QR 678 Vs PRP –A randomised, comparative, prospective study.

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Respected Editor,

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This manuscript reports unpublished work that is not currently under consideration for Publication elsewhere. None of the authors have a financial interest in the subject or techniques described in this manuscript. The Ethical Clearance was given by The Institutional Ethical Committee, The Esthetic Clinics, Mumbai, India.

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Sincerely,

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QR 678 Vs PRP –A randomised, comparative, prospective study.

INTRODUCTION:

Appearance has a prime role to play in social outlook of any human being. Hair, accounts for a significant portion of this appearance and is pivotal in shaping the personality of an individual. Loss of hair can be distressing for a person psychologically and emotionally. It can also make an individual vulnerable to the anxiety which can add to the morbidity and inferior quality of life.[1]

Androgenetic Alopecia (AGA) is the most common form of hair loss noticed in males as well as females. [2,3]It begins to appear by the age of 20 years and is significantly seen in almost 50% of men by the age of 50 years[4] and upto 50% of women over the course of lifetime. [5-7]

It is a gradual and cumulative form of hair loss from the scalp in a specific pattern (Male pattern hair loss). The typical feature being gradual loss of hair line in male often leading to complete baldness as given by Norwood and Hamilton scale. In females there is a diffuse thinning of hair over the top of the head retaining the hairline (Female pattern hair loss). The remaining hair on the scalp is the combination of few terminal healthy hair and numerous vellus hair. Androgenetic Alopecia is complex process and its pathology is a blend of genetic and environmental factors. [8] Increased level of 5 α- reductase activity leading to increased dihydrotestosterone level has been implicated as the prime cause of Androgenetic Alopecia.[9]

Numerous medical treatment modalities have been mentioned in the literature for Androgenetic Alopecia. Platelet Rich Plasma (PRP) is a highly concentrated autologous plasma solution derived from patients own blood. It is rich in factors like Fibroblast growth factor (FGF), Vascular endothelial growth factor (VEGF), Platelet derived growth factor (PDGF), Transforming growth factor beta (TDF-β). [10,11]It has been mentioned in the literature that these growth factors, induces the follicular stem cells to shift from dormant to active state starting the process of hair production.[12]

Recently, Kapoor and Shome have introduced a new formulation called as QR678. It is a plant derivative consisting of biomimetic peptides including Sh-Polypeptide 9 [bio-mimicking Vascular endothelial growth factor (VEGF)], Sh-Polypeptide 1 [bio-mimicking Basic Fibroblast growth factor], Sh-Oligopeptide 2 [bio-mimicking Insulin like growth factor (IGF-1)], Copper tripeptide-1, Sh-Polypeptide 3 [bio-mimicking Keratinocyte growth factor (KGF-1)] Sh-Oligopeptide 4 (bio-mimicking Thymosin Beta-4 (Thymosin β4)]and vitamins, minerals and amino acids. The therapy has been proved effective in treatment of Male as well as female Androgenetic Alopecia.[2]

The aim of the present study is to compare the efficacy of QR678 therapy versus PRP in the treatment of Male Androgenetic Alopecia.

MATERIAL AND METHODS:

Study design:

A prospective, comparative single-blind study was carried after taking the approval from the review board of the Institutional Ethical committee. A total of 50 males, in the age range of 25-50 years, resident of India, were selected for the study. Patients were randomly divided into two groups (Group A –QR678 group and Group B- PRP group) of 25 patients each. Signed, written and informed consent was taken by all the participants.

Inclusion criteria:

- Male patients, in the age range of 25-50 years with Norwood Hamilton grade II-IV were selected.
- Individuals who have not responded to topical minoxidil for a period of one year or more.
- Non responders of oral Finasteride 1mg for 1 year.

Patients had been additionally guided not to change hair style or use any hair colour in the due course of study. Also, patients with hypertension, diabetes and hypercholesterolemia underwent regular monitoring for the same. All the hair growth related medications were withdrawn 6 months before the study and were not allowed during the study.

Exclusion criteria:

- History of hair loss less than 6 months.
- Patients with serious drug allergy diagnosed or suspected malignancy, autoimmune / hematologic disorders.
- Seborrheic dermatitis.
- Patients who had recently started or stopped oral Finasteride and/or minoxidil were also excluded from the study to avoid the bias due to confounding factors.

Injection Technique for Scalp:

All the patients were evaluated at the baseline and standard global photographic and videometric assessment was done to assess the condition of hair. At each visit approx. 1.5 ml solution of QR678 (containing 0.0002 mg/0.1 ml of IGF-1, 0.0002 mg of bFGF, 0.0005mg of VEGF and 0.0001 mg of KGF, 0.001 mg of copper tripeptide, and 1 × 10−6 mg of thymosin β4 in distilled water) was injected in the scalp skin of patients of group A and same amount of PRP was injected on the scalp skin of the patients of group B. On an average 60-70 tiny, intradermal injections were administered covering the visible areas of hair thinning and alopecia. Solution was injected through nappage technique using insulin syringe. Each injection was given 1cm apart with a volume of 0.02ml per injection. A total of 8 sessions were done at an interval of 3 weeks.

Scalp Assessment and Evaluation:

1. Hair pull test:

It was performed by an independent observer before starting each session to evaluate the improvement in hair loss. A bundle of 50-60 hair were grasped between thumb, index finger and middle finger and pulled from the base close to the scalp. Pulled out hair were counted. Results were evaluated at baseline, 6 months and 1 year.

2. Videomicroscopic assessment:

With the help of proscope digital handheld camera, videomicroscopic photographs were taken at the fixed position on the centre of the scalp, 20 cm posterior to glabella. The images were taken to calculate hair counts per cm.^{2} The images were analysed for hair density(cm^2), terminal hair density(cm^2), vellus hair density(cm^2) and shaft diameter(μm) using specialized software(Trilogic company, Moscow<,Russia, Tricho. Science Version 1.5). Unpaired t-test was used to assess the level of significance within the group and between the groups. Graphpad software was used to calculate the results.

3. Global photographic assessment:

Standard clinical photographs of the vertex and the superior frontal area of the head were taken for the clinical assessment at baseline, 6 months and 1 year. Photos were analysed and graded by 2 blinded dermatologist reviewers at baseline, 6 months and 1 year on a scale of 0 to +10 , where 0 represented no growth and 10 indicated full thick hair growth. The mean score was compared and plotted.

4. Patient self-assessment:

Patients completed a validated questionnaire at the end of study comprising 2 sections. 1st section had 5 questions related to the efficacy of the treatment which were to be rated on a scale of 0-5, with 0 being strongly disagree and 5 being strongly agree. The 2nd section had 4

options regarding the adverse effects due to the treatment and patients were asked to tick the appropriate response (multiple ticks were allowed). (Table 1 and 2)

RESULTS:

A total of 50 male patients were included in the study in the age range of 25-50 years. As per the Norwood Hamilton Classification of Male pattern baldness, 16 patients were in grade II, 18 were in grade III and remaining 16 patients were in grade IV of Alopecia. All the patients were equally distributed in both the groups. (Figure 1)

1. Hair pull test:

Before beginning of the treatment, the average number of hair pulled out was 10 in each group. Reduction in hair fall (i.e.: pull test became negative i.e.: number of hair pulled is 3 or less) was noted in all the patients of QR678 group by the end of 8th session (6months) whereas; the hair fall was reduced (pull test negative) in just 50% in PRP group.

The same results were maintained in group A (QR678) at 1 year follow-up. However, the average number of hair pulled was increased in PRP group at the end of 1 year (Table 3, Figure 2).

2. Videomicroscopic assessment:

The baseline and final values for hair density (cm^2), terminal hair density (cm^2), vellus hair density (cm^2) and shaft diameter (μm) at the beginning of the study and 1 year follow up have been mentioned in Table 4. Unpaired t test was done to find out the level of significance within the group. It was noted that there was a significant improvement in all the parameters in the group A (QR678) as $p<0.005$ in group A, whereas; the baseline and the final values in group B (PRP group) were not significant ($p>0.005$). Also, inter group significance was calculated using unpaired t-test. The coefficient of correlation was +0.967 and p value proved to be significant ($p<0.005$). (Table 4-5, Figure 3)

3. Global photographic assessment:

Subjective evaluation of the clinical photographs was done by 2 blinded reviewers (Figure 4 and Figure 5).Reviewers rated each photo on a scale of 0 to +10, with 0 showing no improvement and 10 showing maximum improvement. The assessment was done at baseline, 6 months and 1 year. The mean value at the baseline was 5 for group A as well as group B. Marked improvement was seen in the global assessment score in group A (mean- 7.5) which was maintained for over 1 year (mean-8). Whereas the mean assessment score in PRP group which was 6 at the end of 6 months further decreased to 4 at the end of 1 year follow up highlighting the decrease in the overall appearance of the hair once the treatment is discontinued. (Table 6, Figure 6)

It was also interesting to note that only 1 individual (4%) QR678 group showed no improvement. In PRP group 4 individuals (16%) showed no improvement while 2 patients (8%) experienced worsening. (Table 7, Figure 7)

4. Patient self-assessment:

In section A, 5 questions were asked to assess the efficacy of the treatment and patients were advised to rate it on a scale of 0-5. Higher agreement score was given for the improvement in bald spots by QR678 group (mean=4) compared to PRP group (mean= 2.5). Other factors like improvement in appearance (QR678=4.8, PRP=3), improvement in growth of hair (QR678=4.4, PRP=3.2), overall effectiveness of the treatment (QR678=4, PRP=3.8) and satisfaction with the treatment (QR678=4.5, PRP=3) was also higher in QR678 group as compared to PRP group. (Figure 8)

28% (N=7) of the patients in group A and 88% (N=22) patients in group B reported uncomfortable pain during injection. While no other adverse effects were seen in patients of Group A, the patients in group B reported itchy scalp and unsteadiness in the form of light-

headedness. 88% (N=22) patients of group B additionally reported increase in hair fall post therapy.

DISCUSSION:

Loss of hair can have a substantial influence on the psychology of an individual. It not only increases the stress level but can also be a reason of low self-esteem and depression. [13,14] Non-surgical treatment options of Male Androgenetic Alopecia are limited. To name a few, topical minoxidil and oral finasteride alone or in combination have shown good results. But they may have remarkable side effects like headache and increase in body hair with minoxidil use and loss of libido with the use of finasteride. [13, 14]

PRP is well known in the field of medicine and is defined as a volume of the plasma fraction of the autologous blood with and above baseline platelet concentration.(usually more than 1,000,000 platelets/μL)[15] It consists of growth factors (platelet-derived growth factor (PDGF), vascular endothelial growth factor and transforming growth factor-b (TGF-b) with their isoforms which play a significant role in the elongation of hair shaft.[16] These factors are present inside the Alpha granules of the platelets.[17]

Bulge area of the follicle contains primitive stem cells of ectodermal origin which gives origin to the epidermal cells and sebaceous glands. Matrix at the dermal papilla contain germinative cells of mesenchymal origin. Interaction between these two kinds of cells as well as with binding GFs (PDGF, TGF-β, and V□GF) leads to activation of the proliferative phase of the hair, giving rise to the future follicular unit. [18]

It has been mentioned in the literature that this activation is through the up regulation of transcriptional activity of β-catenin. It also induces *in vitro* proliferation of dermal papilla cells, and increases dermal papilla cell growth by activating ERK signalling. Also, PRP acts by prolonging the anagen phase of hair growth cycle by increased expression of FGF-7 and

increases the cell survival by inhibiting apoptosis (associated with increased Bcl-2 protein levels as well as activated Akt signalling) It also upregulates the perifollicular vascular plexus,by increasing VEGF and PDGF levels, which inturn have an angiogenic potential. [19]

A new formulation named QR678 (US patent 2017, FDA approval 2019) was introduced by Kapoor and Shome in 2018. A QR Code is a code used in medicine derived from "Quick Response" and the number 678 in Morse Code signifies "there is no answer". Hence, the formulation was named as QR 678 which signifies "Quick Response to a disease which earlier had no answer", i.e., to alopecia.[2] It consists of 0.0005mg of Sh-Polypeptide-9 (bio-mimicking VEGF), 0.0002 mg of Sh-Polypeptide-1 (bio-mimicking bFGF), 0.0002 mg/0.1 ml of Sh- Oligopeptide-2 (bio-mimicking IGF-1), 0.0001 mg of Sh-Polypeptide-3 (bio-mimicking KGF), 0.001 mg of copper tripeptide, and 1 × 10−6 mg of Sh-Oligopeptide-4 (bio-mimicking Thymosin β4) in distilled water .

To evaluate its safety and efficacy, the formulation was 1st tried in a preclinical animal trial where it was proved to be relatively free of untoward effects. Later on an open-label, prospective, single-arm interventional pilot study was carried out in which 1000 patients of hair loss were treated with QR678. [2]

In our study we compared QR678 with the standard PRP therapy. Marked improvement was noted with 4th session itself with QR678 and the assessment was done after 8 session and follow up till 1 year.

Kapoor et al in their study with QR678 mentioned reduction in hair pull from 10 in 1st session to 3 after 4th session, suggesting a reduction in hair fall.[2] Our study had similar results with control in hair fall after 8 sessions (6 months) and the results were maintained after 1 year as well. Besti et al in their study on PRP showed that a significant decrease in hair fall was noted with a negative pull test after 3rd session in all the patients. [20] Also Khatu et al showed a negative pull test after 4 sessions.[3] However in our study hair fall was decreased (pull test

of 3 or lesser) in upto 50% of the patients after 8 sessions in PRP group but after 1 year follow-up control in hairfall was maintained in only 30% patients.

As mentioned by Gkini et al[21] in their study with PRP, significant increase in hair density by 19,29% and 9,19% was noted at 3 and 6 months respectively but with large variability in results (from no improvement to significant improvement).Hair density followed an upward curve in the beginning, reached a peak at 3 months. A downward trend started at 6 months, which continued to decrease further at 1 year, although still maintaining its value higher than that at baseline.[21]

Other studies by Khatu et al with PRP have shown a substantial increase in the follicular density with an average of 22.09 follicular units/cm. However these studies had limitations and trichoscopic hair evaluation could not give satisfactory objective results.[3] The trichoscan requires clipping of hair and dyeing it. At times trichoscan is error prone and not precise. [22] In our study we used video microscopic assessment with the photography of the scalp at the fixed position of 20 cm from glabella ad finished the objective assessment test. The inter group as well as intra group results were significant with QR678 group.

PRP has been mentioned in the literature as a safe and effective procedure for the treatment of Androgenetic Alopecia (AGA) in some studies.[21,23] Multiple trails have also been published suggesting the role PRP on hair growth. However, most of these studies shows methodological inadequacy. [15]

One important shortcoming is the lack of standardized device and protocols that define the preferred method for producing PRP. As mentioned by Lynch and Bashir, PRP is usually prepared on a per-patient basis. Approximately 8 to 60 ml of fresh venous blood is drawn, collected and centrifuged. This leads to separation of the erythrocytes from lighter plasma with a buffy coat at the interface. The plasma and buffy coat are then aspirated and mixed.[24]

Other flaws include lack of a reference protocol mentioning the frequency of applications and the amount of PRP to be injected, heterogeneity in mode of application, small sample size, lack of controls, lack of detailed reports in patients' characteristics and used statistical methods.[15]

Apart from this, it is also cumbersome to draw patient's blood at each session. It also adds to the need of extra armamentarium and overall cost of the treatment.[2] No deaths or serious complication have been reported with the use of PRP or QR678 in the past.[2,25] In our study also, no serious side effects were noted. However few patients in group B experienced side effects like itchy scalp, unsteadiness during injection and increase in hair fall. Patients in both the groups experienced some pain during injection, more so in the PRP group (88%). Less pain with QR678 injection may be attributed to the more physiologic pH of the solution.

CONCLUSION:

The bioengineered formulation of QR678 proved to be more beneficial for Male Androgenetic Alopecia (Male pattern hair loss) compared to PRP. The unique combination of growth factors is not only safe and efficacious but the patient is comfortable during and after the procedure. This is one of its kind studies comparing QR678 with PRP showing the maximum follow-up for QR678 till date. Although the efficacy of QR678 have shown promising results for Male as well as Female Androgenetic Alopecia, more comparative studies between QR678 versus PRP and other non-surgical modalities for the treatment of Androgenetic Alopecia with long term follow up are warranted.

CONFLICT OF INTERESTS:

The authors have been awarded a patent from the **United States Patent & Trademark Office (USPTO)** & from the **Indian Patent Office** administered by the Office of the Controller General of Patents, Designs & Trade Marks (CGPDTM) for the invented hair formulation, used in this study.

FINANCIAL DISCLOSURE:

The authors have no financial interest in any of the materials used.

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TABLE LEGENDS:

Table 1: Patient Self-assessment Questionnaire; Section 1

Table 2: Patient Self-assessment Questionnaire; Section 2

Table 3: Hair Pull Test

Table 4: Hair growth parameters showing difference within and between QR678 and PRP groups (N=50)

Table 5: Mean percentage improvement in Hair growth parameters between QR678 and PRP groups (N=50)

Table 6: Global Photographic Assessment; Patients showing Improvement

Table 7: Global Photographic Assessment; Patients showing no improvement and worsening

FIGURE LEGENDS:

Figure 1: Demographic distribution of patients as per Norwood Hamilton Grading.

Figure 2: Hair Pull Test.

Figure 3: A Shows a photograph of videomicroscopic images showing vellus hair count (in red) and terminal hair count (in green). 2B: Shows a photograph of videomicroscopic image showing assessment of mean hair shaft diameter. All measurements shown were multiplied by a factor of 2.77 for conversion to microns.

Ref: https://doi.org/10.1080/14764172.2018.1439965

Figure 4: Photographs of Patient in group A- QR678.

a and b (baseline), c and d(6months) and e and f (after 1 year)

Figure 5: Photographs of Patient in group B- PRP.

a and b (baseline), c and d(6months) and e and f (after 1 year)

Figure 6: Global Photographic Assessement.

Figure 7: Global Photographic Assessment; Patients showing no improvement and worsening.

Figure 8: Patient Self-assessment Questionnaire; Section 1.

Figure 9: Patient Self-assessment Questionnaire; Section 2.

Que. No.	Question	Possible Responses (On a scale of 0-5)
1	Is the Bald spot getting any better?	Strongly disagree > Strongly agree
2.	Is there any improvement in appearance?	Strongly disagree > Strongly agree
3.	Is there any improvement in growth of Hair since start of the therapy?	Strongly disagree > Strongly agree
4.	Is the treatment effective?	Strongly disagree > Strongly agree
5.	Are you satisfied with the treatment?	Strongly disagree > Strongly agree

Table 1: Patient Self-assessment Questionnaire; Section 1

Adverse Effect	Tick the appropriate Response(if noticed)
Itchy Scalp	
Uncomfortable Pain during Injection	
Unsteadiness during injection	
Increase in hair fall	

Table 2: Patient Self-assessment Questionnaire; Section 2

Groups	Number of Hair pulled		
	Baseline	6 months	1 year
QR678	10	2	2
PRP	10	5	7

Table 3: Hair Pull Test

Variables	Outcome	Un-Paired t test					
		QR 678-Group	level of significance	PRP- Group	level of significance	t-value	df
		Mean± SD		Mean± SD			
Hair density [cm²]	Baseline	159.4 ± 47.6	p=0.001	167.2 ± 14.4	p=0.54	1.107	8
	Final	197.1 ± 52.5		176.1 ± 11.6			
Terminal hair density[cm²]	Baseline	142.7 ± 41.8	p=0.001	148.7 ± 19.7	p=0.06		
	Final	179.3 ± 47.0		155.7 ± 7.2			
Vellus hair density [cm²]	Baseline	14.8 ± 9.7	p=0.001	16.9 ± 5.4	p=0.87		
	Final	12.8 ± 8.5		15.9 ± 3.9			
Shaft diameter [µm]	Baseline	30.74±3.01	P<0.001	31.12±2.22	p=0.91		
	Final	41.77±5.64		35.45±1.41			

Table 4: Hair growth parameters showing difference within and between QR678 and PRP groups (N=50)

Hair growth parameters	QR678 Group	PRP Group	Un-Paired t test			
	Mean % distribution		*t value*	*df*	*Correlation coefficient r*	*Level of significance*
Hair density	23.60	17.28	*6.9*	*2*	*+0.967*	*p<0.005*
Terminal hair	25.64	18.15				
Vellus hair	26.23	17.32				
Shaft diameter	35.8	26.76				

**p<0.005 is considered as significant*

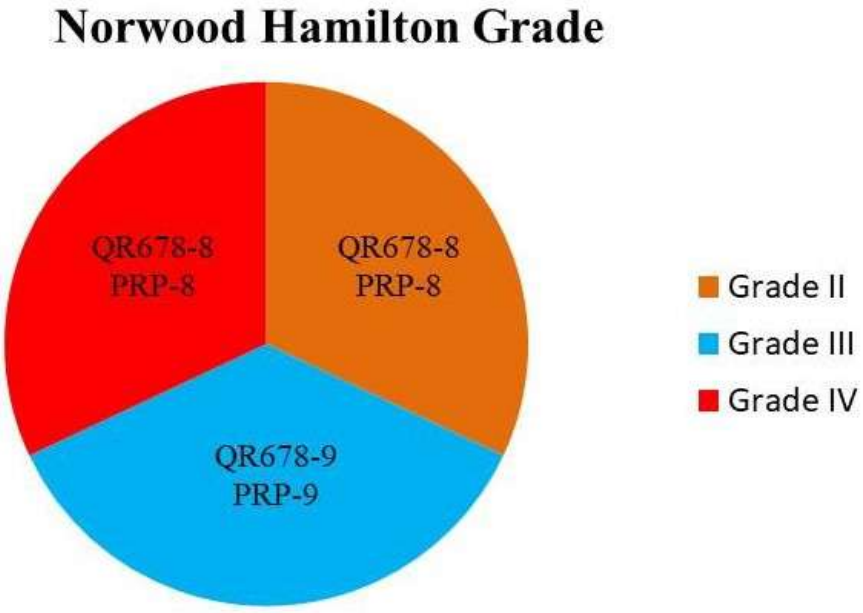
Table 5: Mean percentage improvement in Hair growth parameters between QR678 and PRP groups (N=50)

Reviewer	QR678			PRP		
	Baseline	6 months	1 year	Baseline	6 months	1 year
Reviewer 1	5	8	8	5	6	4
Reviewer 2	5	7	8	5	6	4
Mean	5	7.5	8	5	6	4

Table 6: Global Photographic Assessment; Patients showing Improvement

	No. of Patients showing no Improvement	No. of Patients showing worsening
QR678	1	0
PRP	4	2

Table 7: Global Photographic Assessment; Patients showing no improvement and worsening



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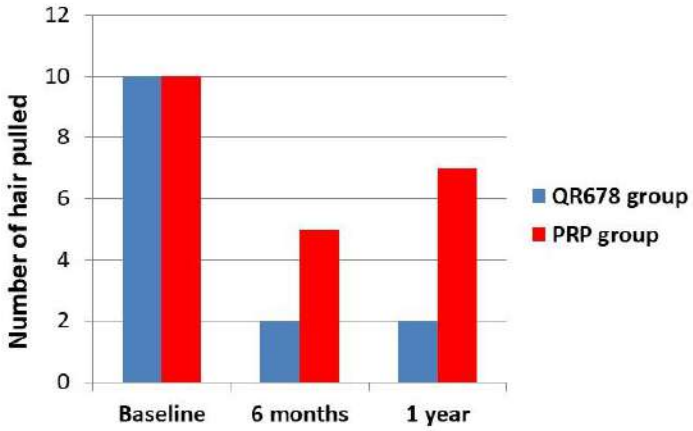

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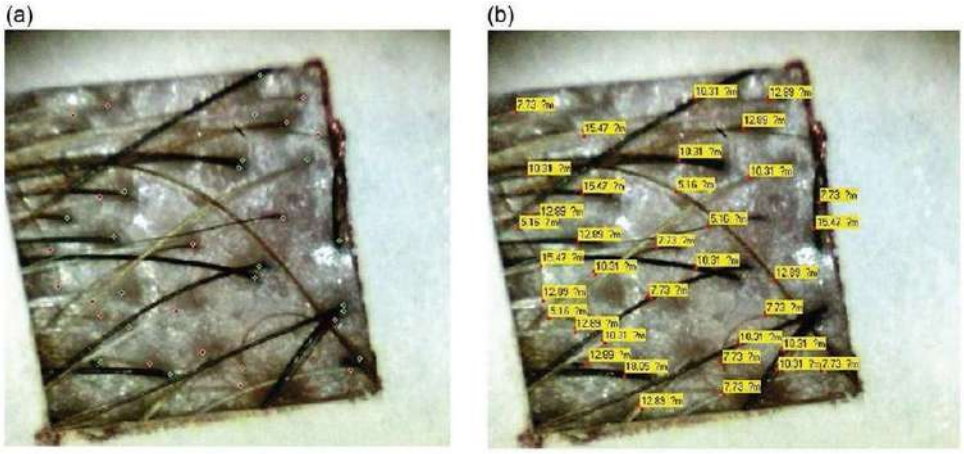

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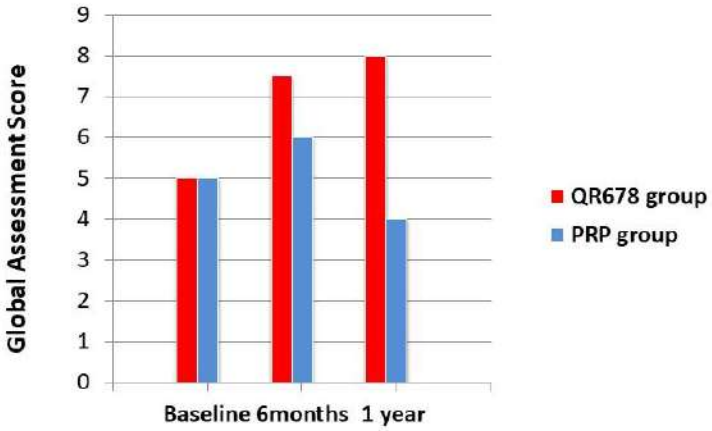
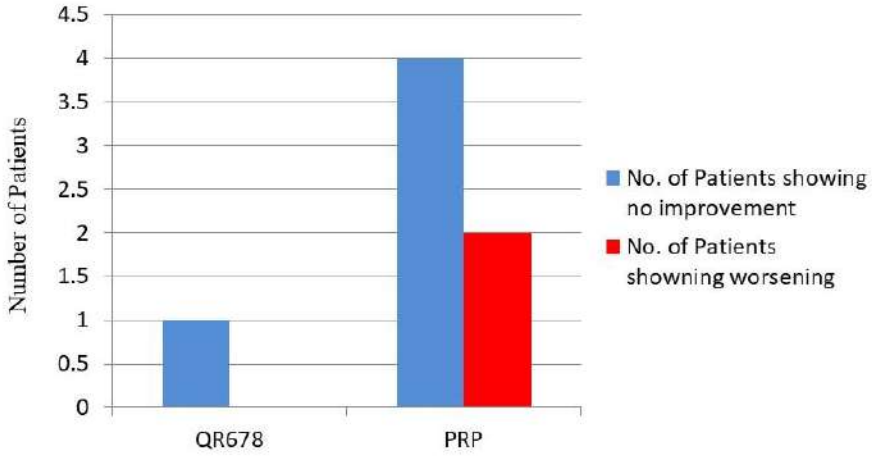
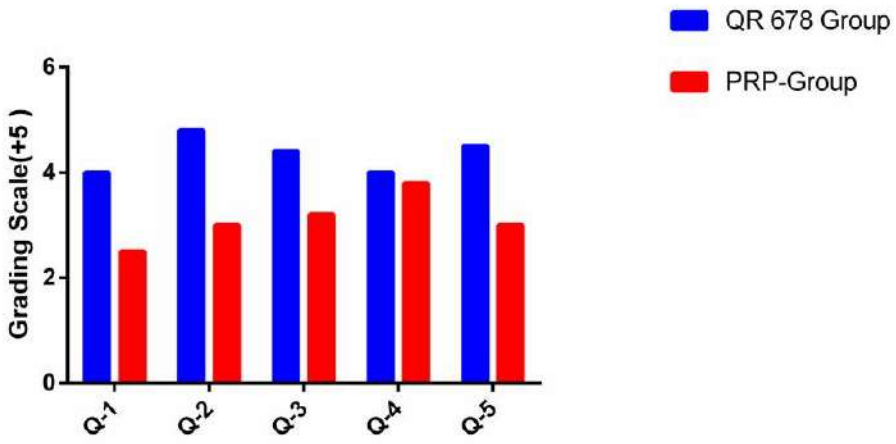


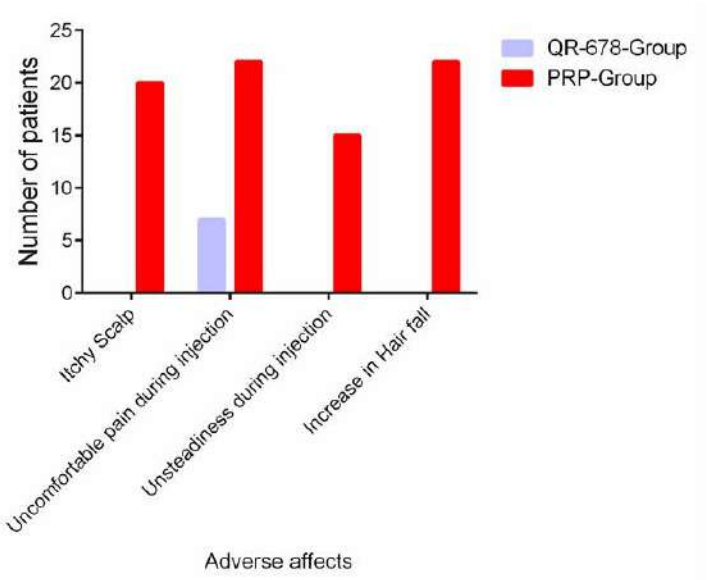
254x190mm (96 x 96 DPI)



254x190mm (96 x 96 DPI)



254x190mm (96 x 96 DPI)



254x190mm (96 x 96 DPI)