



## Application of Fractional Microneedling Radiofrequency and Autologous Platelet-Rich Plasma in Managing Facial Acne Scars

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### Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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### ABSTRACT

**Background:** Many treatment modalities were tried to treat acne scar & still big challenge for dermatologists. Radiofrequency is a non-ionizing electromagnetic radiation used in medicine for nearly 75 years and the application of autologous Platelet-Rich Plasma has been safely used and documented in many fields of medicine. In dermatology and cosmetics they also are used in treating different conditions.

**Aim of Study:** To evaluate the clinical efficacy of fractional microneedling radiofrequency (FMR) and autologous Platelet-Rich Plasma (PRP) for treatment of facial acne scars.

**Patients and Methods:** A therapeutic comparative study carried out in Dermatology Sulaimani Private Center for period from 1<sup>st</sup> of November, 2014 to end of June, 2015. Forty patients with acne scar were included, we divided these patients into 2 groups; group –A (22 patients were treated with FMR alone) & group- B (18 patients treated with both FMR & autologous PRP). Both groups were treated by 3 sessions at 4 weeks interval in between sessions. Clinical assessment of improvement

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was assessed by the patient himself (patient assessment) and two dermatologists by using standardized digital photography (physician assessment).

**Results:** Most of the patients in both groups had showed a good improvement in their facial acne scar with the use of our treatment. Females were more than males with mean age of 30 years. There was a significant association between excellent improvement (by physician assessment and patients assessment) and patients acne scar treated in group- B (FMR & PRP) ( $p=0.02$ ).

**Conclusion:** Combination of FMR & autologous PRP is highly effective method for treating acne scars and it had an excellent satisfaction rate among patients.

*Keywords: FMR; PRP; facial acne scar.*

## 1. INTRODUCTION

Acne vulgaris is a chronic inflammatory disease of the pilosebaceous, follicles, characterized by comedones, papules, pustules and often scars, Acne is primarily a disease of the adolescent, with 85% all teenagers being affected to some degree. It occurs with greatest frequency between the ages of 15 and 18 in both sexes. Generally, involution of the disease occurs before age 25; however, great variability in age at onset and of resolution occurs. Around 12% of women and 3% of men will continue to have clinical acne until 44 years of age [1]. Acne vulgaris is one of the most common skin disorder encountered by dermatologists in practice [2].

All types of acne, from papules, pustules through nodulocystic disease can cause scarring and adequate treatment must be started early. Even with the excellent treatment options available, Scarring may occur early regardless of the severity of acne. Close inspection of acne skin under a bright light can reveal some scarring in up to 90% of patients who attend a dermatologist but significant (socially noticeable) scarring occurs in about 22% of sufferers [3], the scar occur as a result of the severe inflammation in the dermis where lead to destruction of collagen fiber.

Acne scars can be classified as ice pick, rolling, and boxcar scar (shallow & deep). They can be atrophic or hypertrophic. Acne scars can also be classified as mild, moderate, or severe depending on certain criteria such as number, depth, color, and the area of the face involved [4,5].

Facial acne scars are a common long-term cosmetic concern that results from severe acne. Atrophic scars are caused by compromised collagen production during the natural wound-healing process following an inflammatory response to acne, which results in surface

irregularities [6]. Evaluation of scar type and its severity is a very important step to select the most appropriate therapeutic option among the currently available ones. There have been several approaches to classify acne scars in order to evaluate objectively type and severity [7].

Acne scar treatment remains a challenge in the medical literature. Several modalities have been implicated to treat atrophic acne scarring, including invasive: surgical techniques (subcision, punch grafts, and excisions), autologous fat transfer, injection of dermal fillers, dermabrasion, [8-12]. Non invasive: chemical peels, and laser therapy (non-ablative, ablative) [13,14]. The need for cosmetic facial enhancement procedures with minimal down time and low risk has led to the development of methods for non-surgical skin rejuvenation [15].

Fractional microneedle radiofrequency (FMR) is a recently developed, minimally invasive method for delivering thermal energy to the target tissue with minimal destruction, by using rapid penetration with microneedles. FMR treatment has demonstrated excellent efficacy for skin rejuvenation, face lifting, large pores and acne scars [16,17]. It has shown to stimulate the restructuring of collagen and elastin, resulting in better skin quality and reduction of wrinkles [18,19]. Microneedling sometimes called collagen induction therapy is often used for rejuvenation and treatment of scars. This treatment method uses a stamp or a roller, pressing 0.5-2 mm needles into the skin. The small wounds are closed immediately, but the mechanical injury initiates the wound healing process and stimulates the proliferation of fibroblasts and production of collagen and elastin [20]. The formation of new capillaries improves the blood supply to the skin. Microneedling is also used to increase the transdermal delivery of active skin care substances [21]. Radiofrequency (RF) energy generate heat depending on the

resistance of specific tissue, Radiofrequency has been used to provide a controlled and moderate level of tissue heating in different areas of medicine for many years [22]. The advantage of RF is the relatively low-level heat developed by the devices, which diminishes the tissue burn and allows for a surface treatment profile. Previous studies on the wound healing process after FRM treatment have shown that radiofrequency thermal zones containing denatured collagen were maintained in the reticular dermis for longer than 28 days after treatment, although new dermal tissue partially replaced the zones, and that various wound healing genes involved in dermal remodeling, such as tropoelastin and procollagen, steadily increased until 28 days after the procedure [23].

Platelet-rich plasma (PRP) is blood plasma with concentrated platelets. The concentrated platelets found in PRP contain large reservoirs of bioactive proteins, including growth factors that are vital to initiate and accelerate tissue repair and regeneration. Platelet-rich plasma contains autologous growth factors, especially epidermal growth factor, platelet-derived growth factor, transforming growth factor  $\beta$ , and vascular endothelial growth factor, that act synergistically with other growth factors [24-26].

PRP effectiveness in wound healing has prompted its use in the treatment of depressed facial scars, along with the available treatment modalities. The use of fractional laser or light-emitting diode (LED) phototherapy along with PRP has led to substantial improvement with good cosmetic results and skin rejuvenation [27,28]. Platelet-rich fibrin (PRF), the second generation of platelet concentrate has been used with success as filler to correct deep nasolabial folds [29]. PRP also has an adjuvant role in autologous fat transfer procedures as it has booster effect on fat grafts, along with its rejuvenation capacity per se. Growth factors present in PRP promotes recovery of laser-damaged skin & accelerates tissue remodeling with the increased synthesis of collagen. So, PRP holds a promising role in soft tissue augmentation [30,31].

Autologous PRP is the plasma portion of autologously sourced blood with an iatrogenically high platelet concentration. At sites of tissue damage, platelets are the first cells to arrive and are important in mediating tissue repair through the release of growth factors from their  $\alpha$ -granules. Platelet-derived factors may influence

cellular growth, morphogenesis and differentiation and may be used therapeutically to accelerate the natural healing process [32]. Studies have consistently shown that PRP concentrates are an abundant source of GFs; moreover, the proportion of these GFs approaches the human physiological ratio, therefore PRP concentrates are more effective than any single GF. PRP has wound-healing properties, affecting keratinocytes, endothelial cells, erythrocytes, fibroblasts and collagen. Hence, PRP may improve the quality of re-epithelialization and healing. Another study observed a greater proliferation of stem cells when the skin was treated with PRP activated with calcium and thrombin than with non activated PRP. To date, PRP has been confirmed to be useful for cosmetic and plastic surgery [33,34].

## 1.1 Aim of Study

This study has been carried out to assess the efficacy of combined treatment of Fractional microneedle radiofrequency (FMR) and Platelet-rich plasma (PRP) in the improvement of facial acne scar.

## 2. PATIENTS AND METHODS

### 2.1 Study Design and Settings

A therapeutic comparative study carried out in Dermatology Sulaimani Private Center for period from 1<sup>st</sup> of November, 2014 to end of June, 2015.

### 2.2 Population of the Study

All patients with facial acne scars who attended to Dermatology Sulaimani Private Center were the study population.

### 2.3 Consent Paper

An informed consent paper was obtained for each patient before starting the procedure.

### 2.4 Exclusion Criteria for all FMR

1. History of Keloid scar.
2. Active inflammation.
3. Diabetes Miletus.
4. Connective tissue disease.
5. Oral isotretinoin use within the preceding 6 months.

6. Ablative or non-ablative laser skin resurfacing within the preceding 12 months.
7. Pregnancy and/or lactation.
8. Concurrent systemic diseases (e.g., hematologic diseases with bleeding tendency or atopic dermatitis affecting wound healing).
9. Currently taking anticoagulants or anti-platelet agents.
10. Haemodynamic instability (collapse).
11. Sepsis.

## 2.5 Sampling

A sample of 40 patients with acne scar who attended to Dermatology Sulaimani Private Center. These patients were divided into 2 group taking into consideration age, sex, severity of scar to be similarly divided between the 2 groups.

**Group A:** A sample of 22 patients was treated with FMR.

**Group B:** A sample of 18 patients was treated with PRP and FMR.

## 2.6 Data Collection

The data was collected by researcher through direct interview and fulfilling a prepared questionnaire. The patients were diagnosed by a specialist in Dermatology Sulaimani Private Center. After taking full history and examination, The questionnaire included the followings:

1. Sociodemographic characteristics: Age, gender.
2. Acne scar duration.
3. Acne scar types.
4. Physician assessment
5. Patients' assessment.
6. Complication of treatment method.
7. Pain scale during treatment.

Then we divided the patients into 2 groups As follows:

**Group A** (treated by FMR alone): For FMR cases by using RF Machin (Ellisys). We have 3 sessions for each case in interval of 1 month in between .for each session we apply the Emla cream to the face for 40 mints in nylon occlusion then clean the face, by using 49 pin head with 3 parameter (RF flounce, emission time and needle depth) are important in FMR according to

anatomical site of the scar for that we start the FMR protocol for each session with 2 passes:

A-1<sup>st</sup> superficial pass (0.6 mm in RF flounce of 5 mega hertz (mh)) for whole face B-2<sup>nd</sup> parameter pass that according to the device parameter for whole face without overlapping but Applying deep pass on the scar lesion & according to the anatomical site for depth of (1.5-2 mm in RF flounce of 7 mega hertz (mh)),

These session repeated each month for 3 sessions.

**Group B** (treated by FMR + PRP): the patients in this group were treated with the 3 sessions at 1 month interval & each sessions include two part:-

- 1- Each patient had FMR session (the FMR technique as mentioned in group A).
- 2- After half hour we started the PRP application by using Korean PRP kits we start taking 2 cc of Anticoagulant citrate dextrose solution formula A Nothrom co. (ACD-A) adding 18 cc of patients' blood into the Korean tube (e+PRP) then put in centrifuge of Korean origin (NeoGenesis) for 5 mints with speed of 3000 RPM, then we take round 4 cc of separated rich plasma from RBC and then by using mechanical activator device(PRO-PRP) 20 times ,the PRP will be ready to be injected into the scar for depth around 1 mm to 1.5 mm by (30)g needle injection for space of 3 mm in between.

These sessions repeated monthly & for 3 months.

## 2.7 Outcome Evaluation

Three photos were taken before treatment for each patient for both sides and the front of the face with a digital camera (Sony DSC-T99 Cyber-shot Digital Camera, 14.1 megapixel HD) and another set of photos taken in each visit then 1 month post- last treatment using identical camera setting, lighting and patient positioning.

Clinical assessment of improvement was assessed by the following:

- 1- patient himself (patients' assessment)
- 2- The 2 evaluators (dermatologists) asked to perform 2 actions. First, to identify the photograph that showed better scar appearance. Second, to rate the difference

in severity of the acne scars using the above mentioned scale.(physicians assessment)

The dermatologist's assessment and self-assessment level of improvement of patients were evaluated using the following five-point scale:

No improvement, Mild improvement (1-25%), Moderate improvement (26-50%), Good improvement (51-75%), Excellent improvement. (>75%).

### 2.8 Statistical Analysis

All patients' data entered using computerized statistical software; Statistical Package for Social Sciences (SPSS) version 20 was used. Descriptive statistics presented as (mean ± standard deviation) and frequencies as percentages. Multiple contingency tables conducted and appropriate statistical tests performed, Chi-square used for categorical variables and Fishers exact test was used when more than 20% of expected variable was less than 5. In all statistical analysis, level of significance (p value) set at ≤ 0.05 and the results presented as tables and/or graphs. Statistical analysis of the study was done by the community medicine specialist.

### 3. RESULTS

A total of 40 patients with acne scars were included in this study & divided into 2 groups (Group A& Group B).

**Group- A;** 22 patients of them were treated with FMR. Mean age were 29±14 years, and half of them were aging 21-29 years. Females were more than males. All these findings were shown in Table 1.

Mean duration of acne scar for group A were 9±6 years, 54.5% of them had acne scar duration of less than 10 years. These findings were shown in Table 2.

The most common Type of acne scar in Group A patients were shallow boxcar (35%), followed by; deep boxcar (32.5%), ice-pick scar (17.5%) and rolling scar (15%). All these findings were shown in Table 3 and Fig. 1.

Physicians assessing the improvement rate for all the cases completing the study of acne scar

patients treated with FMR by comparing the photos before treatment & 1 month after the 3<sup>rd</sup> & last session, in which show 36% cases with mild improvement , 27% with good improvement ,22% moderate improvement.9% no improvement & 4% excellent improvement shown in Table 4 and Figs. 2, 3.

**Table 1. Sociodemographic characteristics of acne scar patients treated with RF**

Variable	No.	%
<b>Age</b> mean±SD (29±9 years)		
< 20 years	3	13.6
20-29 years	11	50.0
30-39 years	4	18.2
≥ 40 years	4	18.2
Total	22	100.0
<b>Gender</b>		
Male	9	40.9
Female	13	59.1
Total	22	100.0

**Table 2. Acne scar duration of patients treated in group A with FMR.**

Variable	No.	%
<b>Acne scar duration</b> mean±SD (9±6 years)		
< 10 years	12	54.5
≥ 10 years	10	45.5
Total	22	100.0

**Table 3. Acne scar types of group A patients treated with FMR**

Variable	No.	%
<b>Acne scar types</b>		
Rolling scar	6	15.0
Shallow boxcar	14	35.0
Deep boxcar	13	32.5
Ice-pick scar	7	17.5
Total	40	100.0

Patient's assessment showing 27% for each mild, moderate & good improvement & 13% showing excellent improvement & 4% no improvement shown in Table 4 and Figs. 2, 3.

The treatment was generally well tolerated, during the procedure most of the patients have mild pain as well as erythema which last for 2 days. 1 Cases develop exfoliation which lasting for 5 days. All these findings were shown in Table 5 and Fig. 4.

**Table 4. Treatment outcome by physician assessment and patient's assessment for patients in group A treated with FMR**

Variable	No.	%
<b>Physicians assessment</b>		
No improvement	2	9.1
mild improvement	8	36.4
Moderate improvement	5	22.7
Good improvement	6	27.3
excellent improvement	1	4.5
Total	22	100.0
<b>Patient's assessment</b>		
No improvement	1	4.5
mild improvement	6	27.3
Moderate improvement	6	27.3
Good improvement	6	27.3
excellent improvement	3	13.6
Total	22	100.0

**Table 5. Complications after treatment with FMR**

Variable	No.	%
<b>Complication types</b>		
Treatment related pain	13	27.1
Mild erythema	19	39.5
Edema	15	31.3
Exfoliation	1	2.1
Hyperpigmentation	0	-
Scarring and infection	0	-
Total	41	100.0

**Group- B (FMR&PRP):** A total of 18 patients with acne scar were treated with FMR & PRP. Mean age 31±11 years, and half of them were aging 20-29 years. Females were more than males. All these findings were shown in Table 6.

**Table 6. Sociodemographic characteristics of acne scar patients in group B treated with FMR & PRP**

Variable	No.	%
<b>Age</b> mean±SD (31±5 years)		
< 20 years	0	-
20-29 years	9	50.0
30-39 years	8	44.4
≥ 40 years	1	5.6
Total	18	100.0
<b>Gender</b>		
Male	5	27.8
Female	13	72.2
Total	22	100.0

Mean duration in group B of acne scar for FMR & PRP patients was 10±2 years, 61.1% of them

had acne scar duration of ≥10 years. These findings were shown in Table 7.

**Table 7. Acne scar duration with acne types and acne complications acne scar patients in group B treated with RF & PRP**

Variable	No.	%
<b>Acne scar duration</b> mean±SD (10±2 years)		
< 10 years	7	38.9
≥ 10 years	11	61.1
Total	18	100.0

The common acne scar type for FMR & PRP patients in group B were deep boxcar (44.4%), followed by; shallow boxcar (33.4%), ice-pick scar (11.1%) and rolling scar (11.1%). All these findings were shown in Table 8 and Fig. 7.

**Table 8. Acne scar types of patients group B treated with FMR& PRP**

Variable	No.	%
<b>Acne scar types</b>		
Rolling scar	4	11.1
Shallow boxcar	12	33.4
Deep boxcar	16	44.4
Ice-pick scar	4	11.1
Total	37	100.0

Physicians Assessing the Improvement rate for all the cases completing the stud in Group B treated with both FMR & PRP by comparing the photos before treatment & 1 month after the 3<sup>rd</sup> & last session, in which show 44% cases with good improvement, 33% with excellent improvement, 16% moderate improvement & 5% mild improvement.

Patients' assessment showing 61% excellent improvement, 16% for good & mild improvement & 5% for moderate improvement of acne scar patients treated with FMR& PRP in Group B as details shown in Table 9 and Figs. 8, 9.

About two thirds of patients treated in group B had treatment side effects; the main side effects were mild erythema (38.7%), pain (32.2%), edema (22.6%), and ecchymosis at sit of injection in which last for 5- 6 days (6.5%). All these findings were shown in Table 10 and Fig. 10.

In physician assessment There were a significant association between excellent improvement and patients with acne scar treated in group B with both FMR & PRP (p=0.02).

**Table 9. Physician and patients assessments for patients in group B treated with FMR & PRP**

Variable	No.	%
<b>Physician assessments</b>		
No improvement	0	-
Mild improvement	1	5.6
Moderate improvement	3	16.7
Good improvement	8	44.4
Excellent improvement	6	33.3
Total	18	100.0
<b>Patients assessments</b>		
No improvement	0	-
mild improvement	3	16.7
Moderate improvement	1	5.6
Good improvement	3	16.7
Excellent improvement	11	61.1
Total	18	100.0

**Table 10. Complications after treatment in group B patients**

Variable	No.	%
<b>Complication types</b>		
Treatment related pain	10	32.2
Mild erythema	12	38.7
Edema	7	22.6
Severe peeling	0	-
Bleeding	0	-
Ecchymosis at sit of injection	2	6.5
Scarring and infection	0	-
Total	32	100.0

In patients assessment There was a significant association between excellent improvement and patients with acne scar in group B treated with both FMR & PRP (p=0.02). These findings were shown in Table 11 and Figs. 12, 13.

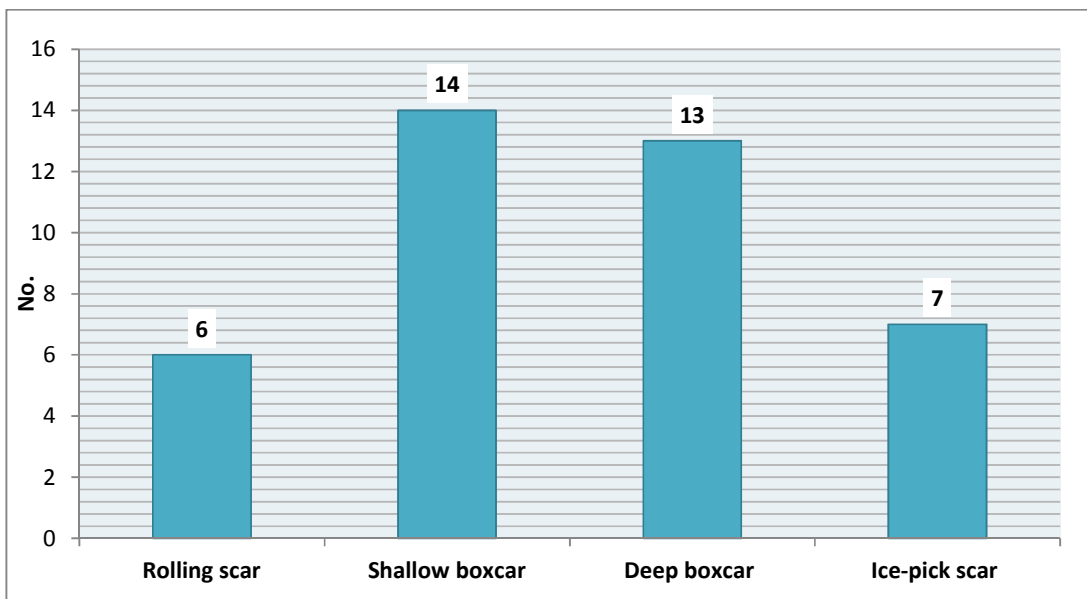
#### 4. DISCUSSION

The scarring process can occur at any stage of acne; however, it is uniformly believed that early therapy in inflammatory and nodulocystic acne is the most effective way to prevent post-acne scarring [7].

To the best our knowledge this is the first study to evaluate the combination of FMR & PRP for the treatment of facial acne scars.

Different studies were done on acne scar by using either FMR or PRP separately or other combination like autologous PRP combined with erbium fractional laser [35] but not this combination (FRM & PRP) like what is in done in our study.

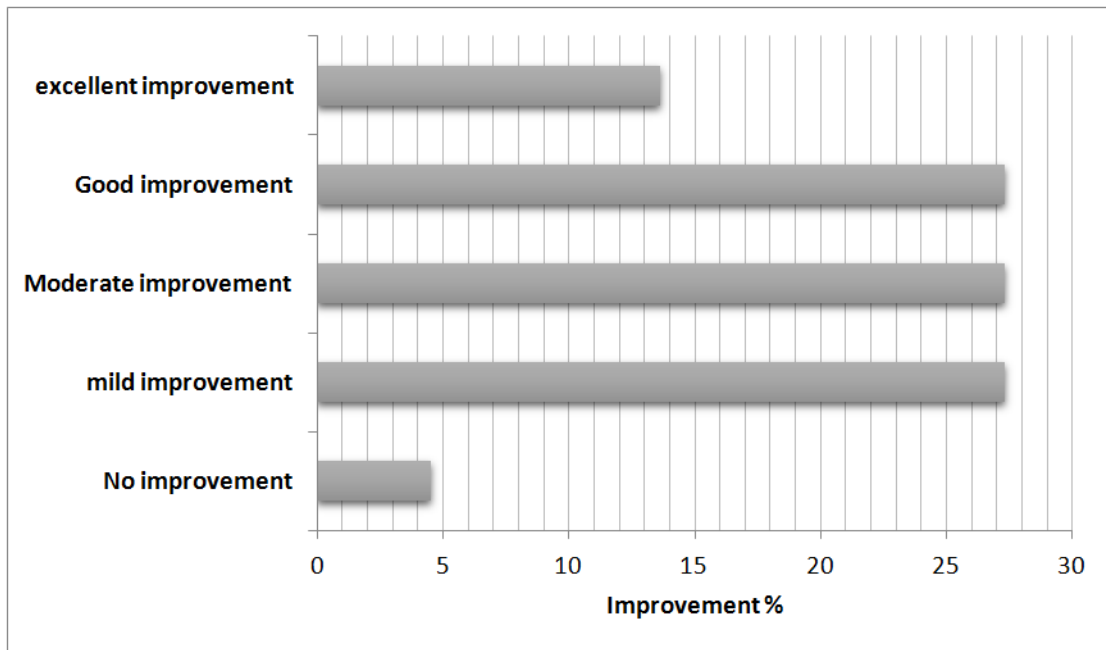
The present study revealed that the improvement in facial acne scar which were assessed by physicians and patients were significantly better among group -B(FMR & PRP) patients than group- A patients (FMR) (p=0.02) due to the synergistic effect of both treatment (FRM & PRP) which were used in group-B.



**Fig. 1. Acne scar types of patients treated with FMR**



**Fig. 2. Physicians assessment in group A**



**Fig. 3. Patients' assessment in Group A**

The mean age (between 18-45 year) in both groups of acne scars patients was close to the results of Al-Hammamy HR, et al study in Iraq [36].

The females were more than males in this study. This finding is consistent with Zhu JT, et al. [35]

study. Female patients with acne scars are more than males in seeking for acne and acne scar treatment and highly adopting cosmetics procedures.

Women are affected to a greater extent than men in post-adolescence [37].



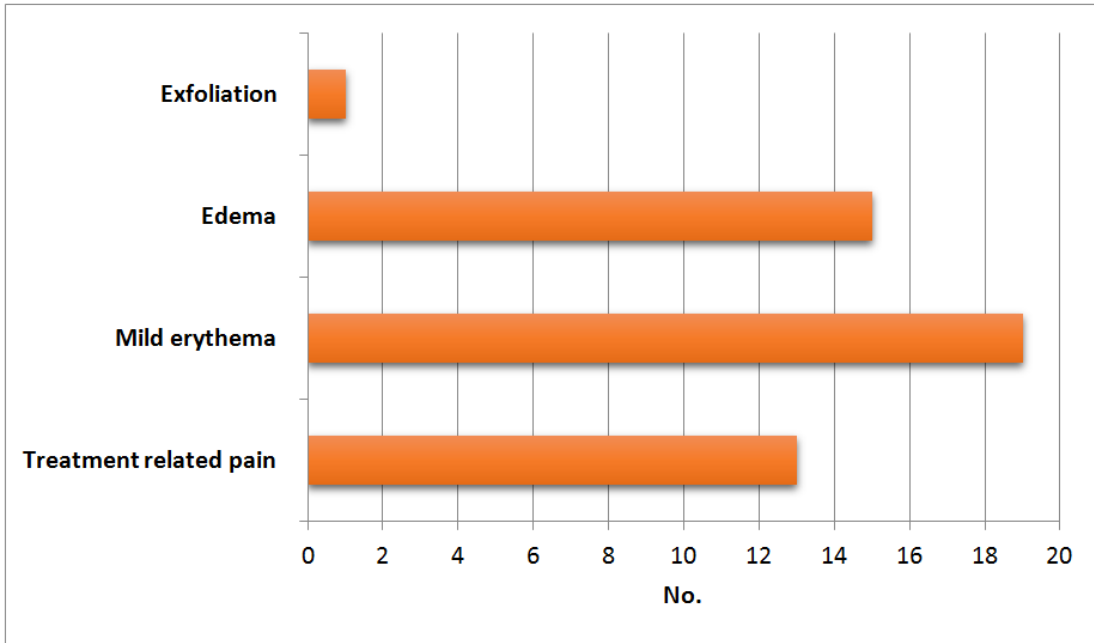
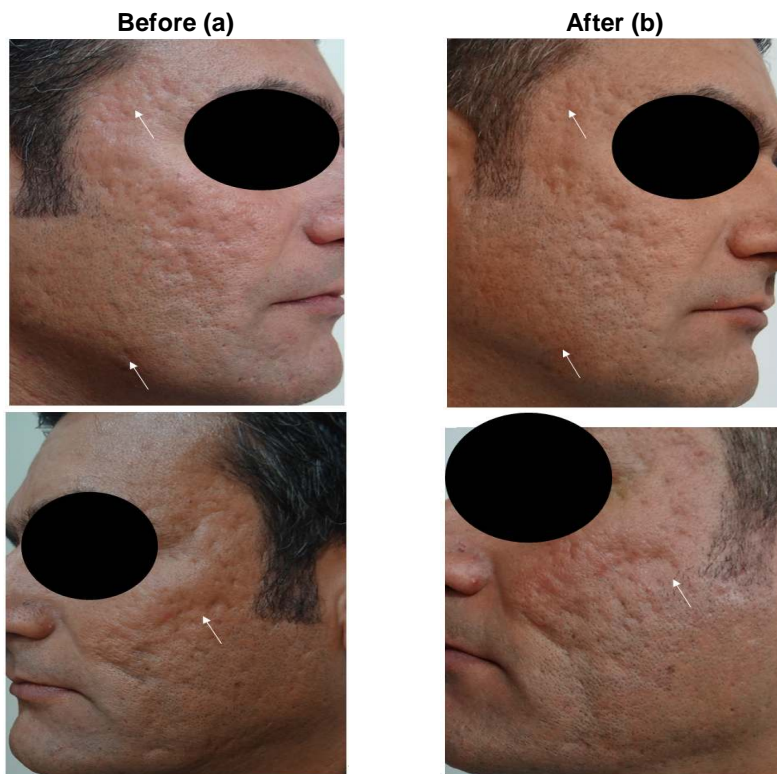


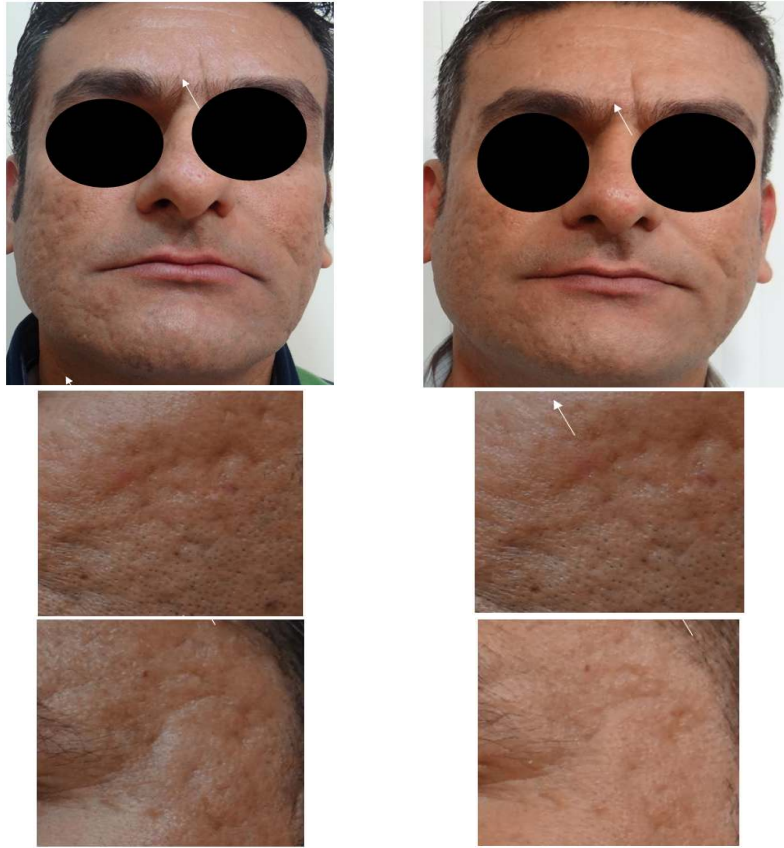
Fig. 4. Group A (FMR) side effects



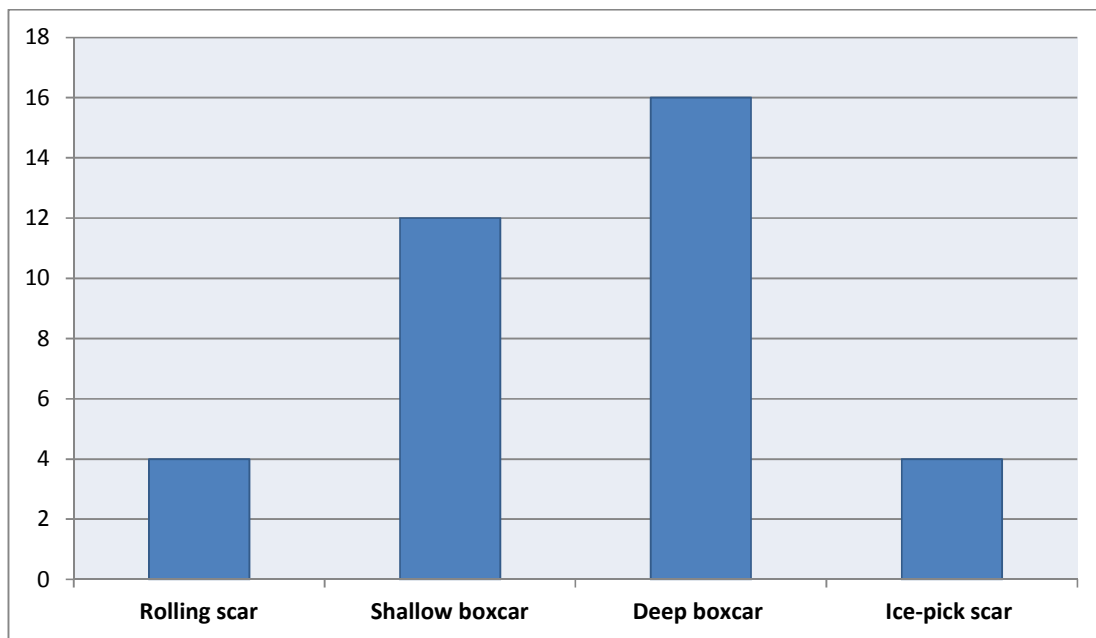


Fig. 5. (Group A) 45 year old male with acne scar pretreatment (a) & 1 month after 3 session's treatment (b) by FMR

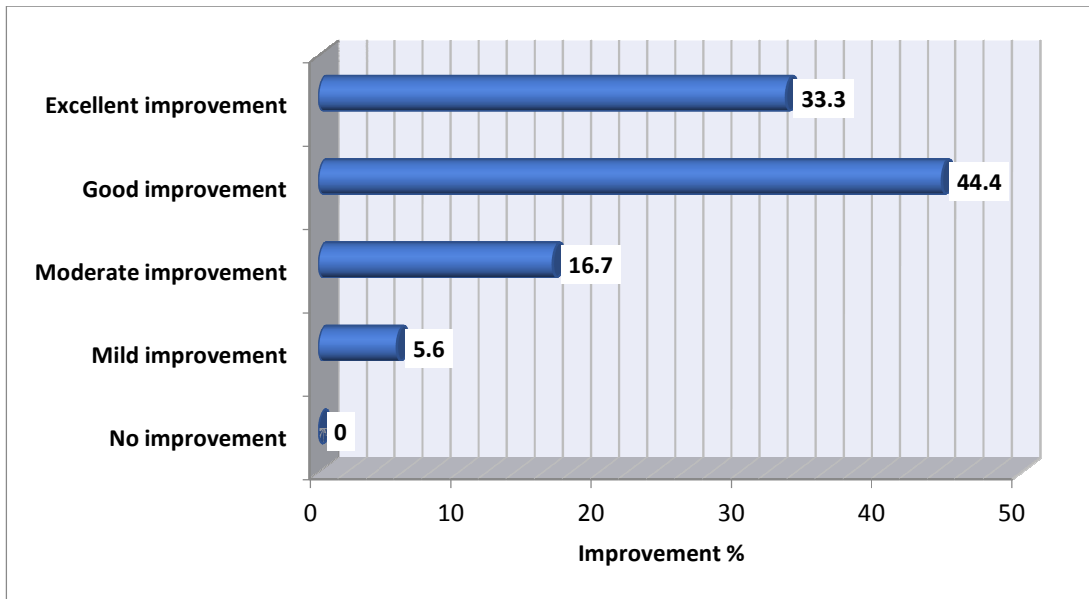




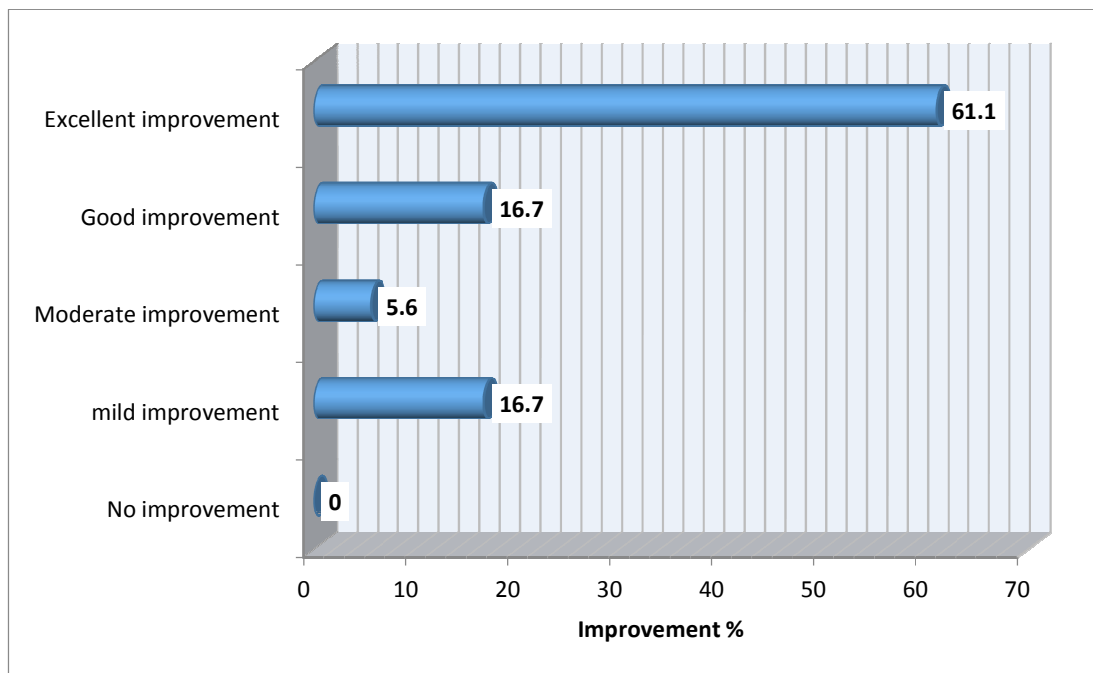
**Fig. 6. Group A) 38 year old male with acne scar pretreatment (a) & 1 month after 3 sessions treatment (b) by FMR**



**Fig. 7. Acne scar types of patients in group B treated with FMR & PRP**



**Fig. 8. Physician assessment in group B (FMR & PRP) by**



**Fig. 9. Patients' assessment in group B (FMR & PRP patients)**

Long duration of acne scars ( $\geq 10$  years) was predominant in both groups of our study. This finding is similar to results of Ramesh M, et al. [38] study in India. A study of 2,133 volunteers aged 18-70 from the general population showed that 56% of studied patients had long duration of scars [39].

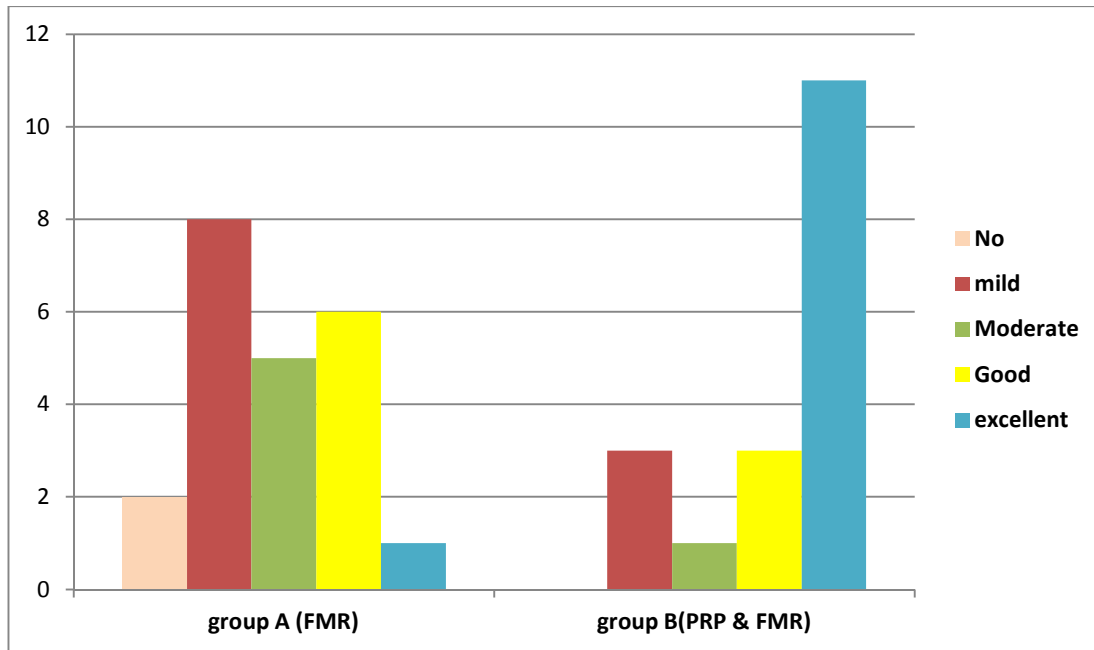
Shallow and deep boxcar types were common acne scar types in both groups. This finding is inconsistent with results of Fabbrocini G, et al. [7] study in Italy which reported that rolling scars represented 60-70% of acne scars. This inconsistency might be attributed to difference in sample size and selection.



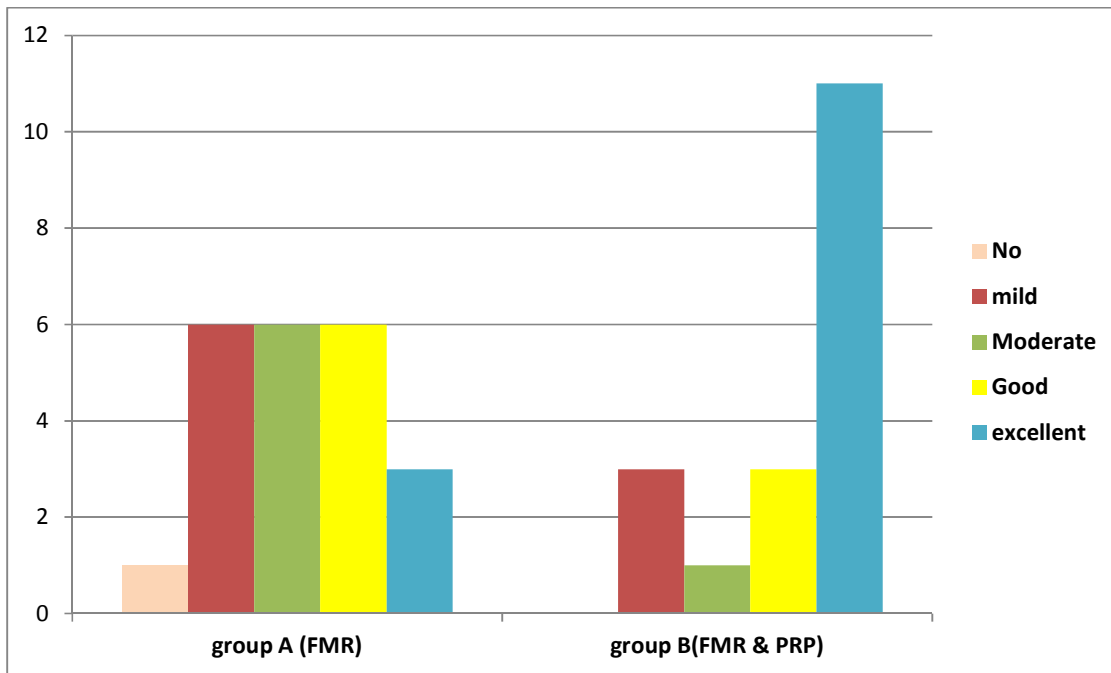
**Fig. 10. (Group B) 40 year old female with acne scar pretreatment (a)& 1 month after 3 session's(b) treatment by both FMR &PRP**



**Fig. 11. (Group B) 33 year old male with acne scar pretreatment (a)& 1 month after 3 session's (b) post treatment by both FMR &PRP**



**Fig. 12. Physician's assessment improvement in Group A & B according to treatment method**



**Fig. 13. Patient's assessment improvement in Group- A & B according to treatment methods**

For group -A (FMR alone), physician assessment show no improvement by 9.1% of acne scar patients, mild (36.4%), moderate (22.7%), good (27.3%) and excellent (4.5%). These findings are close to results of Chandrashekar BS, et al. [40]

study in India which found that FMR results were 2% of patients had no improvement, 58% of the patients had moderate, 29% had minimal, 9% had good and 3% showed very good improvement.

**Table 11. Distribution of treatment outcome according to treatment methods**

Variable	FMR		FMR & PRP		$\chi^2$	P
	No.	%	No.	%		
<b>Physician assessment</b>					11.5*	<b>0.02</b>
No improvement	2	9.1	0	-		
Mild improvement	8	36.4	1	5.6		
Moderate improvement	5	22.7	3	16.7		
Good improvement	6	27.3	8	44.4		
Excellent improvement	1	4.5	6	33.3		
Total	22	100	18	100		
<b>Patient assessment</b>					10.8*	<b>0.02</b>
No improvement	1	4.5	0	-		
Mild improvement	6	27.3	3	16.7		
Moderate improvement	6	27.3	1	5.6		
Good improvement	6	27.3	3	16.7		
Excellent improvement	3	13.6	11	61.1		
Total	22	100	18	100		

\*Fishers exact test

The assessment by patients for group-A (FMR) in our study revealed that no improvement represented by 4.5% of acne scar patients, mild (27.3%), moderate (27.3%), good (27.3%) and excellent (13.6%). This finding is close to results of Harth Y, et al. [41] study which reported that 19 out of 20 patients (95%) of the treated patients experienced some improvement. 25% experienced very good to excellent improvement (defined as >50% improvement) additional 30% experienced good improvement (defined as 26% - 50% improvement) and the rest reported some improvement (up to 25%). Only one patient did not notice any improvement.

The most common side effect of both treatment types (FMR and FMR +PRP) reported in present study was erythema. This finding is similar to results of Cho SI, et al. [17] study in South Korea and Zhu JT, et al. [35] study. Complications reported in group -A was lower than that for group B. This finding is consistent with results of Kim IS, et al study in South Korea [42].

## 5. LIMITATION OF THE STUDY

1. Small sample size.
2. Loss to follow up.
3. Difficulties in publishing female patient's photos.

## 6. CONCLUSIONS

- Fractional radiofrequency micro-needle is effective treatment method for acne scars.

- Combination of Fractional radiofrequency micro-needle and platelets rich plasma is highly effective methods for treating facial acne scars.
- The combination treatment method (FRM & PRP) had high effectiveness and highly satisfied by patients than FRM alone with low side effects.

## 7. RECOMMENDATIONS

- ❖ By using combination treatment (FRM & PRP) as a potent treatment method for patients with acne scars, minimally invasive and allow for quick recovery times that allow patients to return back to their daily routines the same day.
- ❖ Further controlled studies are required to address such comparisons. Additionally, the optimization of treatment protocols and confirmation of the efficacy of treatment should be established by clinical trials involving larger numbers of patients.

## ETHICAL APPROVAL

Ethical approval before we start the procedure we take informed consent from the participants and obtained all necessary Ethical approval from ethical and scientific committee in sulaimani medical school.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.



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