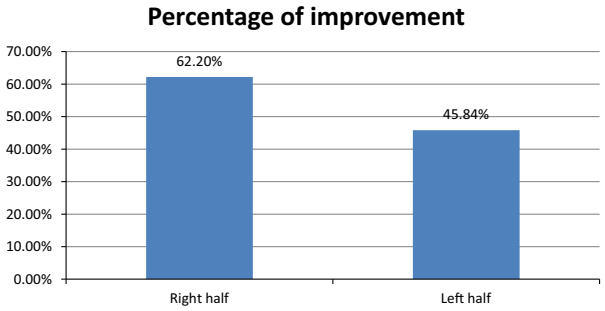

Figure 3 Overall percentage of improvement in acne scars among both halves on evaluation of pre- and post-treatment mean quantitative scores.

in four patients, good response in 11 patients, fair response in 32 patients and poor response in three patients (Fig. 4). "Unpaired *t*-test" showed significant difference in both halves (t 5.915051, P < 0.00001). Right half of the face showed 22%, 39%, 64%

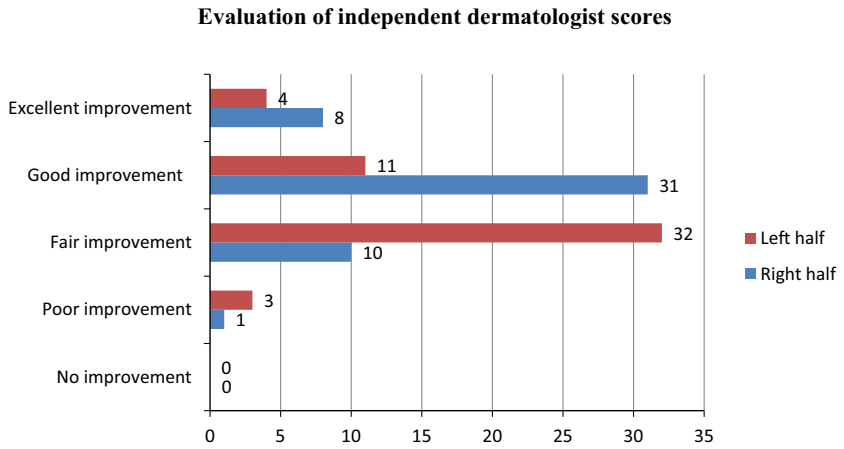

Figure 4 Clinical response on final independent dermatologist scores.

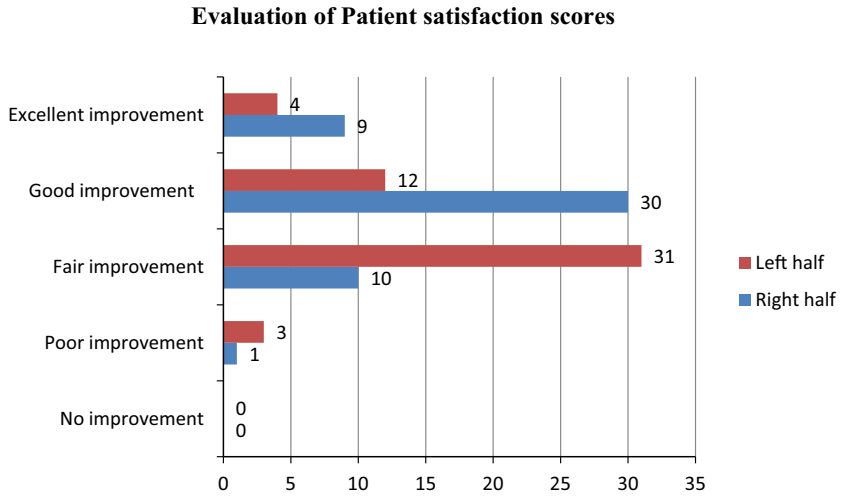

Figure 5 Clinical response on final patient satisfaction scores.

improvement and left half of the face showed 16.6%, 27.4%, 47.6% improvement for 1st, 2nd, and 3rd session respectively.

On evaluation of patient satisfaction scores, right half of the face showed excellent response in nine patients, good response in 30 patients, fair response in 10 patients and poor response in one patient. Left half of the face showed excellent response in four patients, good response in 12 patients, fair response in 31 patients and poor response in three patients (Fig. 5). "Unpaired *t*-test" showed a significant difference in both halves (t 5.837697, $P < 0.00001$). Right half of the face showed 22%, 39%, and 64% improvement and left half of the face showed 16.6%, 27.4%, and 47.6% improvement for 1st, 2nd, and 3rd session respectively (Figs 6–8).

Discussion

This study has shown that PRP can significantly affect the final treatment outcome in the management of atrophic acne scars. A standard centrifugation machine was used during each session. Method employed for spin was based on another study conducted by Amable *et al.*[16], to get maximum platelet count. Method of collection of blood does not significantly affect final platelet count; hence, we took venous blood for each preparation.[17]

Subjective scoring was done by one independent dermatologist to minimize individual bias. Almost all patients stated that the site treated with PRP showed more reduction in visibility of scars in comparison with the site treated with distilled water on the completion

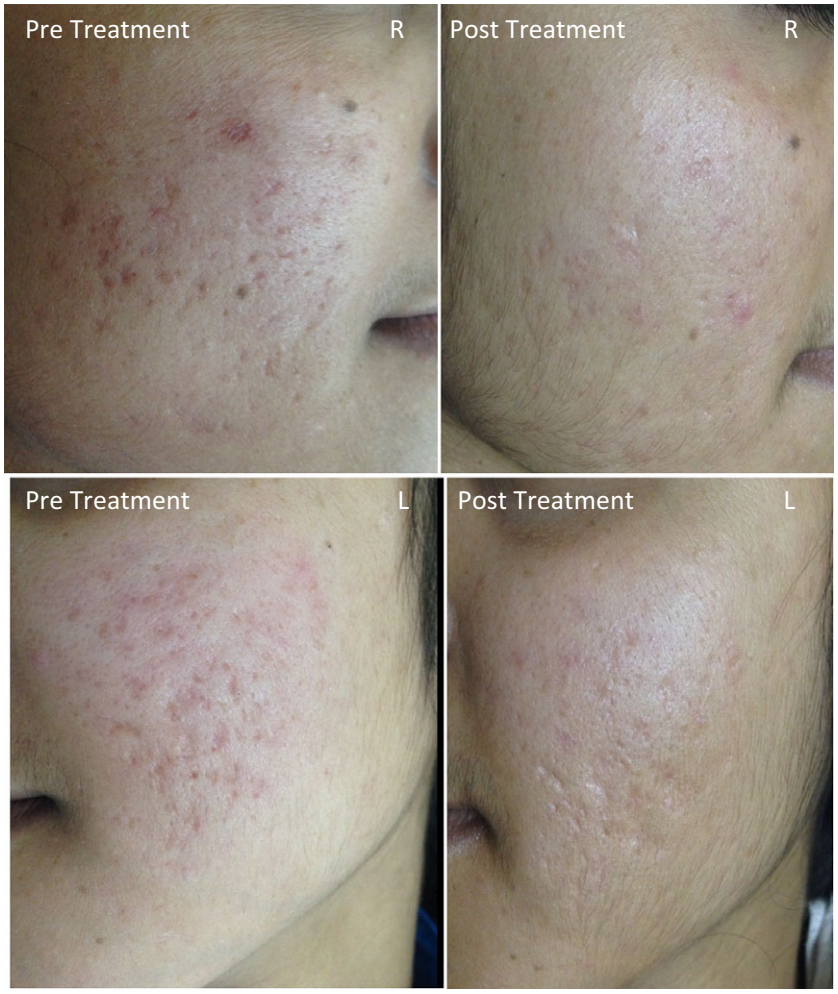

Figure 6 Acne scar of a 23-year-old female with pretreatment and post-treatment right and left half of face showing significant improvement (margin of each scar can still be appreciated in left half).

of the study. They also emphasized that roughness of skin was higher on left half (distilled water) of the face than the right half (PRP) after treatment. We did split-face trial to eliminate individual variations. All patients were treated with microneedling with PRP on both halves of the face for 3 months after the completion of study.

In this study, we did not evaluate the exact duration of erythema, edema, and crusting following the procedure, yet experienced clinically that the site treated with PRP showed early resolution in comparison with the site treated with distilled water in many sessions. A split-face trial conducted *by* Lee *et al.*[18] experienced the same observations.

Goodman Qualitative scores with PRP and microneedling showed excellent response in 40% patients and good response in 60% patients. On the other hand, the site treated with distilled water and microneedling showed excellent response in 10% patients, good response in 84% patients and no improvement in 6% patients. Chawla[19] had conducted a split-face trial comparing microneedling with PRP and microneedling with vitamin C and concluded that vitamin C did not prove to be as efficacious as PRP in acne scar management.

Goodman Quantitative scores for both halves of the face had a mean score of 19.24 before treatment. After treatment, site treated with PRP and microneedling, the mean score was 7.08 and showed 62.20% improvement in acne scars (P value < 0.00001). The site treated with microneedling and distilled water had a mean score of 10.42 and showed 45.84% improvement of acne scars following treatment (P value < 0.00001). Gawdat *et al.*[20] had conducted a

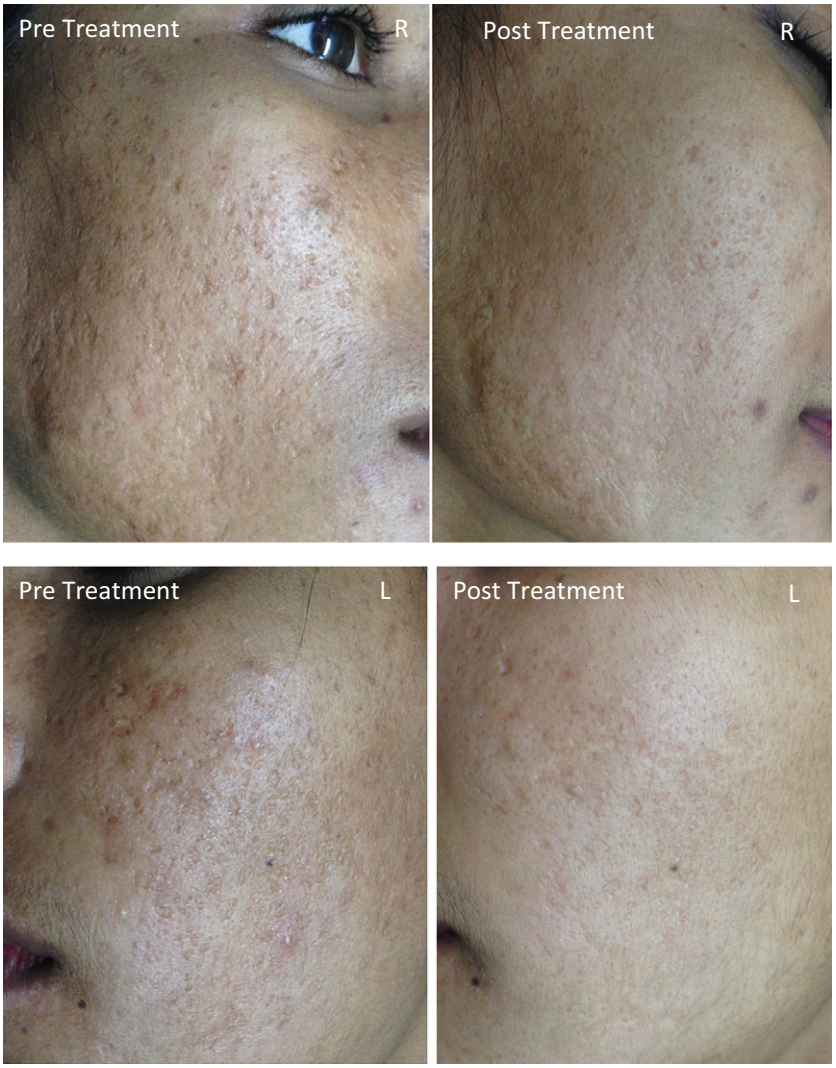

Figure 7 Acne scar of a 25-year-old female with pretreatment and post-treatment right and left half of face showing significant improvement (margin of each scar can still be appreciated in left half).

study using PRP and FCL (Fractional CO_2 Laser) and experienced that combined PRP- and FCL-treated areas had a significantly better response ($P = 0.03$), fewer side effects and shorter downtime ($P = 0.02$) than FCL-treated areas. Zhu *et al.*[21] evaluated the efficacy of platelet-rich plasma and observed that overall degree of clinical improvement was significantly better on the PRP-treated side (2.7 ± 0.7) than on the control side (2.3 ± 0.5) ($P = 0.03$).

In our knowledge, microneedling and PRP with split-face trial has not been studied much in the past. In comparison with former studies, differences can be observed in types of treatments, variations in PRP preparation, mean platelet counts, final results, and individual response to treatment. Thus, more research is required for the standardization of PRP preparation and its uses.

We hypothesized that injury to the dermis by microneedling acts in synergy with activated platelets. Activated platelets modify the natural healing response from the beginning of inflammation to the initiation of collagen induction by releasing cytokines and growth factors. All these factors induce remodeling of acne scars.

We propose that platelet-rich plasma is efficacious in the management of atrophic acne scars. It can be combined with microneedling to enhance the final clinical outcomes in comparison with microneedling alone.

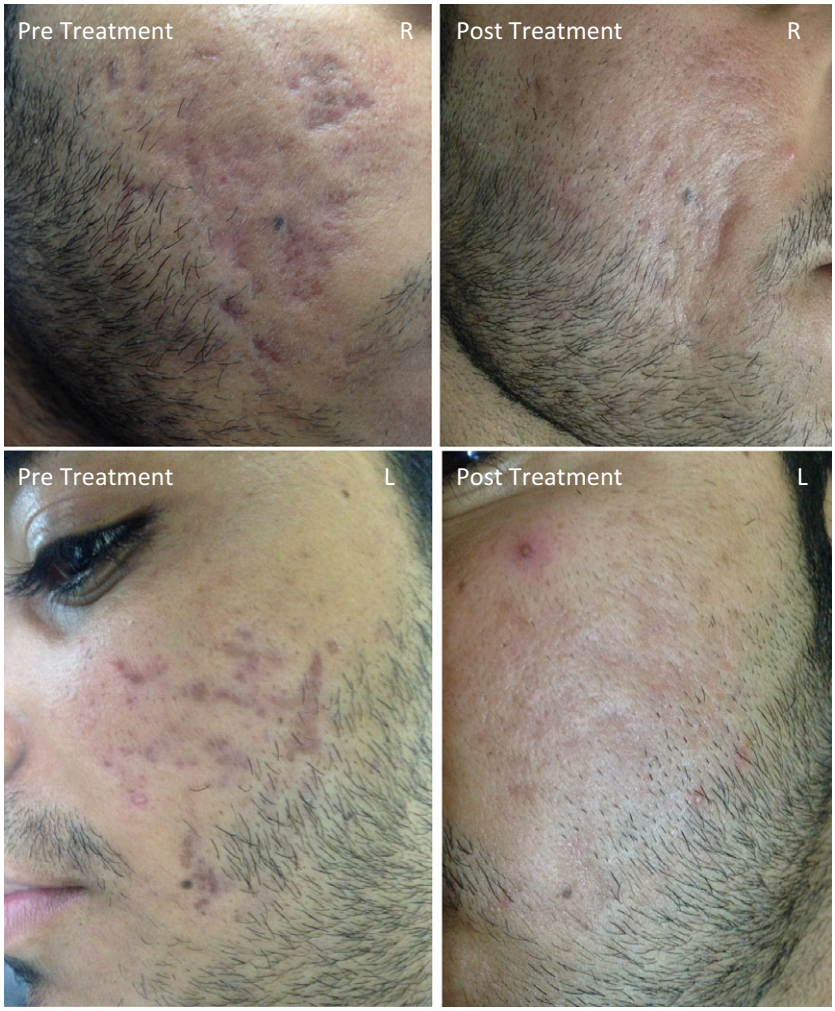

Figure 8 Acne scar of a 25-years-old male with pretreatment and post-treatment right and left half of face showing significant improvement (margin of each scar can still be appreciated in left half).

Acknowledgment

We express deep gratitude to Dr. V.K. Khurana, (M.D, skin and V.D) for encouraging us to take this study. I am very much thankful to Dr. Swati, and Dr. Amarjeet singh verma for their patronage and timely guidance.

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