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ORIGINAL CONTRIBUTION

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Therapeutic effect of microneedling and autologous platelet-rich plasma in the treatment of atrophic scars: A randomized study

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Summary

Background: New treatments and techniques were being added over the last few years to treat atrophic scars with variable results and adverse effects.

Aim of the work: The aim of this study was to evaluate and compare the therapeutic efficacy and safety of microneedling, autologous platelet-rich plasma, and combination of both procedures in the treatment of atrophic scars.

Patients and methods: This study included 90 patients with atrophic scars and were classified randomly into three groups: I: 28 patients treated with microneedling, one session every 4 weeks; II: 34 patients treated with intradermal injection of platelet-rich plasma, one session every 2 weeks; and III: 28 patients treated with alternative sessions of each microneedling and platelet-rich plasma, 2 weeks between each session, for a maximum of six sessions.

Results: There was a statistically significant improvement in the appearance of atrophic scars, with reduction in the scores associated with the clinical evaluation scale for atrophic scarring in all groups, but the improvement was more obvious in group III.

Conclusions: Although a single treatment may give good results, combination between skin needling and platelet-rich plasma is more effective, safe with less number of sessions in all types of atrophic scars.

KEYWORDS
atrophic scars, dermapen, platelet-rich plasma

1 | INTRODUCTION

Atrophic scars are dermal depressions commonly caused by the destruction of collagen after inflammatory acne, chickenpox, other diseases [especially *Staphylococcus* infection], surgery, or accidents.[1] Many treatments have been proposed for cosmetic improvement of atrophic scars such as chemical peeling,[2] subcision,[3] dermabrasion,[4] and fillers.[5] Although significant clinical improvements can be seen with ablative lasers, adverse effects such as prolonged postprocedure erythema and dyspigmentation impede their widespread use especially in patients with darker skin[6] On the other hand, nonablative lasers such as diode, neodymium-doped yttrium aluminum garnet and pulsed dye laser have better safety profiles but lower efficacies.[7] At present, the new generation of a device with bipolar fractional radiofrequency technology is effective in treating acne scars through the process of dermal coagulation and minimal epidermal ablation.[8]

New treatments and techniques were being added over the last few years to overcome these limitations. One of such techniques is microneedling therapy or collagen induction therapy. There are some pathological as well as clinical studies are available in the world literature that have documented a favorable clinical and histopathological response in the skin after microneedling. However, there is a definite paucity of objective clinical trials on the efficacy of microneedling treatment in facial and other types of scars.[9]

Autologous platelet-rich plasma is plasma with a higher concentration of platelets than normally found, it can enhance wound healing,

which has been demonstrated in controlled studies for soft and hard tissues.[10,11] The application of autologous platelet-rich plasma to surgical wounds has been shown to accelerate tissue repair and reduce postoperative pain.[12] The alpha-granules of the platelets release growth factors in response to platelet activation, and stimulate cell proliferation and cell differentiation for tissue regeneration. These growth factors have an important role in the regulation and proliferation of mesenchymal cells, including fibroblasts, and have been shown to induce synthesis of collagen and matrix components; thus, improvement of atrophic scars is expected.[13] So, this study aimed to evaluate and compare the therapeutic efficacy and safety of microneedling, autologous platelet-rich plasma, and combination of both procedures in the treatment of atrophic scars.

2 | PATIENTS AND METHODS

2.1 | Patients

This study was carried out on 90 patients with different clinical variants of atrophic scars diagnosed clinically on the basis of typical appearance of skin lesions and selected from the outpatient clinic of dermatology and venereology department, Tanta university hospitals, Egypt, from June 2013 to June 2014 after obtaining the approval of the research ethics committee of the Tanta university hospitals, Egypt (code no. 220/02/14), and written informed consent from each participant. The studied patients were classified into the following groups regarding to the type of treatment.

Group I (microneedling group): IA included 18 patients with atrophic postacne scars, and IB included 10 patients with atrophic post-traumatic scars. They were treated with microneedling technique using dermapen, one session every 4 weeks.

Group II (platelet-rich plasma group): II A included 18 patients with atrophic postacne scars, and II B included 10 patients with atrophic post-traumatic scars and six with postchickenpox scars. They were treated with intradermal injection of autologous platelet-rich plasma, one session every 2 weeks.

Group III (combination group): III A included 18 patients with atrophic postacne scars, and III B included 10 patients with atrophic post-traumatic scars. They were treated with alternate sessions of each microneedling and intradermal injection of autologous platelet-rich plasma one session every 2 weeks. All patients received treatment for a maximum of six sessions or till patient satisfaction, which is first.

2.2 | Study design

Comparative, simple randomized, noncontrolled study.

2.3 | Inclusion criteria

Newly diagnosed cases, patients with atrophic scars at any site and age more than 12 years and who did not receive previous scar treatment within 6 months before the study.

2.4 | Exclusion criteria

Patients with platelet or blood clotting disorders, chronic liver or autoimmune diseases. Patients on systemic retinoids in last 6 months or topical retinoids in the last 2 weeks. Patients with chronic debilitating diseases, uncontrolled systemic infection, history of malignancy, or keloid tendency or with unrealistic expectations. Pregnant and lactating females or who were using hormonal treatment. Patient who did not complete the treatment sessions or the follow-up period.

2.5 | All the studied patients were subjected to a standard protocol which consisted of:

A written informed consent was obtained from every patient before entering the study. Complete history taking, general and dermatological examinations. Skin phototyping according to Fitzpatrick's skin type classification.[14] All patients were skin phototypes II-IV. Evaluation of the presence of active acne lesions, as well as evaluation of the presence of postinflammatory hyperpigmentation, hypertrophic scars, or keloids was performed. Traumatic scars were evaluated according to scar distribution (on forehead, cheeks, chin, or arms), texture, pigmentation, atrophy, and overall appearance.[15] Atrophic acne scars were evaluated according to the subtype (ice pick, boxcar, and rolling scars).[16] Furthermore, the color of acne scars was noted, whether skin-colored, erythematous, or hyperpigmented color, for detection of any change in the color of scar at the end of treatments. Finally, the grading of atrophic acne scars was made according to Goodman and Baron grading system.[17]

Routine investigations including complete blood picture, bleeding time, fasting blood sugar, and liver and renal function tests were performed. All studied patients were instructed to avoid the use of any other scar therapy during the whole duration of the study and during follow-up period. The patients were informed about the nature of the procedures, number of sessions, and expected side effects of the procedures. Clinical documentation by sequential photographs was done. Photographs were taken for the area of interest in each patient when they first presented (pretreatment), before each session and during follow-up period (3 months after the last session). Photographs were taken using a Sony (Cyber-shot) digital camera (DSC-W350), 14.1 mega pixels.

Three-millimetre punch biopsies were taken from lesional skin of atrophic scars from five patients in each group before treatment and at the end of follow-up period. They were kept in formalin 10% and embedded in paraffin for histopathological examination using hematoxylin and eosin and special stains using van Gieson and orcein stains to demonstrate histopathological skin changes and effect of treatment.

2.6 | Therapeutic regimen

2.6.1 | Platelet-rich plasma separation method

Ten to 20 cc of venous blood has been collected from the anticubital vein under complete aseptic conditions. The whole blood

sample was collected into tubes containing sodium citrate (10:1) as an anticoagulant (to bind calcium and prevents the initiation of the clotting cascade by preventing the conversion of prothrombin to thrombin). Then, the citrated whole blood was subjected to two centrifugation steps. The initial centrifugation ("soft" spin) at 1419 *g* for 7 minutes to separate the plasma and platelets from the red and white cells. The resulting plasma supernatant, which contains the suspended platelets (and may contain a portion of the white cell "buffy coat") was harvested to a second centrifugation step ("hard" spin) at 2522 *g* for 5 minutes, leading to separation of the plasma into two portions: platelet-poor plasma and platelet-rich plasma. Typically, the lower 1-2 cc of the plasma (10% of the initial volume of autologous blood) was yielded as platelet-rich plasma concentrate after centrifugation. Then, it was activated by adding calcium chloride at ratio of 10:1 (0.1 cm of calcium chloride to each 1 cm of platelet-rich plasma) immediately before the injection. Once it was activated, it should be injected rapidly within 10 minutes to avoid clotting of the plasma. The patients were injected in the recumbent position. It was injected using insulin syringe intradermally and subcutaneously in the scars. 0.1 cc of platelet-rich plasma was injected per point under every scar of postacne and chickenpox scars, and with a space of one cm between different points of injections in linear post-traumatic scars.[18]

2.6.2 | Microneedling method

Patient's skin was first cleaned with ethyl alcohol followed by ether to remove all oils on skin surface. Topical anesthetic cream was then applied to skin under occlusion for 30 minutes. An automated microneedling device (dermapen 3) with nine microneedles of 0.25 mm to 2.5 mm length was used. The instrument consists of a rechargeable hand-piece with disposable needles at one end, which uses a motor to drive the movement of the needles.[9]

2.6.3 | Postmicroneedling care

The normal skin care regimen (including sunscreen cream containing (zinc oxide) should continue. The patient can return to work and social life a day after treatment. A bland emollient as panthenol was prescribed to the patient if irritation of the skin occurred. Patients were also instructed to avoid skin rubbing or friction.

2.7 | Assessment of the efficacy of the therapeutic procedure

2.7.1 | Clinical assessment

Physician's opinion

Two dermatologists were asked to record percentage of improvement for each patient after completion of the treatment by comparing before and after digital photographs. Finally, the minimum rate on which the three investigators agreed was considered as investigators' view in the study. The scars were graded on a quartile scale: [15](0) no improvement; (1) 1%-25% improvement (mild); (2) 26%-50% improvement (moderate); (3) 51%-75% improvement (marked); and (4) 76%-100% improvement (very significant).

Patient satisfaction

The degree of improvement according to patient opinion was evaluated; the patients were asked at final visit to rate the overall satisfaction comparing with the pretreatment condition, patients were asked to fill up a questionnaire about their satisfaction using the following grades[19]: 1=very dissatisfied, 2=not satisfied, 3=slightly satisfied, 4=satisfied, and 5=very satisfied.

2.7.2 | Safety assessment

The patients were informed to report any complications occurred; erythema, pain, ecchymosis, infection, postinflammatory hyperpigmentation, or any allergic manifestations. All clinical medical events, whether observed by the investigator or reported by the patients, are considered as adverse events.

2.7.3 | Follow-up assessment

The patients were followed up and evaluated clinically and by colored photography monthly to detect any improvement or worsening of the scars over a period of 3 months after the last session and to detect any complications and maintenance of the response.

2.8 | Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0 (American Cast Iron Pipe Company, Birmingham, AL). Qualitative data were described using number and percent.[20] Quantitative data were described using range (minimum and maximum), mean, standard deviation, and median. Comparison between different groups regarding categorical variables was tested using chi-square test. When more than 20% of the cells have expected count less than 5, correction for chi-square was conducted using Fisher's exact test or Monte Carlo correction. The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-Wilk test, and D'Agstino test; also Histogram and QQ plot were used for vision test. If it reveals normal data distribution, parametric tests were applied. If the data were abnormally distributed, nonparametric tests were used. For normally distributed data, comparison between two independent population was made using independent *t* test while more than two population was analyzed *F* test (ANOVA) to be used. For abnormally distributed data, comparison between two independent population was made using Mann-Whitney test while Kruskal-Wallis test was used to compare between different groups and pairwise comparison was assessed using Mann-Whitney test. Correlations between two quantitative variables were assessed using Spearman coefficient. Significance of the obtained results was judged at 0.5.

3 | RESULTS

3.1 | Clinical results

The clinical data of the patients were demonstrated in Table 1.

3.1.1 | Regarding the postacne scar subtype among the patients

In group IA, six (33.3%) patients had boxcar scars, four (22.2%) patients had rolling scars, and eight (44.5%) patients had ice pick scars. In group IIA, no (0%) patients had boxcar scars, four (22.2%) patients had rolling scars, eight (44.5%) patients had ice pick scars, and six (33.3%) patients had mixed boxcar and ice pick scars. In group IIIA, two (11.1%) patient had boxcar scars, eight (44.5%) patients had rolling scars, four (22.2%) patients had ice pick scars, and four (22.3%) patients had mixed boxcar and ice pick scars (Table 2).

3.1.2 | Regarding the postacne scar grade among the patients before treatment

In group IA, four (22.2%) patients were grade 3 acne scars and 14 (77.8%) patients were grade 4. In group IIA, 12 (66.7%) patients were grade 3 and six (33.3%) patients were grade 4. In group IIIA, four (22.2%) patients were grade 2, six (33.3%) patients were grade 3, and eight (44.5%) patients were grade 4 (Table 2).

3.2 | Evaluation of the clinical response

3.2.1 | Regarding to the degree of improvement

In group I, four (14.3%) patients showed mild improvement, 20 (71.4%) patients showed moderate improvement, and four (14.3%) patients showed marked improvement. The mean improvement score was (39.71±13.06; Figure 1A, B; Table 3). In group II, eight (23.5%) patients showed mild improvement, 14 (41.1%) patients showed moderate improvement, six (17.7%) patients showed marked improvement, and six (17.7%) patients showed very significant improvement. The mean improvement score was (48.82±23.74; Figure 2A, B). In group III, two (7.1%) patients showed moderate improvement, 18 (64.3%) patients showed marked improvement, and 8 (28.6%) patients showed very significant improvement. The mean improvement score was (70.43±13.32; Figure 3A, B). There was statistically highly significant difference between the studied groups with higher response in group III followed by group II and lastly group I (*P* value <.001*; Table 3).

TABLE 1 Comparison between the studied groups as regard different clinical parameters

	Total (No.=90)		I (No.=28)		II (No.=34)		III (No.=28)			
	No.	%	No.	%	No.	%	No.	%	Test of significance	*P*
Gender										
Male	44	48.9	16	57.1	12	35.3	16	57.1	χ^2=2.021	.364
Female	46	51.1	12	42.9	22	64.7	12	42.9		
Age/year										
≤25	34	37.8	4	14.3	20	58.8	10	35.7	χ^2=6.516*	.038*
>25	56	62.2	24	85.7	14	41.2	18	64.3		
Min.-Max.	16.0-40.0		24.0-31.0		16.0-40.0		19.0-39.0		*F*=1.873	.166
Mean±SD	26.33±6.08		26.93±2.06		24.24±7.77		28.29±5.80			
Median	26.0		26.0		22.0		26.0			
Scar type										
Postacne scar	54	64.3	18	64.3	18	52.9	18	64.3	χ^2=3.769	^{MC}P=.427
Postchickenpox	6	6.7	0	0.0	6	17.7	0	0.0		
Post-traumatic	30	33.3	10	35.7	10	29.4	10	35.7		
Scar duration/year										
≤7	34	37.8	10	35.7	14	41.2	10	35.7	χ^2=0.134	.935
>7	56	62.2	18	64.3	20	58.8	18	64.3		
Min.-Max.	2.0-25.0		3.0-25.0		3.0-20.0		2.0-18.0		$^{KW}\chi^2$=0.217	.897
Mean±SD	10.64±5.29		11.29±5.51		10.12±5.12		10.64±5.60			
Median	11.0		12.0		10.0		11.0			
Skin type										
II	10	11.1	2	7.1	0	0.0	8	28.6	χ^2=6.874	^{MC}P=.118
III	44	48.9	12	42.9	18	52.9	14	50.0		
IV	36	40.0	14	50.0	16	47.1	6	21.4		

Chi-square test (χ^2).
MC, Monte Carlo test; *F*, *F* test (ANOVA).
Chi-square test for Kruskal-Wallis test ($^{KW}\chi^2$)
*Statistically significant at $P \leq 0.05$

TABLE 2 Comparison between the studied groups according to postacne scar subtype and grades before treatment

	Total (No.=54)		IA (No.=18)		IIA (No.=18)		IIIA (No.=18)			
	No.	%	No.	%	No.	%	No.	%	χ^2	*P*
Postacne scar subtype										
Boxcar	8	14.8	6	33.3	0	0.0	2	11.1	7.432	^{MC}P=.260
Rolling	16	29.6	4	22.2	4	22.2	8	44.5		
Ice pick	20	37.0	8	44.5	8	44.5	4	22.2		
Boxcar and ice pick	10	18.5	0	0.0	6	33.3	4	22.2		
Grade of acne scar before treatment										
1	0	0.0	0	0.0	0	0.0	0	.0	6.407	.114
2	4	7.4	0	0.0	0	0.0	4	22.2		
3	22	40.7	4	22.2	12	66.7	6	33.3		
4	28	51.9	14	77.8	6	33.3	8	44.5		
Mean±SD	3.44±0.64		3.33±0.50		3.22±0.83		3.78±0.44			

Chi-square test (χ^2); MC, Monte Carlo test.

3.2.2 | Regarding to the grade of postacne scars before and after treatment

In group IA, before treatment there were four (22.2%) cases grade 3 and 14 (77.8%) cases grade 4. After treatment, four (22.2%) cases became grade 2 and 14 (77.8%) became grade 3 (*P* value .003*). In group IIA, before treatment 12 (66.7%) cases were grade 3 and 18 (33.3%) cases were grade 4. After treatment, 10 (55.6%) cases became grade 2 and eight (44.4%) became grade 3 (*P* value .005*). In group IIIA, before treatment four (22.2%) cases were grade 2, six (33.3%) cases were grade 3, and eight (44.5%) cases were grade 4. After treatment, 10 (55.6%) cases became grade 1, eight cases (44.4%) became grade 2 (*P* value .008*). These results were significant meaning that there was upgrading by one grade in acne scars after microneedling and platelet-rich plasma and by two grades in the combination of microneedling and platelet-rich plasma (Table 4).

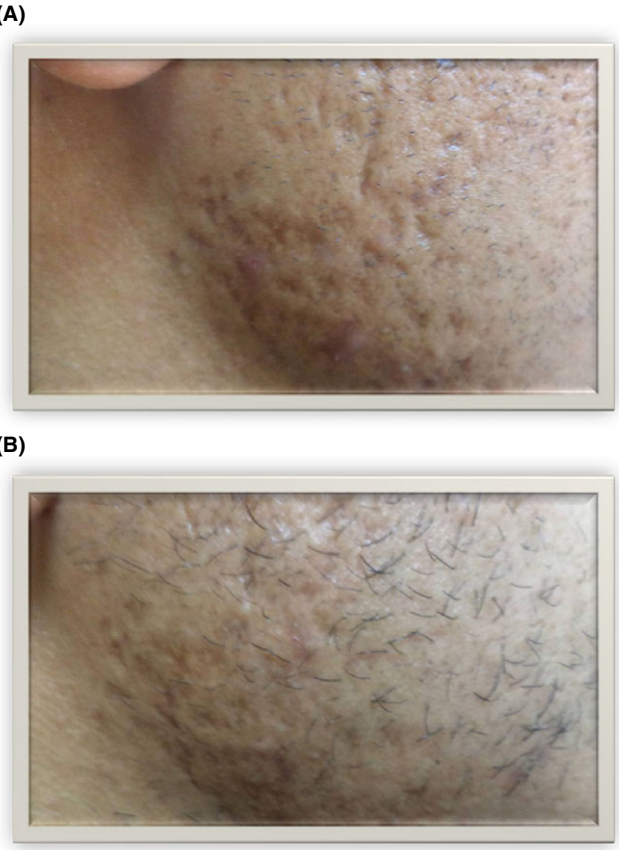

FIGURE 1 (A) Male patient with grade 4 rolling acne scars before microneedling. (B) The same patient after six sessions of microneedling became grade 2(marked improvement)

3.2.3 | Regarding to the side effects

Erythema was associated with pinpoint bleeding and lasted for a maximum of 24 hours. There was highly significant difference between the groups with more severe pain in group I followed by group III and least in group II, also there was highly significant difference between the groups regarding erythema which was more severe in group III followed by group I (*P* value<.001*; Table 5).

3.3 | Assessment of follow-up

Maintenance of the response was detected in all the studied groups.

3.3.1 | Regarding the patient satisfaction

There was statistically significant difference as regards the satisfaction of the patients between the studied groups, patients in group III were more satisfied than group II and I (*P* value .002*) (Table 6).

3.3.2 | Histopathological evaluation

By hematoxylin and eosin stain, pretreatment atrophic scars showed epidermal atrophy with flattening of rete ridges (Figure 4A). Post-treatment demonstrated relatively thickened epidermis with more

TABLE 3 Comparison between the studied groups according to the degree of improvement

	Total (No.=90)		I (No =28)		II (No.=34)		III (No.=28)			
	No.	%	No.	%	No.	%	No.	%	χ^2	^{MC}P
Degree of improvement										
Mild	12	13.3	4	14.3	8	23.5	0	0.0	20.583*	.001*
Moderate	36	40.0	20	71.4	14	41.1	2	7.1		
Marked	28	31.1	4	14.3	6	17.7	18	64.3		
Very significant	14	15.6	0	0.0	6	17.7	8	28.6		
Mean±SD	52.71±21.61		39.71±13.06		48.82±23.74		70.43±13.32			

Value for Chi-square (χ^2).
MC, Monte Carlo test.
*Statistically highly significant at $P \leq .001$.

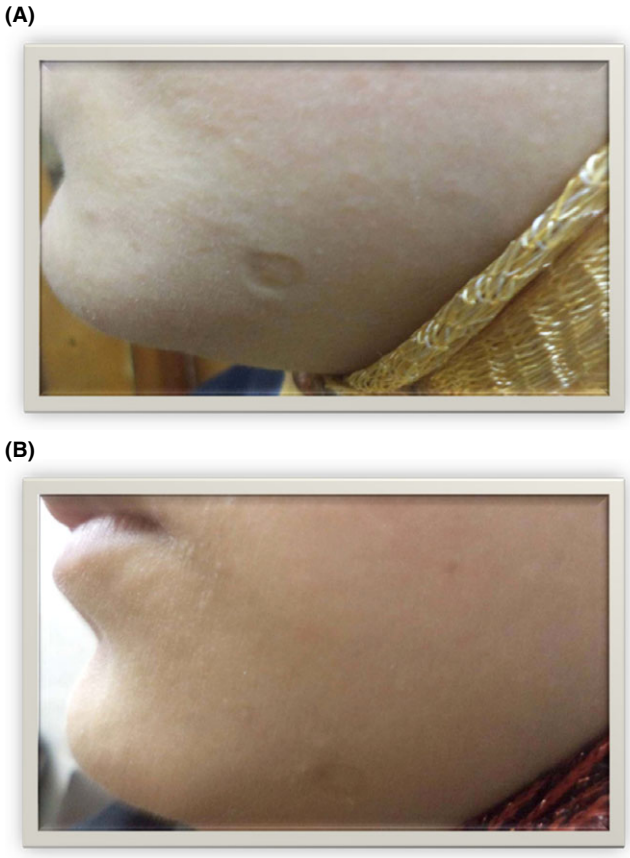

FIGURE 2 (A) Female patient with postchickenpox scar before platelet-rich plasma. (B) The same patient after six sessions of platelet-rich plasma showing very significant improvement

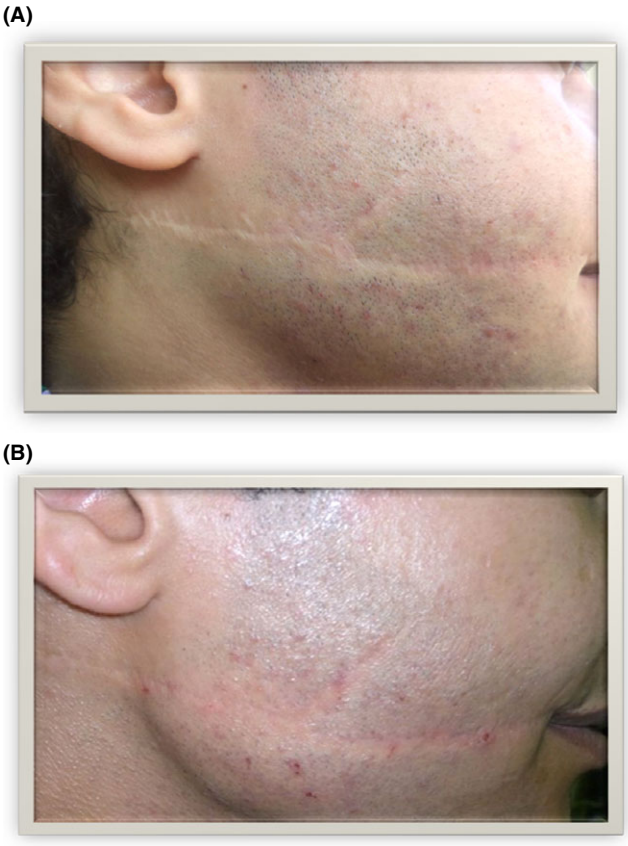

FIGURE 3 (A) Male patient with post-traumatic scar before combined microneedling and platelet-rich plasma treatment. (B)The same patient after six sessions of microneedling and platelet-rich plasma showing very significant improvement

developed rete ridges (Figure 4B). By orcein stain, in the pretreatment biopsies atrophic scars showed few elastic fibers (Figure 5A). After treatment, there were increases in the amount of elastic fibers (Figure 5B). By van Gieson stain, the pretreatment atrophic scars showed compact collagen bundles parallel to the surface (Figure 6A). Post-treatment sections showed increased collagen fiber bundles which appeared to have been laid down in a normal lattice pattern, rather than in parallel bundles as seen in scar tissue (Figure 6B). These post-treatment changes in hematoxylin and eosin and special stains were more observed in group III than II and I.

3.3.3 | Correlation between the degree of improvement and clinical parameters

In group I, there was statistically significant negative correlation between the age of the patient and the response to treatment (*P*

TABLE 4 Comparison between atrophic acne scars in the studied groups according to grade of acne scars before and after treatment

Group						
	Before treatment		After treatment			
Grade	No.	%	No.	%	Z	*P*
Group IA						
1	0	0.0	0	0.0	3.000*	.003*
2	0	0.0	4	22.2		
3	4	22.2	14	77.8		
4	14	77.8	0	0.0		
Mean±SD	3.78±0.44		2.78±0.44			
Group IIA						
1	0	0.0	0	0.0	2.828*	.005*
2	0	0.0	10	55.6		
3	12	66.7	8	44.4		
4	6	33.3	0	0.0		
Mean±SD	3.33±0.50		2.44±0.53			
Group IIIA						
1	0	.0	10	55.6	2.640*	.008*
2	4	22.2	8	44.4		
3	6	33.3	0	0.0		
4	8	44.5	0	0.0		
Mean±SD	3.22±0.83		1.67±1.0			

Z, Z for Wilcoxon signed-rank test before and after treatment.
*Statistically significant at $P \leq .05$.

value .023; Figure 7), which indicates that the younger patient showed higher response to microneedling treatment. In group II and III, the results were nonsignificant. In group I, there was statistically significant negative correlation between the duration of the scar and the response to treatment (*P* value .034; Figure 8), which indicates that new scars respond better to microneedling treatment. In group II and III, the results were nonsignificant.

There was statistically significant relation between the type of the scar and the response to treatment in groups II and III (*P* value .001, .023, respectively; Figure 9 and 10, respectively), which indicates that nonacne scars respond to platelet-rich plasma and combination of platelet-rich plasma and microneedling better than acne scars. However in group I, the results were nonsignificant. There was statistically significant relation between the subtype of acne scars and the response to treatment in group II (*P* value .028; Figure 11), which indicates that response of boxcar and ice pick scars for platelet-rich plasma is better than rolling acne scars.

4 | DISCUSSIONS

In the current study, all patients in the microneedling group showed improvement, better response was observed in nonacne scars than acne scars, although the difference was statistically insignificant but this may be due to low number of patients. The current study was agreed with Majid who stated that facial scars due to etiologies other than acne also respond to microneedling with good to excellent response.[21] In previous studies, it was revealed that dermaroller improved atrophic acne scars in 100% of patients.[22,23]

The response of rolling acne scars was better than boxcar and ice pick scars; however, the difference was statistically insignificant, but this may be due to the few number of patients. These results were in agreement with Sharad[24] It was presumed that shallow boxcar and rolling scars responded well to dermaroller, while ice pick scars were the least responsive to the microneedling.[21,23]

The technique of microneedling has been shown to increase the remolding of the skin by creating thousands of microscopic channels through the epidermis to the dermis. In response to the multiple cutaneous injuries and breaking the old collagen strands, a cascade of growth factors (stimulating migration and proliferation of

TABLE 5 Comparison between the studied groups according to side effects

	Total (No.=90)		I (No.=28)		II (No.=34)		III (No.=28)			
Side Effect	No.	%	No.	%	No.	%	No.	%	χ^2	^{MC}P
Pain										
No	8	8.9	0	0.0	8	23.5	0	0.0	29.348*	<.001*
Mild	20	22.2	2	7.1	18	53	0	0.0		
Moderate	28	31.1	8	28.6	8	23.5	12	42.9		
Severe	34	37.8	18	64.3	0	0.0	16	57.1		
Erythema										
No	34	37.8	0	0.0	34	100.0	0	0.0	49.433*	<.001*
Mild	6	6.7	4	14.3	0	0.0	2	7.1		
Moderate	32	35.5	18	64.3	0	0.0	14	50.0		
Severe	18	20.0	6	21.4	0	0.0	12	42.9		

Value for Chi-square (χ^2).
MC, Monte Carlo test.
*Statistically highly significant at $P \leq .001$.

TABLE 6 Comparison between the studied groups according to patient satisfaction

	Total (No.=90)		I (No.=28)		II (No.=34)		III (No.=28)			
	No.	%	No.	%	No.	%	No.	%	χ^2	^{MC}P
Patient satisfaction										
Not satisfied	30	33.3	16	57.2	12	35.3	2	7	19.182*	.002*
Slightly satisfied	8	8.9	6	21.4	2	5.9	0	0.0		
Satisfied	26	28.9	6	21.4	12	35.3	8	28.6		
Very satisfied	26	28.9	0	0.0	8	23.5	18	64.4		

Value for Chi-square (χ^2).
MC, Monte Carlo test.
*Statistically significant at $P \leq .05$.

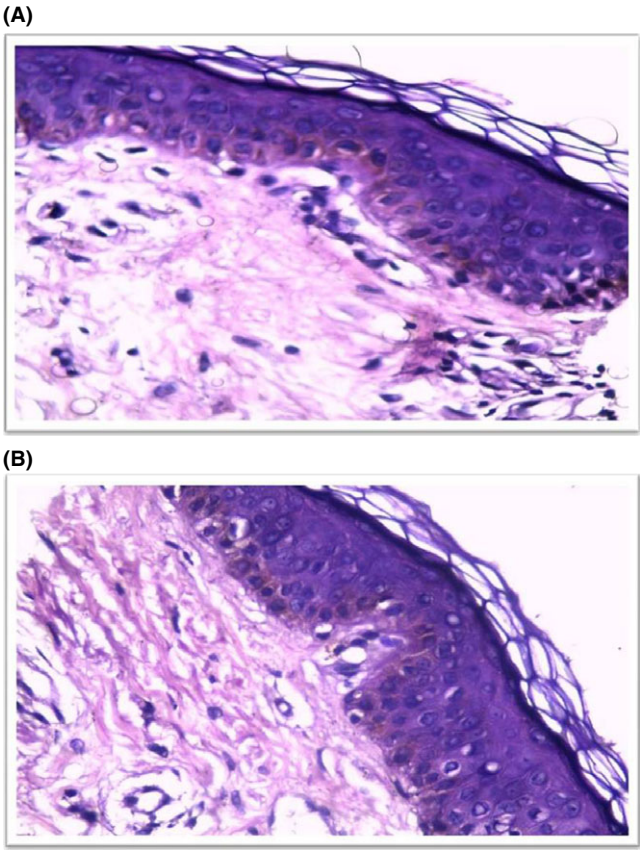

FIGURE 4 (A) Acne scar showing thin epidermis and flat rete ridges (H&E ×400). (B) Acne scar after treatment showing thickened epidermis and normal rete ridges (H&E ×400)

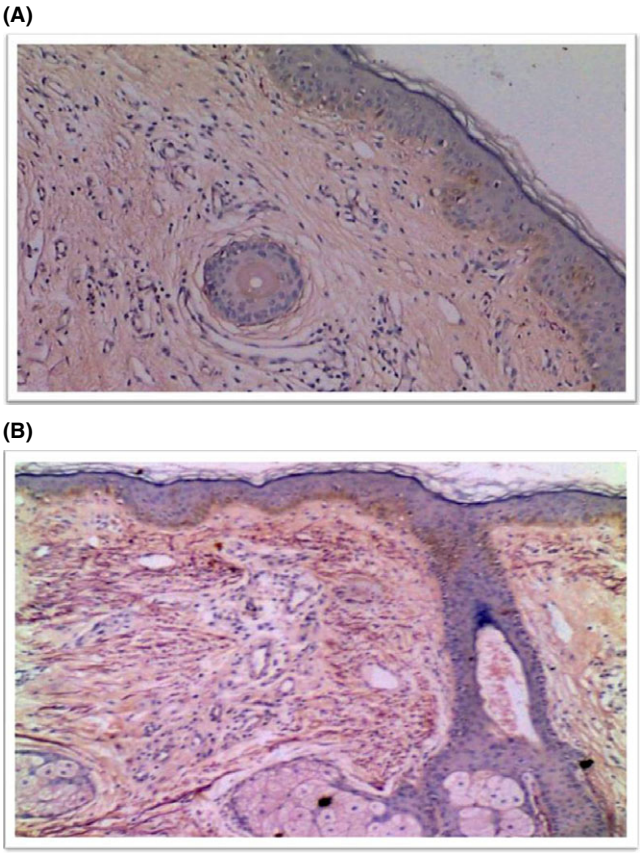

FIGURE 5 (A) Acne scar before microneedling showing scanty fragmented elastic fibers (Orcein ×100). (B) Acne scar after microneedling showing increase in elastic fibers (Orcein ×100)

fibroblasts) leads to collagen production. Thus, architectural and histopathologic changes take place in the lesional area, and scars are attenuated.[25]

Transforming growth factor-β (TGF-β) plays an enormous role in scar formation. While TGF-β1 and TGF-β2 promote scar collagen, TGF-β3 appears to promote scarless wound healing with a normal collagen lattice. Microneedling leads to an initial upregulation of TGF-β1 and TGF-β2 2 and 4 weeks after treatment followed by a strong downregulation at 8 weeks postneedling for TGF-β1 and TGF-β2.[26]

Furthermore, there is a strong upregulation of TGF-β3 2 weeks postneedling without any downregulation at level 4 and 8 weeks postoperatively. Based on that research, they can postulate that microneedling offers a novel modality to rejuvenate and improve both skin appearance and quality by lessening or preventing scarring.[27]

It was demonstrated that collagen induction therapy resulted in a 140% increase in epidermal thickness, an increase in gene and protein expression of collagen I, glycosaminoglycans, and growth factors such vascular endothelial growth factor, fibroblast growth factor 7,

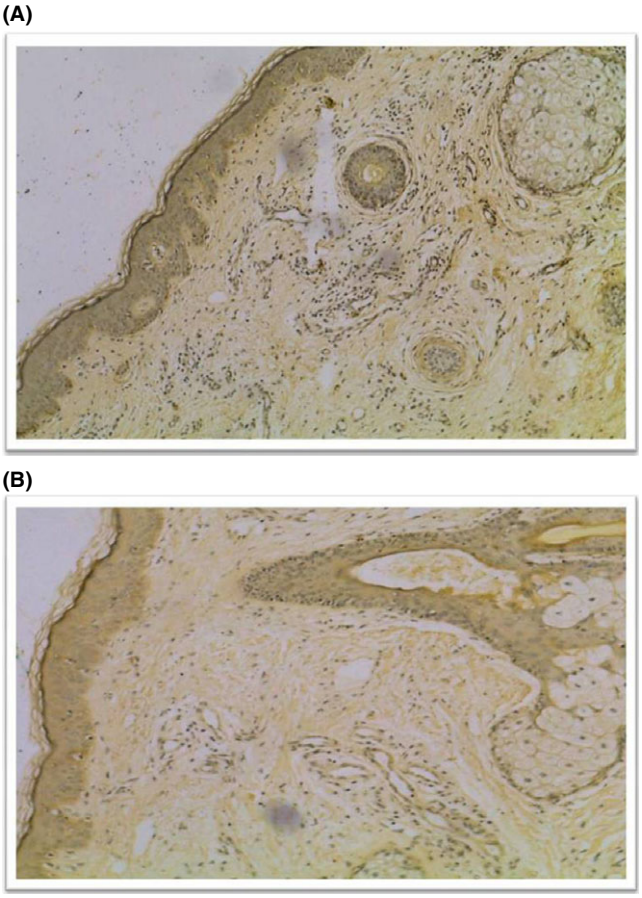

FIGURE 6 (A) Acne scar before combined treatment showing compact collagen bundles(van Gieson ×100). (B) Acne scar after combined treatment showing increase in the collagen bundles amount and thickness arranged in a normal lattice pattern (van Gieson ×100)

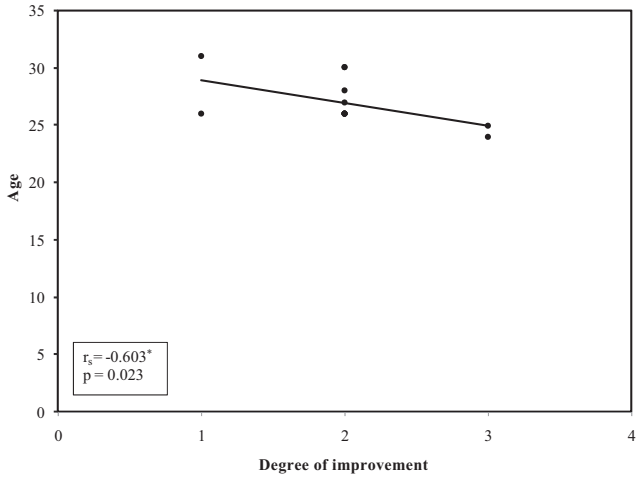

FIGURE 7 Significant negative correlation between degrees of improvement with age in group I

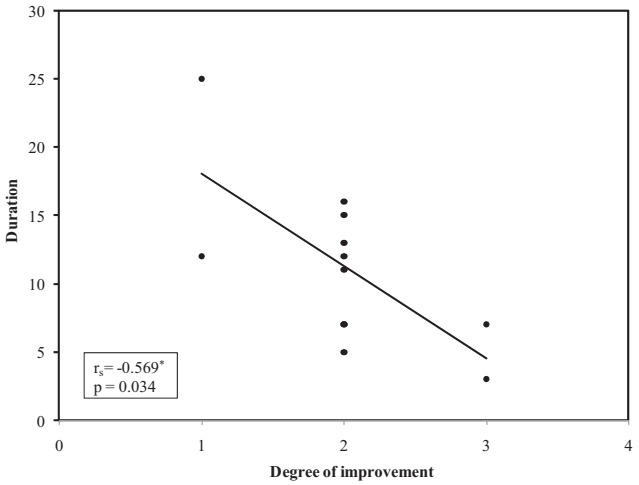

FIGURE 8 Significant negative correlation between degrees of improvement with duration in group I

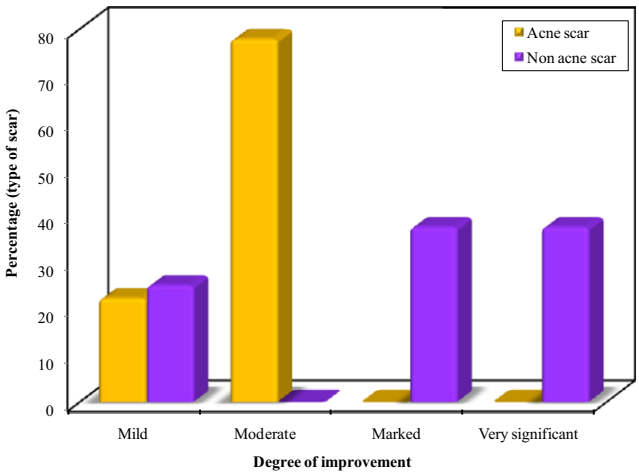

FIGURE 9 Significant relation between degrees of improvement with type of scar in group II

and epidermal growth factor which are all relevant for skin regeneration. The collagen fiber bundles were found to be qualitatively increased, thickened, and more loosely woven in both the papillary and reticular dermis after medical needling. The epidermal-dermal interface showed regular dermal papillae; cellular polarity and normal epidermal differentiation appeared to be maintained; and the connective tissue network within the reticular dermis was regularly thickened and organized what is shown in a healthy collagen matrix.[28]

The body has its own bioelectric system, so during collagen induction therapy microneedles penetrate the skin, cells react to this intrusion with a current that is additionally increased by the needles own electrical potential. The membrane of a living cell has been shown to have a resting electrical potential of –70 mV. The electrical potential depends greatly on the transport mechanisms. If a single acupuncture needle comes close to a cell, the inner electrical potential quickly increases to –100 mV. Cell membranes react to the local change with an electrical potential that creates increased cell activity and a release of potassium ions, proteins, and growth factors.[29] According to that hypothesis based on bioelectricity, collagen

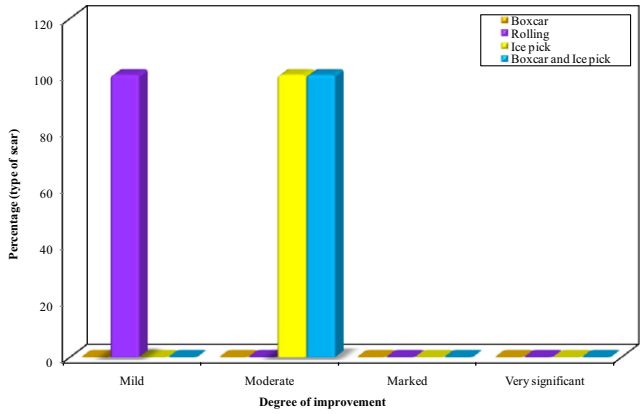

FIGURE 10 Significant relation between degrees of improvement with subtype of acne scar in group IIA

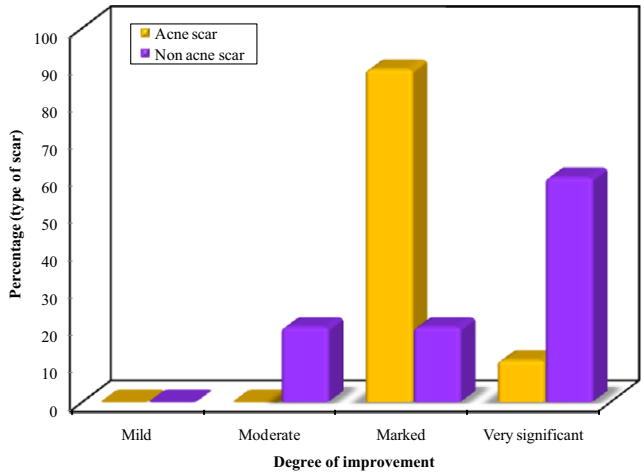

FIGURE 11 Significant relation between degrees of improvement with type of scar in group III

induction therapy triggers a cascade of growth factors that stimulate directly the maturation phase of wound healing.[30]

An obvious advantage with this procedure is the fact that the epidermis, the protective layer of the skin, is preserved. Another advantage is the reduced incidence of adverse effects. The microscopic channels made by microneedling close spontaneously within a few hours, and there is only minimal histological damage.[31]

There was statistically significant negative correlation between the age of the patients and duration of scars with the response to treatment, indicating that the younger patient with new scars showed higher response to microneedling treatment than old patients with old scars. This was in agreement with Sharad.[24]

As regards group II, all patients in the platelet-rich plasma intradermal injection group showed improvement, and the nonacne scars respond better than acne scars.

Oh et al. treated a woman with a single erythematous, atrophic, depressed, using autologous platelet-rich plasma injection with a substantial improvement of the lesion and symptoms, with good cosmetic results.[32] Many authors reported that acne scars have been significantly reduced in most of the patients after injection of platelet-rich plasma.[33,34]

In our study, ice pick and boxcar acne scars responded better than rolling scars to platelet-rich plasma injection. Lee, et al. reported that platelet-rich plasma injections when combined with ablative CO_2 fractional resurfacing cause significant improvement in acne scars than laser alone.[35]

The platelet-rich plasma may actively correct atrophic scarring, through the release of growth factors from their alpha-granules. They contain storage pools of numerous growth factors, including platelet-derived growth factor, transforming growth factor, vascular endothelial growth factor, insulin growth factor 1, fibroblast growth factor, epidermal growth factor, and keratinocyte growth factor, as well as many cytokines, chemokines, and than grade 4. The current study disagreed with Nofal and colleagues who resulting metabolites, which could serve in rebuilding the lost collagen and elastic fibers, improving the atrophic scars.[36,37]

Another pathway that explains the effect of platelet-rich plasma in the treatment of acne scars could be through the accelerating effect it has on the generation of hyaluronic acid which is known to draw water into the hyaluronic acid matrix, causing it to swell, which creates volume and skin turgor and lubricates tissues. There are also indications that native hyaluronic acid promotes cell proliferation and extracellular matrix synthesis and modulates the diameter of the collagen fibers, improving atrophic scars.[38]

There was a statistically significant negative correlation between the grade of the scar before treatment and the response to treatment, which indicates that grade 3 acne scars responds to platelet-rich plasma injection better reported more improvement in patients with grade 4 acne scar.[33]

As regards group III, all patients showed improvement. The nonacne scars respond better than acne scars to combined microneedling (using dermapen) and platelet-rich plasma injection. This result was in agreement with Nofal and colleagues.[33]Fabbrocini and colleagues used dermaroller combined with topical application of platelet-rich plasma for treatment of acne scars with significant improvement.[23]

In group III, 100% of boxcar, ice pick and combined acne scars showed marked improvement. Regarding rolling acne scars, 75% showed marked improvement and 25% showed very significant improvement. It could be suggested that platelet-rich plasma contains autologous growth factors that act synergistically with these induced by skin needling to enhance the wound healing response.

Histopathological examination by hematoxylin and eosin in the present study showed thin epidermis with flat rete ridges at baseline, after treatment procedures, the epidermal thickness increased with well-developed rete ridges. This was in accordance with Fernandes and Signorini.[39] Aust et al. showed that microneedling left the epidermis intact without any damage to stratum corneum or any other layer of the epidermis or the basal membrane.[9] Na et al. demonstrated thicker epidermis and better organized stratum corneum in platelet-rich plasma-treated side than control side.[40]

As regards to increased epidermal thickness, Fernandes explained the difference between standard wounds healing and healing after

microneedling. In standard wounds, the main cells are the keratinocytes, which change in morphology and become mobile to cover the gap in the basement membrane. Peripheral cytoplasmic actin filaments also are developed to pull keratinocytes together to close the wound. These actin filaments, however, are not an important factor in microneedling because re-epithelialization, or the closure of needle holes, occurs within a few hours after needling because the gap is small. A day or two after microneedling, the keratinocytes start proliferating and act more in thickening the epidermis than in closing the defect.[31]

By orcein stain, in this study at baseline there were thin frayed elastic fibers arranged in parallel orientation. After treatment, there was an increase in elastic fibers in the upper dermis, and they appeared thicker and more randomly distributed than untreated scars. By van Gieson stain, at baseline collagen fibers were arranged in parallel pattern; after treatment, collagen deposition was increased and appeared to be laid down in normal lattice pattern. These changes were more evident in group III (combined platelet-rich plasma and microneedling). These results were in agreement with many studies.[9,39]

No major adverse effects were observed in our study among the three groups. Tolerable pain was the most common adverse effect in all groups, and it was more evident in group I and III than group II. Pain was present only during the procedures and did not need analgesics after treatment or discontinuation of sessions. This was in agreement with Nofal and colleagues.[33]

In the present study, erythema was more severe in group III followed by group I, and this may be because patients in group III were of lighter skin phototype. This was in agreement with Fabbrocini et al. No patient of group II showed erythema; however, Redaelli and colleagues reported that 80% of their patients experienced mild erythema after platelet-rich plasma injection.[34]

Regarding pigmentation: In this study, no cases showed hyper- or hypopigmentation even patients of skin type IV. This agreed with Aust et al. who reported that there was no change in the number of melanocytes after microneedling. In their study, they demonstrated that suppressor factor (interleukin -10) is increased during the first 2 weeks after microneedling and melanocyte stimulating hormone gene is downregulated which suggests that needling reduces risk of dyspigmentation through significant downregulation of melanocyte stimulating hormone in the postinflammatory response, so it can be safely performed on Asian and darker skins.[9] The current study also agreed with Fabbrocini et al.[23] However, the present study disagreed with Sharad, as they reported three cases of postinflammatory hyperpigmentation in the microneedling group.[24]

There were many limiting factors in this study, such as small number of the patients, and we could not estimate the exact number of platelets. The follow-up period is needed to be more longer, up to 1 year.

5 | CONCLUSION

Automated microneedling is a simple minimally invasive procedure with rapid healing and low downtime, platelet-rich plasma is effective safe cheap procedure, each procedure has advantages in the treatment of atrophic scars, but combination of both techniques potentiates their effect with more improvement of the atrophic scars of different etiologies. The treatment is well tolerated in skin types III and IV, with no side effects, minimal downtime, and economic cost. Microneedling as individual treatment is advised in young age patients with short history of atrophic scars, and in rolling type postacne scars. For older age, long duration, ice pick, deep boxcar acne scars, and grade 4 postacne scars.

5.1 | Recommendations

Further controlled studies with larger number of patients regarding the number of sessions and interval between sessions are recommended. Further histopathological and special stain studies to evaluate the effect of microneedling and platelet-rich plasma on collagen growth are recommended.

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