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Article *in* Dermatologic Surgery · July 2014

DOI: 10.1111/dsu.0000000000000091 · Source: PubMed

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Platelet-Rich Plasma Versus CROSS Technique With 100% Trichloroacetic Acid Versus Combined Skin Needling and Platelet Rich Plasma in the Treatment of Atrophic Acne Scars: A Comparative Study

Eman Nofal, MD, Afaf Helmy, MD, Ahmad Nofal, MD, Rania Alakad, MSc, and Mohammad Nasr, MD*

BACKGROUND Platelet-rich plasma (PRP) is an autologous preparation of platelets in concentrated plasma that may be beneficial in the treatment of atrophic acne scars by promoting collagen deposition. Skin needling is a technique that uses a sterile dermaroller to puncture the skin and release growth factors. The combination of skin needling and PRP could enhance the efficacy of both modalities. Chemical reconstruction of skin scars technique consists of focal application of high concentration of trichloroacetic acid (TCA) on the acne scars to stimulate collagen production.

OBJECTIVE To evaluate the efficacy and safety of intradermal injection of PRP, 100% focal TCA, and combined skin needling plus topical PRP in the treatment of atrophic acne scars.

PATIENTS AND METHODS Forty-five patients with atrophic acne scars were randomly assigned to 3 equal groups; Group A received intradermal injection of PRP, Group B received chemical reconstruction of skin scars technique with TCA 100%, and Group C was treated by combined skin needling and PRP. Each patient underwent 3 sessions at 2-week interval.

RESULTS All the patients completed the study. The 3 groups showed statistically highly significant improvement in the degree of acne scars after treatment ($p < .001$). No major adverse effects were observed in the studied groups.

CONCLUSION This is the first study to use intradermal injection of PRP alone for the treatment of atrophic acne scars. The 3 modalities showed a promising efficacy and safety in the treatment of atrophic acne scars.

The authors have indicated no significant interest with commercial supporters.

Permanent scarring is an unfortunate complication of acne vulgaris. Having acne scars can be emotionally and psychologically distressing to patients.[1] Acne scars can be classified into atrophic and hypertrophic. Atrophic type includes ice picks, boxcar, and rolling scars. Different treatment modalities have been used to ameliorate acne scars such as laser resurfacing, chemical peeling, subcision, augmentation by fillers, dermabrasion, skin needling, and punch excision. However, no simple and definitive solution for acne scarring has been explored to date.[2]

Platelet-rich plasma (PRP) is an autologous concentration of human platelets in a small volume of plasma. Platelet-rich plasma contains multiple autologous growth factors, especially epidermal growth factor, platelet-derived growth factor, transforming growth factor-β, and vascular endothelial growth factor. It has been used in many areas of medicine, including the acceleration of healing of tendon injuries, the treatment of osteoarthritis, and in cardiovascular diseases.[3]

Chemical reconstruction of skin scars (CROSS) technique was first described by Lee and colleagues.[4] It

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 • Published by Lippincott Williams & Wilkins •
ISSN: 1076-0512 • Dermatol Surg 2014;40:864–873 • DOI: 10.1111/dsu.0000000000000091

consists of application of high concentration of trichloroacetic acid (TCA) focally on the scar. It improves acne scars by focusing on the dermal thickening and collagen production that increase with high TCA concentrations. It also avoids complications by sparing adjacent normal skin and adnexal structures.

Skin needling (microneedling) is a technique that involves using a sterile dermaroller that punctures the skin with a series of fine sharp needles. The skin develops multiple microbruises in the dermis that initiate the complex cascade of wound healing and growth factor release, and finally results in collagen production.[5] Combination of skin needling and topical PRP act synergistically to improve the acne scars. The ability of skin needling to create a way for PRP absorption and the ability of platelets to contribute to wound healing and growth factor release induced by skin needling are the basis of using this combination therapy.[6] The aim of this study was to evaluate the efficacy and safety of PRP injection, CROSS technique, and the combined use of skin needling and PRP in the treatment of atrophic acne scars.

Patients and Methods

This study was performed at the outpatient clinics of Dermatology Department, Faculty of Medicine, Zagazig University Hospitals, from December 2011 to October 2012 after approval of the Institutional Review Board was obtained.

Patients

The study included 45 patients with atrophic acne scars of different durations, types, and severities. Patients were subjected to full history taking, including onset, course, and duration of scars, previous acne and acne scar treatments, and post-treatment complications such as hyperpigmentation or keloid formation.

Dermatologic examination to assess the skin type, the scar type (ice pick, boxcar, and rolling type), the scar severity (Grade 2, 3, or 4) according to the qualitative global acne scarring grading system[7] (Table 1) was performed for every patient. Complete blood picture, prothrombin time, and partial thromboplastin time were performed for patients subjected to combined PRP and needling. The exclusion criteria were patients with active acne, herpes labialis, or bacterial infection; warts on the face, actinic keratosis, or skin cancer; systemic retinoids intake in the previous 6 months, diabetes, pregnancy, history of keloidal scarring; or patients with severe systemic illness or malignancy. Patients on anticoagulant therapy or aspirin, patients with hemoglobin <10 g/dL, or platelets <105/μL were excluded from PRP injection and combined needling and PRP groups.

Participants were subdivided into 3 groups (A, B, and C), each included 15 patients. Group A received intradermal injection of PRP, Group B underwent CROSS technique with TCA 100%, and Group C was treated with skin needling combined with topical PRP. Each patient underwent 3 sessions at 2-week intervals. Before treatment, informed consent was obtained from all patients. We emphasized to the patient that there is no quick, easy, and standard therapy for acne scars. Possible side effects of each procedure such as erythema, edema, pain, prolonged downtime, and hyperpigmentation were also explained.

Methods

Group A (PRP Injection)

Platelet-rich plasma was obtained by double-spin method, followed by the collection of 10 mL of autologous whole-blood into tubes containing trisodium citrate as anticoagulant. The collected blood was first centrifuged at 150 to 200 *g* for 10 minutes at room temperature to separate the red blood cells at the bottom of the tube, the buffy coat (containing the white blood cells) in the middle and the plasma above (soft spin). Then, the upper plasma was pipetted above the buffy coat to undergo another centrifugation at 1500 to 2000 *g* for 15 minutes (hard spin) to obtain a platelet pellet in the bottom of the tube (with a platelet count 4–4.5 times higher than that of baseline), and a platelet-poor plasma (PPP) in the upper part. The PPP is partly removed

TABLE 1. Qualitative Global Scarring Grading System (Goodman and Baron)[7]

Grade		Clinical Features
1	Macular	Macular erythematous, hyperpigmented, or hypopigmented flat marks
2	Mild	Mild atrophic or hypertrophic scarring that may not be obvious at social distances of 50 cm or greater and easily covered by makeup or beard hair in men
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 beard hair in men, but is still able to be flattened by manual stretching of the skin
4	Severe	Severe atrophic or hypertrophic scarring not flattened by manual stretching of the skin

and partly used to resuspend the platelets to finally produce 2 mL of PRP.[6]

Platelet-rich plasma was activated by adding 10% calcium chloride 0.1 mL per 0.9 mL plasma. Local anesthetic cream (eutectic mixture of lidocaine and prilocaine) was applied to the face for approximately 45 to 60 minutes before the procedure. After sterilization of the face with alcohol, 0.1 to 0.3 mL PRP was injected intradermally into the atrophic scars using insulin syringe with a total of 1 mL PRP in each side of the face. Gentle massaging was performed after the procedure, followed by topical antibiotic for 3 days after treatment, but application of sunscreen was not required.

Group B (CROSS Technique With 100% TCA)
The skin was first cleansed and degreased with acetone. Patients were treated by 100% TCA applied focally on the scars by a cotton-tipped wooden applicator until frosting occurred (usually within 10 seconds). Patients were instructed to apply antibiotic cream until focal crust formation, to avoid disturbing the crusts, and to apply sunscreen.

Group C (Combined Skin Needling and PRP)
Local anesthetic cream was applied to the face as previously mentioned in Group A. The face was sterilized with alcohol, and 0.5 to 1 mL of PRP was applied topically to the treated area, followed by needling the skin using the needling tool (Dermaroller MT 20; Shanghai Astiland Technology Co., Ltd, Shanghai, China). It consists of 24 circular arrays of 8 stainless steel needles; 2 mm long (total 192 needles) in a cylindrical assembly. The treatment was performed by rolling the dermaroller over the areas affected by acne scars 6 times in 4 directions (vertically, horizontally, and diagonally right and left) without pressing too hard. The skin was stretched perpendicular to the dermaroller movement to reach the base of the scar. Topical antibiotic and sunscreen were prescribed after the procedure.

Clinical Assessment

Digital color facial photographs were taken at baseline, at each session, 2 weeks after the last session, and at the end of follow-up (2 months after the last session). The results were assessed using the qualitative global scarring grading system (Table 1),[7] quartile grading scale, and patient satisfaction. In the last 2 methods, therapeutic response was classified according to the degree of improvement of acne scars into excellent (>75%), very good (50%–74%), good (25%–49%), and poor (<25%). The results were assessed by 2 blinded dermatologists who evaluated the photographs taken before treatment and 2 weeks after the last session. Side effects were recorded at each session. Pain was graded on a scale of 0 (none) to 9 (maximum).

Statistical Analysis

Data were checked, entered, and analyzed using SPSS (version 19). Data were represented as mean ± SD for quantitative variables. Numbers and percentages were used for categorical variables. Chi-square (χ^2) or Fisher exact test analysis of variance (F test) and McNemar χ^2 test were used when appropriate. $p < .05$ was considered statistically significant.

TABLE 2. Clinical Data of the Studied Groups

	Group A	*Group B*	*Group C*	*F*	*p*
Age (years)					
Mean ± SD	25.1 ± 3.7	25.5 ± 5.6	25.8 ± 5.3	0.09	.9
Range	19–32	19–40	20–35		NS
Gender	N (%)	N (%)	N (%)	χ^2	*p*
Male	5 (33.3)	5 (33.3)	4 (26.7)	0.21	.9
Female	10 (66.7)	10 (66.7)	11 (73.3)		NS
Skin type	N (%)	N (%)	N (%)	χ^2	*p*
III	3 (20.0)	4 (26.7)	5 (33.3)		
IV	8 (53.3)	7 (46.7)	7 (46.7)	0.77	.94
V	4 (26.7)	4 (26.7)	3 (20.0)		NS
Scar type	N (%)	N (%)	N (%)	χ^2	*p*
Ice pick	4 (26.7)	4 (26.7)	5 (33.3)		
Rolling	4 (26.7)	3 (20.0)	7 (46.7)	1.78	.77
Boxcar	7 (46.7)	8 (53.3)	3 (20.0)		NS
Scar duration				F	*p*
Mean ± SD	5.9 ± 2.0	6.3 ± 2.1	5.7 ± 1.9	0.4	.6
Range	3–10	3–11	2–10		NS

NS, nonsignificant.

Results

Clinical data of the studied patients are illustrated in Table 2.

Therapeutic Response

- Qualitative global scarring grading system: The 3 groups showed highly significant improvement in the severity of acne scars after treatment ($p < 0.001$) (Figure 1). Before treatment, there were 34 patients with Grade 4 acne scarring. After treatment, 23 patients improved to Grade 2 and 3 acne scarring. There was no statistically significant difference in the degree of improvement between the 3 groups ($p = .87$) (Table 3). After 2 months of follow-up, there was no further improvement in the clinical response.
- Quartile grading scale (Table 4): There was no statistically significant difference in the therapeutic response between the 3 groups ($p = .49$).
- Patient satisfaction (Table 5): There was no statistically significant difference in the therapeutic response between the 3 groups ($p = .6$).

No significant correlations were found between the therapeutic response and the different clinical variables including age, gender, skin type and scar duration, severity, and type in the 3 groups.

Side Effects

Side effects among the studied patients are shown in Table 6. Pain was reported in all patients of the study. Pain was noticed only during the procedure. It was quite tolerable, without the need for analgesics after treatment or discontinuation of the sessions. No difference in pain severity was noticed between male and female patients.

Discussion

Because of the prevalence of acne scarring and the strong negative emotions it causes in affected patients, dermatologists are frequently presented with the challenge of evaluating and providing treatment recommendations to patients with acne scars.[1] This study was conducted to evaluate the efficacy and the safety of 3 different modalities in the treatment of atrophic acne scars: PRP injection, CROSS technique, and the combined use of skin needling and topical PRP. To the best of our knowledge, this is the first study that evaluates the efficacy and safety of PRP intradermal injection alone in the treatment of atrophic acne scars.

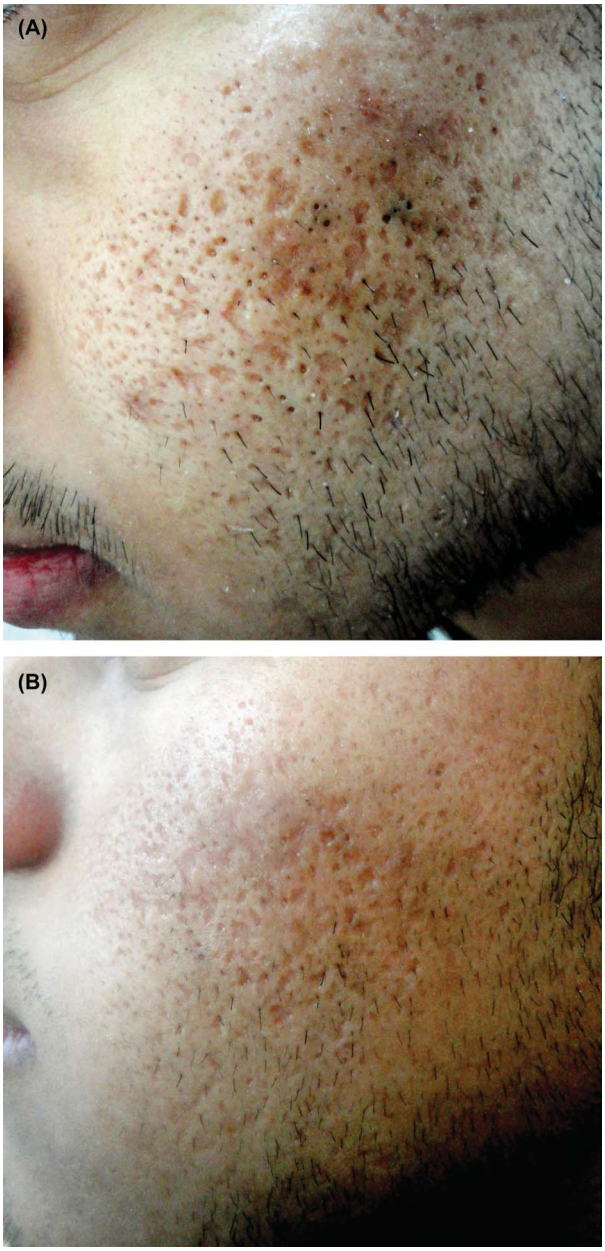

Figure 1. Group A (PRP injection): a male patient with Grade 4 acne scars. (A) Before treatment. (B) Excellent response 2 months after the last session.

Platelet-rich plasma is an autologous preparation of platelets in concentrated plasma. It has recently attracted much attention in various medical fields, including orthopedic, plastic, and dental surgeries and dermatology for its wound-healing ability. Platelets release various cytokines and growth factors that promote angiogenesis, tissue remodeling, and wound healing. Platelet-rich plasma works by the degranulation of α granules in platelets, which contain the synthesized and prepackaged growth factors. Many growth factors have short half-lives, therefore greatest effectiveness may result if they are activated at or just before injection. Fibroblasts accumulate at the site of injection and start to lay down collagen.[8]

Activated platelets release several growth factors, cytokines, and chemokines, including vascular endothelial growth factor, platelet-derived growth factor, epidermal growth factor, fibroblast growth factor, transforming growth factor-β, insulin-like growth factor, IL-8, macrophage inflammatory protein-1α, and platelet factor-4.[9]

Platelet-rich plasma separation involves centrifugation of the whole-blood by single-spin or double-spin method. The single-spin method separates the whole-blood into 3 basic components: red blood cells (bottom of the tube), PRP (middle of the tube), and PPP (top of the tube).[10]

Marx[11] has suggested that PRP with a single spin would not produce a true PRP. Instead, it would produce a mixture of PRP and PPP with low platelet counts. Regardless of the rate of centrifugation or the time of centrifugation, a single spin cannot adequately concentrate platelets because the red blood cells will interfere with the fine separation of the platelets. The device must use a double centrifugation technique. The first spin will separate the red blood cells from the plasma, which contains the platelets, and the second spin will finely separate the platelets from the PPP.

The second centrifugation produces a platelet pellet that can be easily resuspended with maximum platelet concentration and the least platelet loss in the above PPP. The produced PRP contains almost no blood-derived cell types other than platelets. Leukocytes are absent in PRP prepared by this method as they should be avoided in PRP preparations because of their potential proinflammatory effect.[12]

In this study, we have used, for the first time, PRP intradermal injection alone in the treatment of different types of acne scars. Promising results have been achieved using this method, where the severity of acne

TABLE 3. Therapeutic Response According to the Qualitative Global Scarring Grading System

	Group A, N (%)	*Group B, N (%)*	*Group C, N (%)*	χ^2	*p*
Before					
Grade 2	2 (13.3)	1 (6.7)	2 (13.3)		
Grade 3	1 (6.7)	2 (13.3)	3 (20.0)	1.64	.81
Grade 4	12 (80.0)	12 (80.0)	10 (66.7)		NS
After					
Grade 1	0 (0.0)	0 (0.0)	1 (6.7)		
Grade 2	6 (40.0)	5 (33.3)	6 (40.0)	2.42	.87
Grade 3	5 (33.3)	6 (40.0)	5 (33.3)		NS
Grade 4	4 (26.7)	4 (26.7)	3 (20.0)		
p	<.001, HS	<.001, HS	<.001, HS		

HS, highly significant improvement in the severity of acne scars after treatment in each group.
NS, no statistically significant difference in the degree of improvement between the 3 groups.

scars has been significantly reduced after treatment with PRP in most of the patients of Group A, especially patients with Grade 4 acne scars.

Redaelli and colleagues[7] noticed improvement of acne scarring by PRP intradermal injection, while using PRP for skin rejuvenation. They were the first to recommend further trials to examine the benefit of injecting PRP in acne scars. Lee and colleagues[13] postulated that using PRP injection immediately after carbon dioxide laser resurfacing enhances the recovery of laser damaged skin and synergistically improves the clinical appearance of acne scars. Therefore, PRP seems to be a promising new non-surgical aesthetic modality; however, larger and well-controlled studies with prolonged follow-up periods should be performed to investigate its efficacy as a biostimulator and certify its long-term effects.

The CROSS technique was effective in the treatment of acne scars in patients of Group B. The difference in the qualitative global score before and after treatment was highly significant. Our results were consistent with those reported in many previous studies.[4,9,14,15] Lee and colleagues[4] were the first to describe CROSS technique for the treatment of atrophic acne scars. They compared the efficacy of TCA 65% versus TCA 100% concentration and reported that TCA 100% was more effective with higher patient satisfaction than TCA 65% with no increase in the frequency of complications. They have also observed that the clinical response was proportionally related to the numbers of sessions. Similar to Lee and colleagues,[4] we did not use pretreatment application of topical retinoids before CROSS method to avoid excess TCA penetration that may be associated with more complications.

Although CROSS method was found to be more effective on ice-pick scars,[16,17] frequent applications can normalize other types such as deep rolling and deep boxcar types.[4] Some authors have used

TABLE 4. Therapeutic Response According to the Quartile Grading Scale

	Group A, N (%)	*Group B, N (%)*	*Group C, N (%)*	χ^2	*p*
Poor	5 (33.3)	6 (40.0)	4 (26.7)	5.37	.49*
Good	3 (20)	5 (33.3)	2 (13.3)		
Very good	4 (26.7)	4 (26.7)	7 (46.7)		
Excellent	3 (20)	0 (0.0)	2 (13.3)		

*No statistically significant difference in the therapeutic response between the 3 groups.

TABLE 5. Therapeutic Response According to Patient Satisfaction

Patient Satisfaction	*Group A, N (%)*	*Group B, N (%)*	*Group C, N (%)*	χ^2	*P*
Poor	0 (00.0)	0 (00.0)	0 (00.0)		
Good	5 (33.3)	6 (40.0)	5 (33.3)	2.73	0.6
Very good	7 (46.7)	3 (20.0)	5 (33.3)		NS
Excellent	3 (20.0)	6 (40.0)	5 (33.3)		

NS, no statistically significant difference in the therapeutic response between the 3 groups.

CROSS technique for the treatment of acne scars in comparison with other modalities such as microneedling,[16] fractional laser,[17] and subcision.[18] However, no statistically significant difference was observed in these studies as was the case in this study. Kang and colleagues[19] have also reported significant improvement of atrophic acne scars after the use of triple combination therapy (CROSS technique, subcision, and fractional laser), suggesting that combination therapy could be associated with better response than isolated modalities.

Combined microneedling and topical PRP was effective in the treatment of acne scars in patients of Group C. The difference in the qualitative global score before and after treatment was highly significant (p = .001). Skin needling induces neocollagenosis and triggers a cascade of growth factors that directly stimulate the maturation phase of wound healing. It was suggested that dermal tethering, the main obstacle in rolling scars, can be overcome by greater collagen and elastin deposition induced by needling.[16] Furthermore, it provides a clear channel for topical agent such as PRP to be absorbed more effectively through the top layer of the skin. Platelet-rich plasma contains autologous growth factors that act synergistically with growth factors induced by skin needling to enhance the wound-healing response.[6]

The only trial that used the dermaroller combined with topical application of PRP was that of Fabbrocini and colleagues.[6] They compared in a split-face study the effectiveness of skin needling alone and the combined use of skin needling plus topical PRP. Their results showed that the scar severity grade was greatly reduced on both sides of the face, but the improvement was more prominent on the side treated with both skin needling and PRP. A similar efficacy has also been observed using this combination in our study.

Any concerns of immunogenic reactions or disease transfer are eliminated because PRP is prepared from autologous blood. No studies have documented that PRP promotes hyperplasia, carcinogenesis, or tumor growth. Growth factors act on cell membranes rather than on the cell nucleus and activate normal gene expression and normal wound-healing feed-back control mechanisms.[20] Despite this proclaimed safety,

TABLE 6. Adverse Effects in the Studied Groups

Side Effects	*Group A, N (%)*	*Group B, N (%)*	*Group C, N (%)*
No	14 (93.3)	11 (73.3)	0 (0.0)
Mild bruises	1 (6.7)	0 (0.0)	0 (0.0)
Hyperpigmentation	0 (0.0)	4 (26.7)	0 (0.0)
Erythema and edema	0 (0.0)	0 (0.0)	15 (0.0)
Pain			
Mild	6 (40.0)	15 (100)	2 (13.3)
Moderate	3 (20.0)	0 (0.0)	7 (46.7)
Severe	6 (40.0)	0 (0.0)	6 (40.0)

more controlled studies are still needed to evaluate its safety when applied with enhanced transepidermal delivery systems such as microneedling.

In our study, it was an interesting observation that one of the patients in Group C who had both acne and traumatic scars showed remarkable improvement in the depth and width of both types of scars after only 3 sessions of combined skin needling and PRP (Figure 2). This may open the way for further trials to investigate the efficacy of both needling and PRP in traumatic scars.

There is no consensus about the numbers of sessions or the interval between treatment sessions in each modality. We preferred to choose a 2-week interval period to ensure the patient compliance. However, better results might be achieved by more sessions and longer interval to allow more increase in dermal collagen that can take several months.[4]

Adverse Effects

No major adverse effects were observed in our study among the 3 groups. Tolerable pain was the most common adverse effect in all patients. In Group A, only 1 patient had mild bruises at the injected sites that disappeared spontaneously the next day without treatment. This was also observed by Redaelli and colleagues[7] who reported that 3% of their patients experienced well-tolerated bruising after PRP injection for face rejuvenation. Unlike our study, 70% of their patients experienced a burning sensation and 80% experienced mild erythema. They suggested that the burning sensation was probably due to calcium chloride.

Platelet-rich plasma can be used safely in dark skin without the need for sun avoidance or using topical sunscreen. Furthermore, patients could resume their work at the same day with no downtime. The problems with PRP injection were the disapproval of some patients to the process of blood withdrawal and pain during injection.

In few patients who were treated with CROSS technique, cosmetic discomfort was noticed because of the presence of brownish crusts that appeared within 2 days and lasted for 2 to 7 days. As previously reported,[4,16] transient postinflammatory hyperpigmentation occurred in 4 patients who were treated with CROSS, all of them were skin type V. They were treated efficiently with topical hydroquinone 2% for 2 weeks.

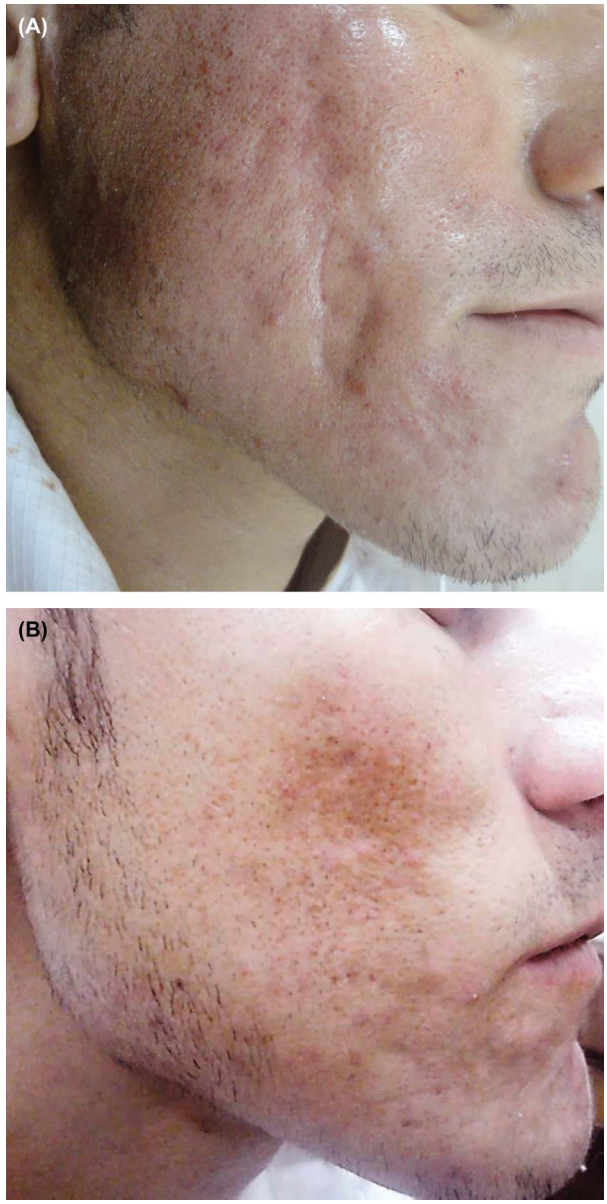

Figure 2. A male patient with Grade 4 acne scars and traumatic scars in Group C (combined skin needling and PRP). (A) Before treatment. (B) Excellent response 2 months after the last session.

The risk of postinflammatory hypopigmentation or hyperpigmentation with skin needling (Group C) is minimal because melanocytes are not directly

targeted[21] and the number of melanocytes in the skin neither increased nor decreased.[22] This could explain the absence of hyperpigmentation in our cases. However, some authors reported hyperpigmentation in few patients[23,24] and attributed it to increased melanogenesis from a stable number of melanocytes rather than to increase in the melanocytes number. So, it has been proposed that skin needling can be safely performed on the skin of people of Asian descent and darker tones, as well as on skin that has been previously treated with lasers or dermabrasion.[16,21]

All patients in Group C tolerated the procedure well except for a temporary erythema and edema that lasted for 2 or 3 days. This was reported in the previous studies that used skin needling. It is noteworthy that no scarring occurred in our study, as well as previous microneedling studies.[6,16,21,22] This could be explained by the fact that needling upregulates the expression of TGF-β3, which is an essential marker preventing scarring.[21,22] However, post-treatment scarring (tram track scarring) was only reported in 1 patient by Pahwa and colleagues[25] who believed that this scarring may be due to the use of a large needling device or strong pressure by the device.

Conclusion

In conclusion, the 3 modalities used in this study represent comparable effective and safe treatment options for atrophic acne scars with no statistically significant difference between them. However, the relatively small number of subjects in each study arm makes meaningful statistical analysis challenging.

Platelet-rich plasma injection is a new and promising modality for the treatment of atrophic acne scars without the risk of hyperpigmentation or scarring. It can be used alone or combined with needling for patients who cannot tolerate prolonged downtime. Further trials to evaluate the efficacy of PRP injection in larger number of patients with acne scars, and in association with other techniques such as TCA, fractional laser, and subcision are recommended.

Chemical reconstruction of skin scars technique is more suitable for patients who cannot tolerate pain, patients with fair skin, and patients with deep scars that cannot be fully reached with a dermaroller. In addition, it is less invasive and more economic modality especially in developing countries.

Assessment of the acne scar type, skin phototype, history of hyperpigmentation or scarring, pain tolerance, and appropriate downtime is critical to allow picking up the most appropriate modality. Because it is evident that some modalities are more effective in certain types of acne scars, and because almost all types of scars are present in almost every patient, combination therapy seems to be the best practical approach for the treatment of atrophic acne scars.

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